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Review Article

SARS-CoV 2 Viral Clearance in 1276 Patients: Associated Factors and the Role of Treatment with Hydroxychloroquine and Azithromycin

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Abstract

Background: The role of hydroxychloroquine (HCQ) in lowering the viral load of patients with COVID-19 is controversial.

Methods: In a retrospective observational study of data collected during care, we aimed to compare viral clearance as determined by qPCR in patients who were treated with hydroxychloroquine (HCQ) and those who were not. As a new feature, we adjusted the data according to the most significant confounding factors (age, initial viral load, and timescale between the onset of symptoms and treatment).

Results: Of the 1 276 patients selected within the hospital database, 776 were treated with HCQ and 500 were not. In the crude analysis, the time from treatment onset to viral clearance was significantly lower in the HCQ group than in the untreated group (log-rank test p<.001). When adjusted for age, initial viral load and time from symptom onset to treatment onset, the adjusted hazard ratio of viral clearance for the HCQ group remained statistically significant (hazard ratio 95% CI 1.18 [1.01-1.38], p = .037). We then performed a meta-analysis of 9 similar studies, including this one, collecting a total of 1461 HCQ-treated patients and 958 controls. It showed a shortened SARS-CoV-2 viral clearance in the HCQ group on day 7 and 14, OR 1.54 (95% CI [1.26;1.89]), OR 2.47 (95% CI [0.55;11.17] respectively.

Conclusion: although age, initial viral load, and time to treatment do influence the viral load in patients with COVID-19, hydroxychloroquine (HCQ) associated with azithromycin (AZ) still independently significantly lowered viral load more rapidly than other treatments, including azithromycin alone. As the reduction of viral load is associated with the outcome, these data strongly suggest that this treatment would be beneficial in patient with COVID-19.

Keywords: COVID-19; SARS-CoV-2; Viral Load; Viral Clearance; Hydroxychloroquine; Azithromycin

Introduction

Since the discovery of HIV and its *in vitro* culture, clinical observations have been supplemented or even replaced by monitoring the blood or plasma viral load of patients with chronic infections such as HIV and hepatitis viruses. Indeed, it is commonly accepted that a decrease in the viral load attests to an improvement in the infection or even to a cure [1]. This makes it possible to use it to judge the therapeutic effectiveness of new antiviral drugs. Thus, monitoring viral load through quantitative polymerase chain reaction (qPCR) has been recommended as a way of monitoring therapeutic efficacy [2]. In addition, this method is widely used as a gold standard marker in randomized clinical trials [3]. For example, viral load monitoring has been applied to monitor the effectiveness of treatments for viral infections such as cytomegalovirus (CMV) [4] and Epstein–Barr virus (EBV) [5]. The effectiveness of EDP-938, a nonfusion replication inhibitor of respiratory syncytial virus (RSV), was evaluated in a randomized controlled trial involving volunteers who were intranasally inoculated with the RSV-A Memphis 37b strain. This trial concluded that all EDP-938 regimens were better than placebo in terms of lowering the viral load [6]. More recently, several randomized trials on the treatment of COVID-19 have used viral load as the primary outcome, demonstrating that

ivermectin [7] reduced SARS-CoV-2 viral load in comparison with convalescent plasma [8]. Metformin glycinate has been reported to reduce SARS-CoV-2 load in a double-blind phase IIb clinical trial [9]. SARS-CoV-2 load was also shown to be associated with patient outcomes [10].

Hydroxychloroquine (HCQ) display broad-spectrum antimicrobial activity *in vitro*, including against many bacteria, fungi, and viruses. In humans, it has been used successfully to cure bacterial diseases such as chronic Q fever and Whipple's disease. In Q fever endocarditis, HCQ treatment lasts for at least 18 months, with target therapeutic levels between 0.8 µg/mL and 1.2 µg/mL [11]. Many studies have evaluated the use of hydroxychloroquine in COVID-19. Most retrospective observational studies demonstrate a benefit of using HCQ on mortality, but not most randomized clinical trials (RCT) [12]. In a very preliminary paper, we reported that 26 patients with COVID-19 treated with hydroxychloroquine (HCQ) with or without azithromycin (AZ) had a significantly shorter virus shedding period than 16 untreated patients with COVID-19 [13]. This paper was severely criticized, and we published an additional paper responding to these criticisms, in which we confirmed the reduction of the viral load in patients treated with HCQ [14]. Subsequently, in another observational study, we reported that the persistence of viral shedding over ten days was more frequent in patients who were not treated with HCQ-AZ [15]. This led us to treat COVID-19 Patients with HCQ and AZ "off label", waiting new drug development. While RCT suggested that this treatment was not efficient, we reported that is our experience, on 30.000 patients treated "of label" the retrospective analysis showed that HCQ and AZ reduced mortality in patient with COVID-19 [12]. Several confounding factors may affect the outcome. The viral clearance of SARS-CoV-2 has been reported to depend upon age, given that the duration of shedding is shorter in younger patients [16-18]. Other confounding factors include the timescale between the onset of symptoms and admission [19], being immunocompromised, and the initial viral load [20]. Armed with this new knowledge, we aim here to reanalyze the data, investigating the role of HQC in the viral shedding of patients with COVID-19.

Material and Methods

This is a retrospective, observational cohort study. Data provided during epidemiological interviews and clinical and biological assessments were recorded in the hospital information system (HIS). For the purposes of this study, data from patients hospitalized between 3 March 2020 and 13 March 2021 were extracted from the HIS.

Data collection

We first selected all patients hospitalized in our hospital within the one-year study period. To avoid bias or the use of inappropriate data, we excluded 440 patients, those who were immunocompromised such as HIV, diabetes, ongoing cancer (3), those misdiagnosed as having COVID-19 (3), those treated with ivermectin alone (48), one minor patient (under the age of 18), 273 patients who were hospitalized for less than three days, 95 patients for whom treatment started more than four days after admission, and 17 patients for whom the timescale between admission and the first qPCR test was more than 15 days (Figure 1). Day zero "D0" was defined as the date treatment started (first treatment received). We then retained those for whom a positive qPCR was obtained between D0-1 and D0+1. For patients who were treated with HCQ and/or AZ. For patients who were not treated with HCQ or AZ, "D0" was defined as the date of admission to the Institut Méditerranée Infection (IHU). Finally, we included those who had a second (positive or negative) qPCR test between D0+1 and D0+10. All qPCR tests were performed in the same laboratory. When the Cycle threshold (Ct) of the qPCR was over 35 cycles, it was considered to be negative [21]. Explicative variables such as age, initial viral load, date of onset of symptoms, treatment, and death were extracted from the HIS in compliance with the provisions of the General Data Protection Regulation (GDPR). HCQ was proposed at 200mg per day for 10 days and AZ at 250mg twice the first day and 250mg per day for 5 days. We identified four treatment groups: those who were treated with the HCQ regimen; those who did not receive HCQ; those treated with a combination of HCQ and AZ; and those treated only with AZ. We conducted an initial analysis of treatment with HCQ (with or without AZ) compared to treatment without HCQ (AZ alone or nothing) and a second analysis comparing patients treated with HCQ and AZ to those receiving AZ alone (excluding those receiving HCQ alone).

Statistics

Patients for whom data were available for 30 days after the onset of treatment (from D0 to D0+30) were selected. Patients who did not become PCR-negative during the follow-up period were censored on the date of their last available positive PCR test during the follow-up period. The survival function was estimated by nonparametric Kaplan–Meier survival analysis.

Confounding factors such as age, baseline SARS-Cov-2 viral load, and the time from onset of disease to the onset of treatment were controlled by using the multivariable Cox proportional hazards

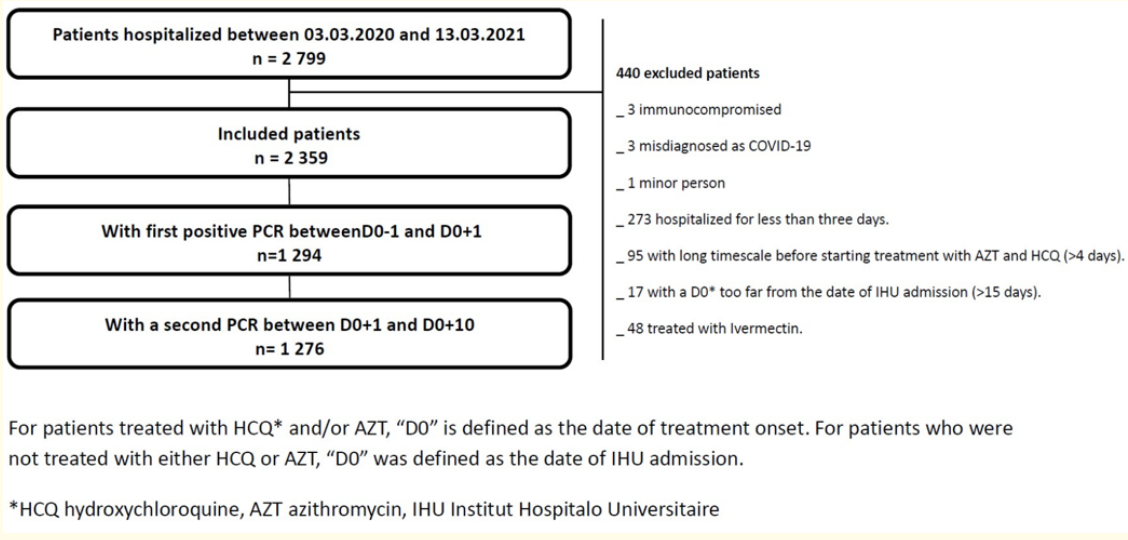

Figure 1: Study flowchart.

model and the multivariable Fine Gray sub distribution hazard model.

The multivariable Cox proportional hazards model was used to identify factors associated with the probability of having a negative viral load during follow-up. Based on the available literature (see above), the model was adjusted for age, baseline SARS-CoV-2 load assessed through qPCR, and the time from the onset of symptoms to the onset of treatment.

We also performed a sensitivity analysis using a competing risk approach. For patients who did not become qPCR negative during the follow-up period, when death occurred before the end of the follow-up period, it was considered a competing event. When patients were still alive at the end of the follow-up period, they were censored on the date of their last available positive qPCR test. The time-cumulative incidence of patients with a negative viral load according to treatment group was estimated by nonparametric competing risk analysis. We then used the multivariable Fine-Gray sub-distribution hazard model [36] to identify factors associated with the probability of having a negative viral load during follow-up.

A two-sided α value of less than 0.05 was considered to be statistically significant. Competing risk analysis was carried out using the LIFETEST and PHREG procedures in the Statistical Analysis System (SAS) 9.4 statistical software (SAS Institute, Cary, NC).

Random effect meta-analyses of binary outcomes were performed using the 'metabin' function from the 'meta' package in R [22]. Treatment success in these studies was defined as negative PCR for SARS CoV-2 on day 7 (first meta-analysis) or day 14 (second meta-analysis) from enrollment in hospitalized patients.

The inverse variance weighting was used to calculate the pooled odds ratio, and the Paule-Mandel estimator was used to calculate the between-study heterogeneity variance. Sensitivity analyses were performed using different pooling methods (Peto, Mantel–Haenszel) and variance estimators (restricted maximum-likelihood, maximum-likelihood).

Ethics and regulation

The retrospective nature of the study was approved by our independent ethics committee (No. 2021–15). To comply with the General Data Protection Regulation (GDPR), all patients were informed that their personal and medical data might be used for research purposes unless they refused. The investigators' declaration to comply with methodology reference MR 004 was filed prior to the onset of this study and was the subject of a declaration in GDPR Register of Assistance Publique Hôpitaux de Marseille No. 2020-152. The end of data use for analysis and treatment (for the meta-analysis) was 2023, October 12.

Results

Inclusion and exclusion

Of the 2 799 patients hospitalized during the study period, we excluded 440 for the reasons described above. Of the 2 359 patients included, we selected those with a first PCR test result obtained within 48 hours of admission (n = 1 294) and those who had a PCR within the first ten days of care (n = 1 276) (Figure 1). Of them, 747 were qPCR negative (> 35 Cycle threshold) (Ct) within 30 days of follow-up, and 529 were censored at the date of their last positive qPCR during follow-up (Table 1).

	HCQ	No HCQ	p
	N=776	N=500	
Number of patients with negative viral load within 30 days	480	267	
Censors*	296	233	
n at risk – Survival probability (% positive) with 95%CI			
D0	776	500	
D5	317 - 50.0 [46.0-53.9]	247 - 63.2 [58.4-67.6]	
D10	76 - 23.0 [19.0-27.1]	75 - 33.4 [27.8-39.2]	
D15	23 - 10.3 [7.0-14.4]	29 - 21.7 [16.3-27.7]	
D20	8 - 5.2 [2.7-9.0]	9 - 7.1 [3.0-13.6]	
D30	1	1	
Time from treatment onset to viral clearance			p<.0001 (Log-Rank)
Q1 95%CI	3**	4 [3-4]	
Median 95%CI	6 [5-6]	8 [7-8]	
Q3 95%CI	10 [9-11]	13 [11-16]	
Mean (std)	7.3(0.3))	9.2 (0.4)	
Crude Hazard ratio 95%CI***	1.39 [1.20-1.61]	Ref.	<.0001
Adjusted Hazard ratio 95%CI****	1.18 [1.01-1.38]	Ref.	.0375

Table 1: Time from treatment onset to viral clearance (HCQ/No HCQ, n=1 276). HCQ hydroxychloroquine.

*: Patients were censored at the time of their last available positive PCR.

**: Confidence interval not estimable.

***: Univariate Cox Proportional-Hazards model.

****: Multivariable Cox Proportional-Hazards model. Hazard ratio is adjusted on age, baseline PCR SARS-CoV-2 (CT), viral load and time from symptom onset to treatment onset (see table 1).

Comparison of treatment with HCQ versus no HCQ

The population analyzed included 776 people who received HCQ and 500 who did not receive hydroxychloroquine. Patients in the HCQ-treated group were significantly younger than those in the group not treated with HCQ; they had a longer time from symptom onset to treatment onset and a lower baseline viral load (Table 2). It should be noted (see above) that these three factors were likely to affect viral clearance in favor of treatment. In the crude analysis, the time from treatment onset to viral clearance was significantly lower in the HCQ group than in the untreated group (log-rank test p < .001) (Table 1, Figure 2). For example, on D0+5 days, 50.0% (95% CI [46.0%-53.9%]) of patients in the HCQ group were still qPCR positive, compared to 63.2% (95% CI [58.4%-67.6%]) of patients in the non-HCQ group. At D0+10 days, 23.0% (95% CI [19.0%-27.1%]) of patients in the HCQ group were still positive, compared to 33.4% (95% CI [27.8%-39.2%]) in the non-HCQ group (Table 1). Overall, the probability of viral clearance was significantly higher in the group treated with HCQ (hazard ratio 95% CI 1.39 [1.20–1.61], p < .0001).

When adjusted for age, initial viral load and time from symptom onset to treatment onset, which were potential confounding factors, the adjusted hazard ratio of viral clearance for the HCQ group remained statistically significant (hazard ratio 95% CI 1.18 [1.01-

	HCQ	No HCQ	p-value*
	N=776	N=500	
Age - (col %)			
<50	16.8	6.2	<.001
50-59	18.8	10	
60-69	22.9	20.6	
70-79	18.7	22.6	
>79	22.8	40.6	
Time from symptom onset to treatment onset (days) - Mean(std)	6.4(4.3)	5.4(4.8)	<.001
Baseline PCR SARS-CoV-2 (CT) viral load - Mean(std)	26.1(5.3)	25.2(5.5)	.004

Table 2: Study population characteristics according to treatment groups (HCQ/No HCQ, n=1 276) HCQ : hydroxychloroquine.

*: Chi-square test / Wilcoxon Two-Sample Test.

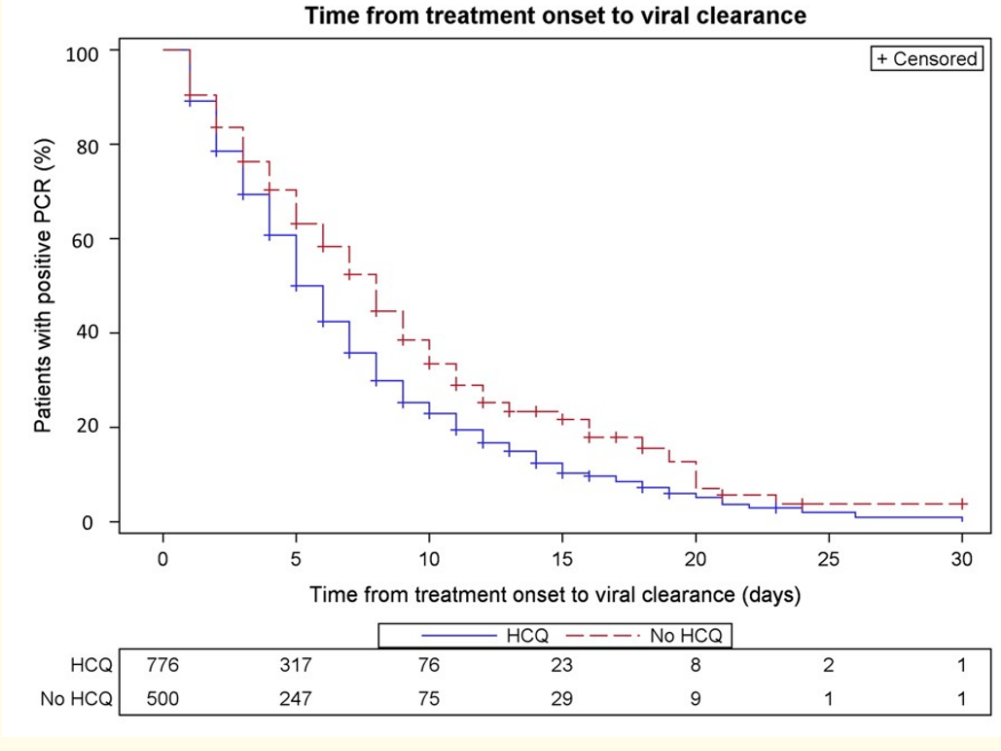

Figure 2: Time from treatment onset to viral clearance according to treatment groups (HCQ/NO HCQ) – Kaplan-meier curves (n = 1276). *HCQ hydroxychloroquine.

1.38], p = .037), suggesting that HCQ treatment had a significant impact on the probability of viral clearance within 30 days of the onset of treatment (Table 3). We noted a decrease in the probability of negativization as age increased (hazard ratios = 1, 0.90, 0.72, 0.59, and 0.50 for patients aged <50, 50-59, 60-69, 70-79 and >79, respectively). When the Ct of the first qPCR increases by one unit, the probability of negativization increases by 12% (hazard ratio = 1.12), i.e., the lower the initial viral load is, the greater the probability of a negative result. Finally, an increased likelihood of negativization was observed when the time to treatment was longer (a longer time was associated with a lower viral load at the time of treatment).

		Hazard ratio 95% CI			p
Treatment (ref. No HCQ)	HCQ	1.18	1.01	1.38	0.037
Age (ref.<50)	50-59	0.90	0.69	1.17	0.438
	60-69	0.72	0.56	0.92	0.008
	70-79	0.59	0.45	0.76	<.0001
	>79	0.50	0.39	0.64	<.0001
Baseline PCR SARS-CoV-2 (CT) viral load		1.12	1.11	1.14	<.0001
Time from symptom onset to treatment onset (days)		1.03	1.02	1.04	<.0001

Table 3: Factors associated with negative viral load – Multivariable Cox Proportional-Hazards model (n=1 276). HCQ : hydroxychloroquine.

When treatment with both HCQ and AZ was compared to treatment with AZ alone, similar results were obtained, suggesting that the essential element in lowering the viral load is treatment with HCQ (supplementary data, Figure 1S, Table 1S, Table 2S). The sensitivity analysis, using a competitive risk approach (death was considered a competing event), yielded similar results (supplementary data, Figure 2S, Table 3S, Table 4S, 5S).

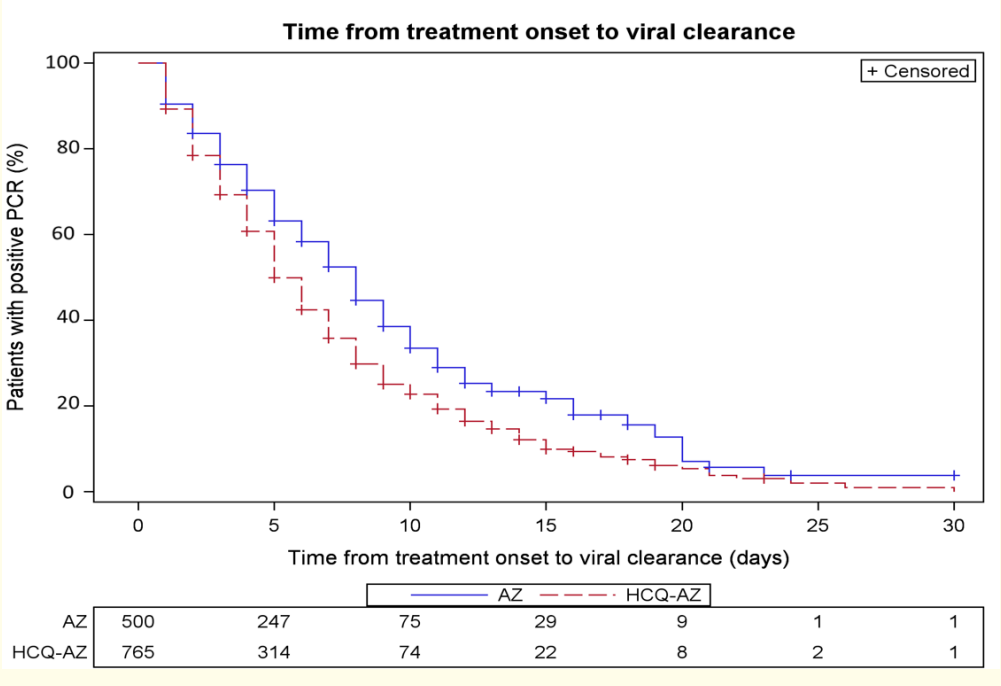

Figure 1S: Time from treatment onset to viral clearance according to treatment groups (HCQ-AZ/AZ) – Kaplan-Meier curves (n=1 265) *HCQ hydroxychloroquine, AZ azithromycin.

	HCQ-AZ	AZ	p
	N=765	N=500	
Number of patients with negative viral load within 30 days	474	267	
Censors*	291	233	
n at risk – Survival probability (% positive) with 95%CI			
D0	765	500	
D5	314 - 49.9[45.9-53.9]	247 - 63.2[58.4-67.6]	
D10	74 - 22.7[18.8-26.9]	75 - 33.4[27.8-39.2]	
D15	22 - 9.9[6.7-14]	29 - 21.7[16.3-27.7]	
D20	8 - 5.4[2.7-9.2]	9 - 7.1[3.0-13.6]	
D30	1	1	
Time from treatment onset to viral clearance			p<.0001 (Log-Rank)
Q1 95%CI	3**	4 [3-4]	
Median 95%CI	5 [5-6]	8 [7-8]	
Q3 95%CI	10 [9-11]	13 [11-16]	
Mean (std)	7.3(0.3)	9.2(0.4)	
Crude Hazard ratio 95%CI***	1.39 [1.20-1.62]	Ref.	<.0001
Adjusted Hazard ratio 95%CI****	1.18 [1.01-1.38]	Ref.	.0403

Table 1S: Time from treatment onset to viral clearance (HCQ-AZ/AZ, n=1 265).

*: Patients were censored at the time of their last available positive PCR.

**: Confidence interval not estimable.

***: Univariate Cox Proportional-Hazards model.

****: Multivariable Cox Proportional-Hazards model. Hazard ratio is adjusted on age, baseline PCR SARS-CoV-2 (CT), viral load and time from symptom onset to treatment onset (see table 1).

		Hazard ratio 95% CI			p
Treatment (ref. AZ)	HCQ-AZ	1.18	1.01	1.38	0.040
Age (ref.<50)	50-59	0.89	0.68	1.16	0.391
	60-69	0.71	0.55	0.91	0.007
	70-79	0.58	0.45	0.76	<.0001
	>79	0.49	0.38	0.63	<.0001
Baseline PCR SARS-CoV-2 (CT) viral load		1.12	1.11	1.14	<.0001
Time from symptom onset to treatment onset		1.03	1.01	1.04	0.001

Table 2S: Factors associated with negative viral load – Multivariable Cox Proportional-Hazards model (n= 1 265).

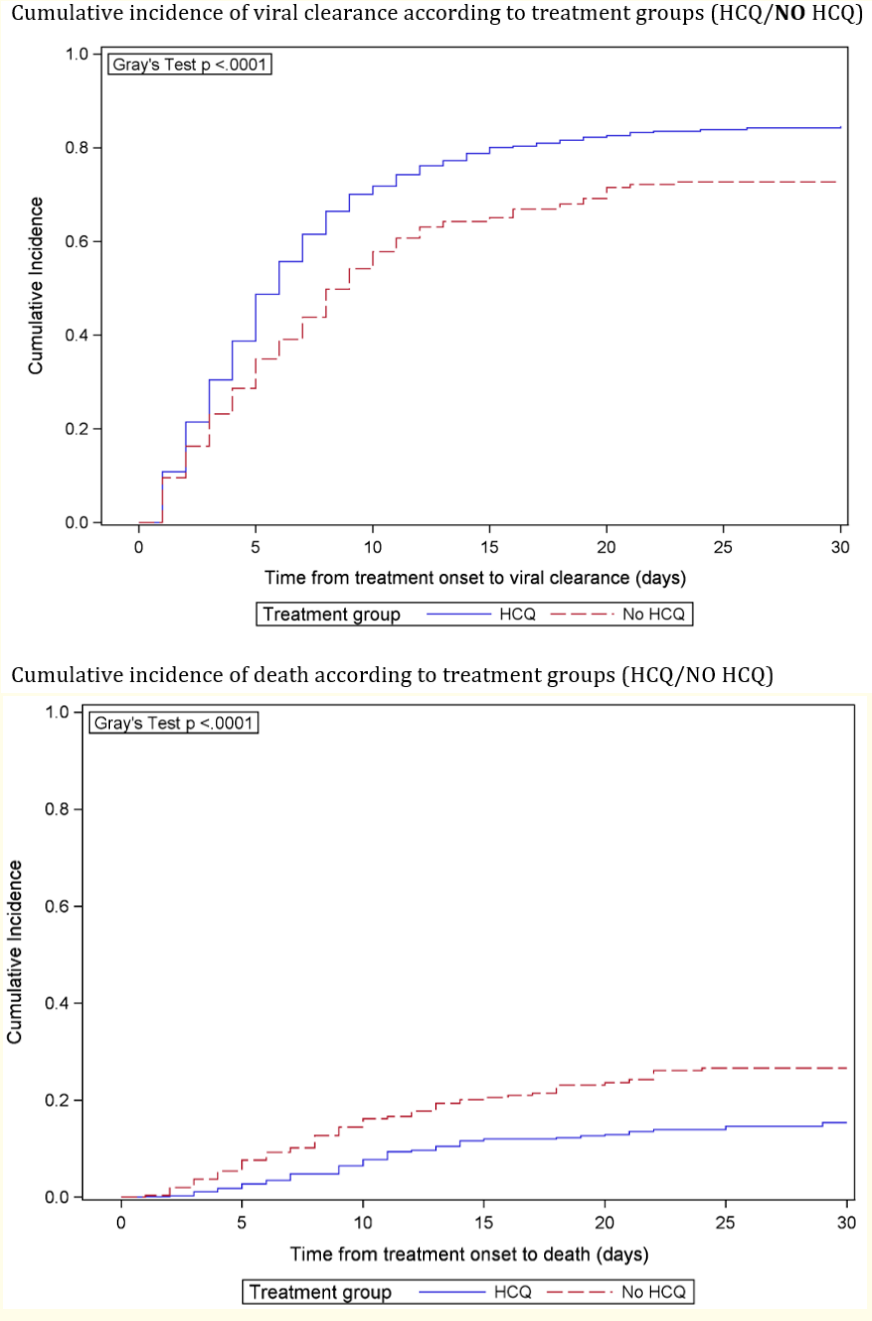

Figure 2S: Non-parametric competing risk analysis (n=1 276).

	HCQ	No HCQ	p-value
	N=776	N=500	
Number of patients with negative viral load within 30 days	480	267	
Number of patients with competing event (death)	64	83	
Number of censored patients	232	150	
Cumulative incidence of patients with negative viral load (% with 95% CI)			p<.0001
D0	0.0%	0.0%	
D5	48.8% [44.8-52.6]	35.0% [30.6-39.4]	
D10	71.8% [67.7-75.5]	57.8% [52.6-62.7]	
D15	80.1% [76.1-83.5]	65.1% [59.8-69.9]	
D20	82.6% [78.8-85.8]	71.6% [66.1-76.3]	
D25	83.9% [80.2-87.0]	72.7% [67.3-77.4]	
D30	84.7% [80.8-87.8]	72.7% [67.3-77.4]	
Crude Hazard ratio 95%CI	1.46 [1.27-1.69]	Ref.	<.0001
Adjusted Hazard ratio 95%CI*	1.20 [1.03-1.40]	Ref.	.0205

Table 3S: Time-cumulative incidence of patients with negative viral load according to treatment groups (HCQ/No HCQ) – Non-parametric competing risk analysis (n=1 276).

*: Hazard ratio is adjusted on age, baseline PCR SARS-CoV-2 (CT) viral load and time from symptom onset to treatment onset (see table 1).

		Hazard ratio 95% CI			p
Treatment (ref. No HCQ)	HCQ	1.20	1.03	1.40	.0205
Age (ref.<50)	50-59	0.91	0.71	1.16	.4378
	60-69	0.74	0.58	0.94	.0127
	70-79	0.56	0.44	0.72	<.0001
	>79	0.45	0.35	0.58	<.0001
Baseline PCR SARS-CoV-2 (CT) viral load		1.12	1.11	1.14	<.0001
Time from symptom onset to treatment onset (days)		1.04	1.02	1.06	<.0001

Table 4S: Factors associated with negative viral load – Multivariable Fine-Gray sub distribution hazard model (n=1 276).

	HCQ (n=776)					No HCQ (n=500)				
Day	At risk	n censored	n negatives	n negatives (cumulated)	Survival probability (% positive)	At risk	n censored	n negatives	n negatives (cumulated)	Survival probability (% positive)
0	776	0	0	0	100,0%	500	0	0	0	100,0%
1	776	23	84	84	89,2%	500	13	48	48	90,4%
2	669	67	80	164	78,5%	439	41	33	81	83,6%
3	522	58	61	225	69,3%	365	38	32	113	76,3%
4	403	36	50	275	60,7%	295	25	23	136	70,3%
5	317	31	56	331	50,0%	247	27	25	161	63,2%
6	230	22	35	366	42,4%	195	23	15	176	58,3%
7	173	13	27	393	35,8%	157	13	16	192	52,4%
8	133	13	22	415	29,9%	128	13	19	211	44,6%
9	98	7	15	430	25,3%	96	8	13	224	38,6%
10	76	9	7	437	23,0%	75	5	10	234	33,4%
11	60	2	9	446	19,5%	60	5	8	242	29,0%
12	49	4	7	453	16,7%	47	2	6	248	25,3%
13	38	4	4	457	15,0%	39	3	3	251	23,3%
14	30	2	5	462	12,5%	33	4	0	251	23,3%
15	23	1	4	466	10,3%	29	4	2	253	21,7%
16	18	1	1	467	9,7%	23	3	4	257	17,9%
17	16	0	2	469	8,5%	16	1	0	257	17,9%
18	14	1	2	471	7,3%	15	2	2	259	15,6%
19	11	1	2	473	6,0%	11	0	2	261	12,7%
20	8	0	1	474	5,2%	9	0	4	265	7,1%
21	7	0	2	476	3,7%	5	1	1	266	5,7%
22	5	0	1	477	3,0%	5	0	0	266	5,7%
23	4	1	0	477	3,0%	3	0	1	267	3,8%
24	3	0	1	478	2,0%	2	1	0	267	3,8%
25	2	0	0	478	2,0%	1	0	0	267	3,8%
26	2	0	1	479	1,0%	1	0	0	267	3,8%
27	2	0	0	479	1,0%	1	0	0	267	3,8%
28	2	0	0	479	1,0%	1	0	0	267	3,8%
29	2	0	0	479	1,0%	1	0	0	267	3,8%
30	1	0	1	480	0,0%	1	1	0	267	3,8%

Table 5S: Time from treatment onset to viral clearance - Number at risk/censored/negatives from D0 to D30 (HCQ/No HCQ, n=1 276).

Discussion

The role of HCQ with or without AZ on SARS-CoV-2 clearance was assessed several times in the literature as the main or secondary outcome in both RCTs and observational retrospective studies [23-32]. Although all these studies were not perfectly comparable in terms of methods, nine of them evaluated qPCR negativity at days 7 and 5 at 14 days (table 4 and 5). The only large study that included 349 HCQ-treated patients and 151 controls concluded that there was no difference in viral clearance. While this is true on day 14, the general conclusion is erroneous, as the number of qPCR-negative patients on day 7 was 182/349 (52.1%) in the HCQ group versus 54/151 (35.8%) in the control group, which is highly

significant (p = 0.001) [26]. This suggests that the critical period for viral clearance in treated patients is within the first 7 days. This is in accordance with our previous findings and our results herein with a median viral clearance at 6 days in the HCQ-treated patient and 8 days in untreated controls [13,15]. Most of the published studies included a very small number of patients and controls, and all of them reported a nonsignificant improvement in viral clearance. This suggests that the nonsignificant differences reported in previous studies should be likely interpreted as "nonconclusive" in relation to the underpowered nature of the study. Singh., *et al.* in 2021 published the first meta-analysis on clearance of SARS-CoV-2 in HCQ-treated patients. With a total of 92 and 46 patients analyzable at days 7 and 14, respectively, no difference was found in terms of viral clearance [33]. At the same time, we updated the meta-analysis and demonstrated that viral load was shortened by HCQ [34]. To go further, we have combined all these studies in a new meta-analysis (Table 4 and 5). The meta-analysis regrouping 1461 HCQ-treated patients and 958 controls showed a shortened SARS-CoV-2 clearance in the HCQ group on day 7 and 14, OR 1.54 (95% CI [1.26;1.89]), OR 2.47(95% CI [0.55;11.17] respectively (Table 4 and 5). In the current study, we wanted to evaluate SARS-CoV-2 clearance in patients who could be analyzed according to the different treatments they received, including treatment with and without HCQ and treatment with the combination of HCQ plus AZ. The results presented herein confirm those of the first study we carried out: the virus disappeared more rapidly in the nasopharynx of patients treated with HCQ and AZ than in other patients.

			HCQ			NO HCQ		
Study	**Study DOI**	**Study type**	**Events**	**Total**	**%**	**Events**	**Total**	**%**
Chen J., *et al.*	https://doi.org/10.3785/j.issn.1008-9292.2020.03.03	RCT	13	15	86,7%	14	15	93,3%
Tang W., *et al.*	https://doi.org/10.1136/bmj.m1849	RCT	37	75	49,3%	35	75	46,7%
Dabbous., *et al.* °	https://doi.org/10.21203/rs.3.rs-83677/v1	RCT	27	50	54,0%	24	50	48,0%
Kamran., *et al.*	https://www.medrxiv.org/content/10.1101/2020.07.30.20165365v2	RCT	182	349	52,1%	54	151	35,8%
Ulrich., *et al.*	https://doi.org/10.1093/ofid/ofac567	RCT	8	67	11,9%	10	61	16,4%
Lecronier M., *et al.*	https://doi.org/10.1186/s13054-020-03117-9	RO*	7	38	18,4%	2	22	9,1%
Byakila kibwika., *et al.***	https://doi.org/10.1186/s12879-021-06897-9	RCT	20	55	36,4%	19	50	38,0%
Rodrigues C., *et al.* **	https://doi.org/10.1016/j.ijantimicag.2021.106428	RCT	13	36	36,1%	9	34	26,5%
This study		RO*	393	776	50,6%	192	500	38,4%
* Retrospective observational, ** outcome at day 6, ° retracted paper								

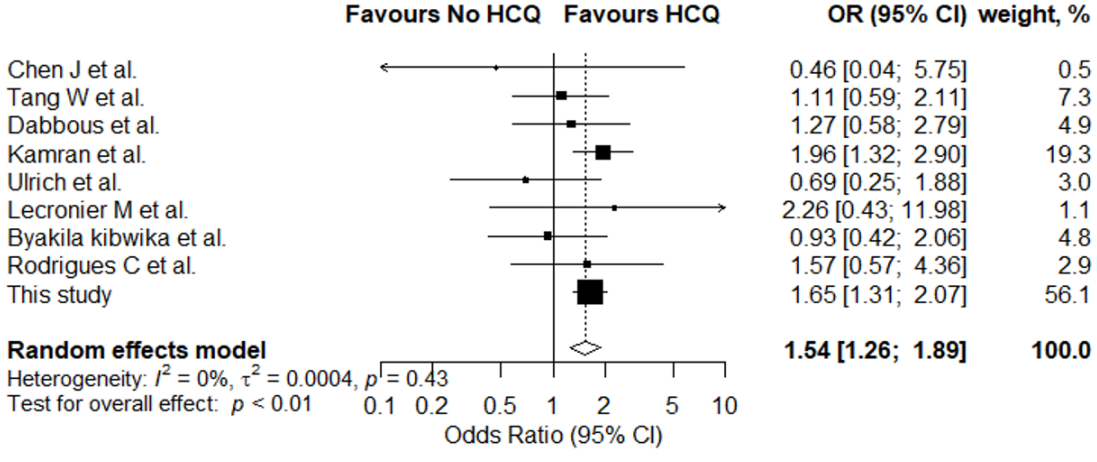

Table 4: HCQ versus no HCQ for treatment outcome negative PCR for SARS CoV2 at day 7 from enrolment in hospitalized patients.

			HCQ			NO HCQ		
Study	**Study DOI**	**Study type**	**Events**	**Total**	**%**	**Events**	**Total**	**%**
Chen CP., *et al.*	https://doi.org/10.1371/journal.pone.0242763	RCT	17	21	81,0%	2	12	16,7%
Tang W., *et al.*	https://doi.org/10.1136/bmj.m1849	RCT	13	75	17,3%	10	75	13,3%
Kamran., *et al.*	https://www.medrxiv.org/content/10.1101/2020.07.30.20165365v2	RCT	244	349	69,9%	110	151	72,8%
Huang M., *et al.*	https://doi.org/10.1093/nsr/nwaa113	RCT	189	197	95,9%	140	176	79,5%
This study		RO*	462	776	59,5%	251	500	50,2%

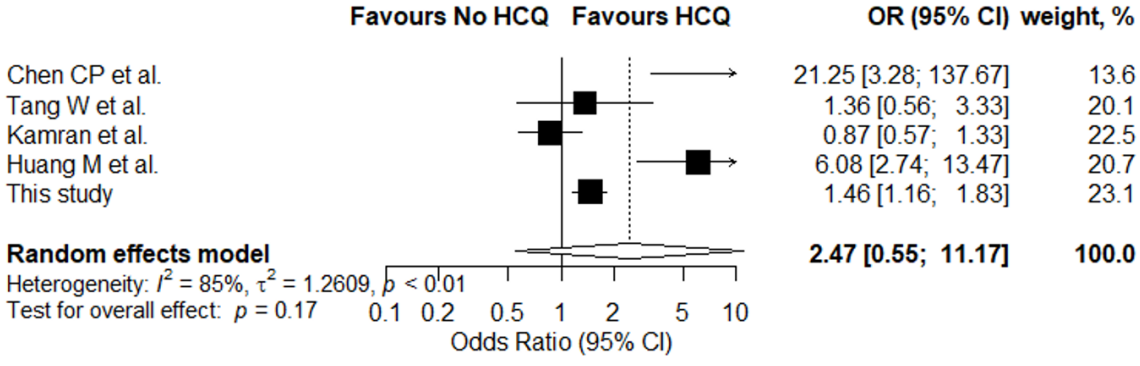

Table 5: HCQ versus no HCQ for treatment: outcome negative PCR for SARS CoV2 at day 14 from enrolment in hospitalized patients.

*Retrospective observational

This study avoids a certain number of biases. The treatment dosage was always the same at 200 mg three times a day. Patients were all treated in the same place by the same team, and therefore, the standard of care (control patients) was the same for all patients, reinforcing the internal validity of the study. The cutoff points for defining qPCR negativity were the same at 35 cycles thresholds, a cutoff that has been confirmed by the fact that at 35 cycles onwards, there is no longer any virus alive in the inoculated samples [35]. As age, time from symptoms to treatment, and initial viral load are recognized as confounding factors [16-20], we included these variables in the multivariate model. The weakness of this study is due to its retrospective nature. As selection bias, at the time of care, the discharge of patients from the hospital isolation facilities was based upon 2 negative qPCR results. To comply with guidelines and be able to free the bed as soon as possible, we repeated the PCR sampling every day until negativation. Even under these conditions, only 54% of patients (1276/2359) had a first PCR within the first 24 hours and a second PCR within 10 days. One can imagine that the excluded patients were the less severe patient not needing a viral load follow up reinforcing the internal validity of the study. Nevertheless, as a single-center study, our findings may not be generalizable to other health care settings or patient populations. Finally, as memory bias, this was controlled by the mean of a standardized medical questionnaire. Potential confounders were controlled by multivariable Cox proportional hazards model and the multivariable Fine Gray sub distribution hazard model.

In conclusion, we found that HCQ treatment significantly increased the probability of viral clearance by 20% independent of age, time to symptoms and initial viral load. This was confirmed after accounting for the difference in mortality between the HCQ-treated and untreated groups by a multivariable Fine-Gray sub distribution hazard model [36] with a similar 20% risk difference. The median time to negative qPCR was decreased by 2 days (6 vs 8 days), which may have important consequences for the individual (decreased risk of virus-related complications) and public health level (contagiousness, epidemic dynamics). In addition, we were able to show that this statistical effect was specific to HCQ treatment and not to HCQ-AZ dual therapy, in favor of a specific biological effect of HCQ for nasopharyngeal viral clearance. These data confirmed the efficacy of HCQ and AZ on viral clearance of SARS-CoV2 in patient with COVID-19 and are in accordance with clinical observational studies on treatment efficacy published in the literature. CT.

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Data Availability Statement

Raw data with survival are available in supplement data files.

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