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Abstract

During the Covid-19 pandemic, many intensive care unit (ICU) patients received hydroxychloroquine. The primary objective of this study was to assess the effects of hydroxychloroquine according to its plasma concentration in ICU patients. A single-center retrospective study was performed from March to April 2020 in an ICU of a university hospital. All patients admitted to the ICU with confirmed Covid-19 pneumonia and treated with hydroxychloroquine were included. The study compared 17 patients in whom the hydroxychloroquine plasma concentration was in the therapeutic target (on-target) and 12 patients in whom the plasma concentration was below the target (off-target). The follow-up of patients was 15 days. No association was found between hydroxychloroquine plasma concentration and viral load evolution (P = 0.77). There was no significant difference between the two groups for duration of mechanical ventilation, length of ICU stay, in-hospital mortality, and 15-days mortality. These findings indicate that hydroxychloroquine administration for Covid-19 patients hospitalized in ICU is not associated with improved outcomes. Larger multicenter studies are needed to confirm these results.

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Keywords: Hydroxychloroquine, Intensive care unit, Covid-19 pneumonia

Introduction

In March 2020, the World Health Organization announced the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak [1]. Many patients were admitted to intensive care units (ICUs) for acute respiratory failure in the context of Covid-19 [2]. The usefulness of antivirals and other drugs used in these patients is not based on strong evidence.

Hydroxychloroquine, a drug mainly used to prevent and treat malaria [3], stops viruses entering the cells by inhibiting glycosylation of host receptors, proteolytic processes and endosomal acidification, and it has immunomodulatory effects by decreasing the cytokine storm [4]. Hydroxychloroquine has an antiviral activity for SARS-CoV-2 in vitro [5]. Gautret et al. reported that hydroxychloroquine and azithromycin were associated with viral load reduction in nasopharyngeal samples in patients after six days of treatment [6]; however, ICU patients were not included in this study. The Surviving Sepsis Campaign guidelines on the management of Covid-19 patients concluded there was insufficient evidence to recommend the use of antiviral drugs and hydroxychloroquine in ICU patients [7]. In addition, the use of two different dosing regimens of this drug did not affect the outcomes of critically ill patients [8]. The aim of the current study was to determine the effects of hydroxychloroquine in ICU patients by measuring plasma concentrations of hydroxychloroquine and comparing patients whose concentrations were within the therapeutic target (on-target) to patients whose concentrations were below the therapeutic target (off-target).

Methods

Design

This single-center, retrospective, observational study was performed in ICU at North Hospital of Marseille from 16th March 2020 to 19th April 2020.

Ethical considerations

The study was approved by the Committee for Research Ethics of French Society of Anesthesia & Intensive Care Medicine (CERAR no. IRB 00010254 - 2020 - 059). Patients were informed regarding the use of their data. Strategies were considered standard care; consent was not required.

Population

Confirmed Covid-19 patients with acute respiratory failure were included in the study if they met the following criteria: i) aged at least 18 and; ii) polymerase chain reaction (PCR)-documented SARS-CoV-2 in nasopharyngeal samples upon ICU admission. Exclusion criteria were known allergy to hydroxychloroquine; a contraindication to

treatment like retinopathy, glucose-6-phosphate dehydrogenase deficiency or QT prolongation; preexisting treatment that might interact with hydroxychloroquine, and treatment with another drug. Two groups were identified: i) patients with hydroxychloroquine plasma concentration above the target concentration of 0.1 µg/mL and a full treatment ("on-target group") between [5]; ii) patients with hydroxychloroquine plasma concentration below the target or treatment discontinuation ("off-target group").

Study protocol

Upon ICU admission, patient demographic, clinical and biological data for each patient were collected, and the Simplified Acute Physiology Score II (SAPS II) and the Sepsis-related Organ Failure Assessment (SOFA) score were calculated. Covid-19 features, onset of disease, and respiratory and systemic symptoms were reported. Use of catecholamines and duration of mechanical ventilation were also recorded. All patients underwent an electrocardiogram for the detection of QT prolongation. Virus load was determined from nasopharyngeal swab samples collected every 72 h. Recovery was defined as two consecutive negative nasopharyngeal swab samples [9]. Follow-up for each patient was 15 days.

Treatment consisted of an 800-mg loading dose of hydroxychloroquine and maintenance dose of 400 mg for 9 days. Plasma concentration of hydroxychloroquine was measured every 72 h to adjust dose in the Laboratory of Pharmacokinetics and Toxicology (Timone Hospital – Marseille). The analytical method was previously validated according to European Medicine Agency guidelines and was linear in the 0.015–2.00 µg/mL range [10]. An additional treatment consisted of a 500-mg loading dose of azithromycin and 250-mg maintenance dose and cefotaxime (6 g continuous infusion) for 5 days. Early treatment discontinuation and side effects were recorded.

Outcomes

The primary endpoint was the reduction/disappearance of SARS-CoV-2 in patient samples at Day 15. The secondary endpoints were the number of days before obtaining a negative PCR, length of ICU and hospital stays, length of mechanical ventilation, use of vasopressor and 15-days mortality.

Statistical analysis

No statistical samples were performed a priori, and sample size was equal to the number of treated patients during the period. The X^2, Fisher's exact test, t test and Mann Whitney test were used to compare variables between on-target and off-target groups, as appropriate. For viral load, the data were analysed to confirm whether the first

endpoint was reached at Day 15. Statistical significance was defined as $P < 0.05$. Analyses were performed using Prism 7 (GraphPad Software, San Diego, CA, USA).

Results

From 16th March to 19th April 2020, 35 Covid-19 confirmed cases were referred to the ICU, 6 of whom were excluded (5 patients received other antiviral drugs and 1 patient had missing data). Finally, 29 patients (17 in the on-target group and 12 in the off-target group) received hydroxychloroquine and azithromycin according to the protocol (Figure 1A). Upon ICU admission, no significant differences in demographic characteristics, severity scores and clinical symptoms were observed between the two groups (Table 1).

Plasma concentrations of hydroxychloroquine in the two groups are shown in Figure 1B. Hydroxychloroquine was discontinued in 75% of patients in the off-target group and 6% of patients in the on-target group ($P < 0.001$). Side effects, notably cardiac conduction disorders, were reported in 1 (6%) patient in the on-target group and 6 (50%) patients in the off-target group ($P = 0.01$).

Primary outcome

On Day 15 after ICU admission, nasopharyngeal swab PCR results were negative in 8 (67%) patients in the off-target group and 11 (65%) patients in the on-target group ($P = 0.77$). At Day 1, the viral load was 25 ± 12 Ct in the on-target group and 30 ± 4 Ct in the off-target group ($P = 0.43$). At Day 15, no statistical difference was found between the two groups (Figure 2).

Secondary outcomes

PCR results were negative on Day 7 in the on-target group and on Day 6 in the off-target group ($P = 0.71$). From Day 1 to Day 15, viral load reduction was similar in the on-target group (-15.2 ± 16.2 Ct) and the off-target group (-19.9 ± 18.0 Ct) ($P = 0.45$). The numbers of patients still in ICU and in hospital at Day 15 were similar in the two groups ($P > 0.05$; Table 1). Duration of mechanical ventilation and use of vasopressors were also similar ($P = 0.92$ and $P = 0.95$, respectively). No statistical difference was found in 15-day mortality rate (0 [0%] patient in the on-target group and 2 [17%] patients in the off-target group, $P = 0.16$) (Table 1).

Discussion

The current study compared patients in whom the hydroxychloroquine plasma concentration reached the therapeutic target to those in whom it did not. Viral load at Day 15, viral clearance and clinical endpoints did not differ significantly between the two groups.

The benefits of hydroxychloroquine for Covid-19 patients are still debated. Due to potential side effects, its indication should be carefully balanced. In ICU patients, the use of antiviral drugs is also discussed. Oseltamivir, which is used to treat or prevent influenza, appears to have no benefits for critically ill patients [11]. In the current study, the mean duration between symptom onset and treatment initiation was seven days, which probably made this treatment ineffective [12]. Antiviral drugs seem to be effective at the onset of infection, and their beneficial effects diminish as the disease progresses [11].

In the current study, patients in whom hydroxychloroquine did not reach the therapeutic concentration were used as controls. The pharmacokinetics of hydroxychloroquine have been described [5]. The clinical and viral courses of the disease were similar regardless of the plasma concentration of hydroxychloroquine, indicating a low probability of efficacy in these patients [13]. Moreover, an 800-mg bolus dose followed by daily 400-mg doses did not reach a plasma therapeutic concentration in 14 (82 %) patients between Days 4 and 6. Furthermore, there were a significant number of side effects. These side effects may have been related to the medical histories and comorbidities of the patients and to interactions with other drugs [14]. They resulted in treatment discontinuation in seven patients and were not associated with plasma concentrations.

The current study has several limitations. It is a retrospective series with a small patient sample and no placebo group. The effects of azithromycin, which also prolongs QT interval, were not clearly considered as an accompanying factor. Moreover, although the two groups were similar in most demographic and clinical variables, undetermined variables may have resulted in differences between them. The negative results of PCR were meaningful, but the comparison of viral load is controversial because of the limitation of the technical problem to collect samples. Finally, the plasma concentration was arbitrarily determined to reach the therapeutic value between Days 4 and 6, which seems reasonable if an effect is to be expected by Day 15. The choice was based on in vitro data and is debatable [5].

In conclusion, the current study results show there was no association between hydroxychloroquine plasma concentration and viral and clinical evolution in Covid-19 patients admitted to the ICU. This finding indicates that the

use of hydroxychloroquine at this stage of disease would be not useful. Randomized controlled trials are required to show whether this drug could be useful in ICU patients admitted for Covid-19 [15].

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Declarations

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Competing Interests: ML served as lecturer for MSD, Octapharma and 3M and consultant for Gilead, Aguettant and Amomed. All other authors report no conflicts of interest.

Ethical Approval: The study was approved by the Committee for Research Ethics of French Society of Anesthesia & Intensive Care Medicine (CERAR no. IRB 00010254 - 2020 – 059).

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Table 1. Demographic and Clinical Findings

Figure 1:

(A) Flow chart

(B) Plasma concentration of hydroxychloroquine in the two groups

The median and [IQR] plasma concentrations of hydroxychloroquine (HCQ) were 0.18 [0.14-0.25] vs 0.06 [0.04-0.8] ($P < .001$) µg/mL for the on-target and off-target group, respectively (Unpaired t test with Welch's correction).

We did not represent patients who were a treatment discontinuation.

Figure 2: Viral load in nasopharyngeal swab at Day 15 in the two groups

The viral load (in cycle threshold [Ct] of PCR assay) between the on-target and off-target groups ($P = 0.98$*).*

Figure 1A: Flow chart

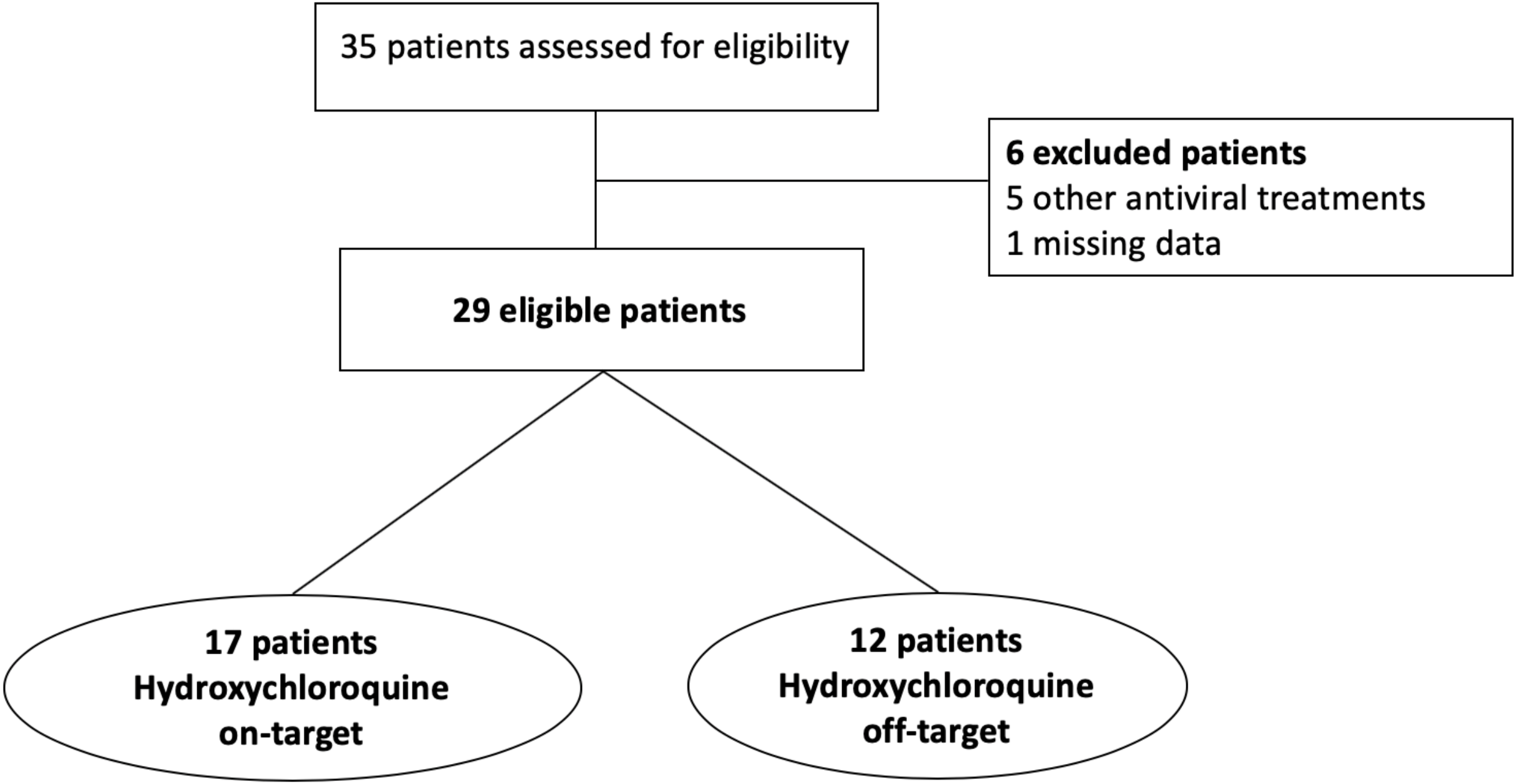

Figure 1B: Plasma concentration of hydroxychloroquine in the two groups

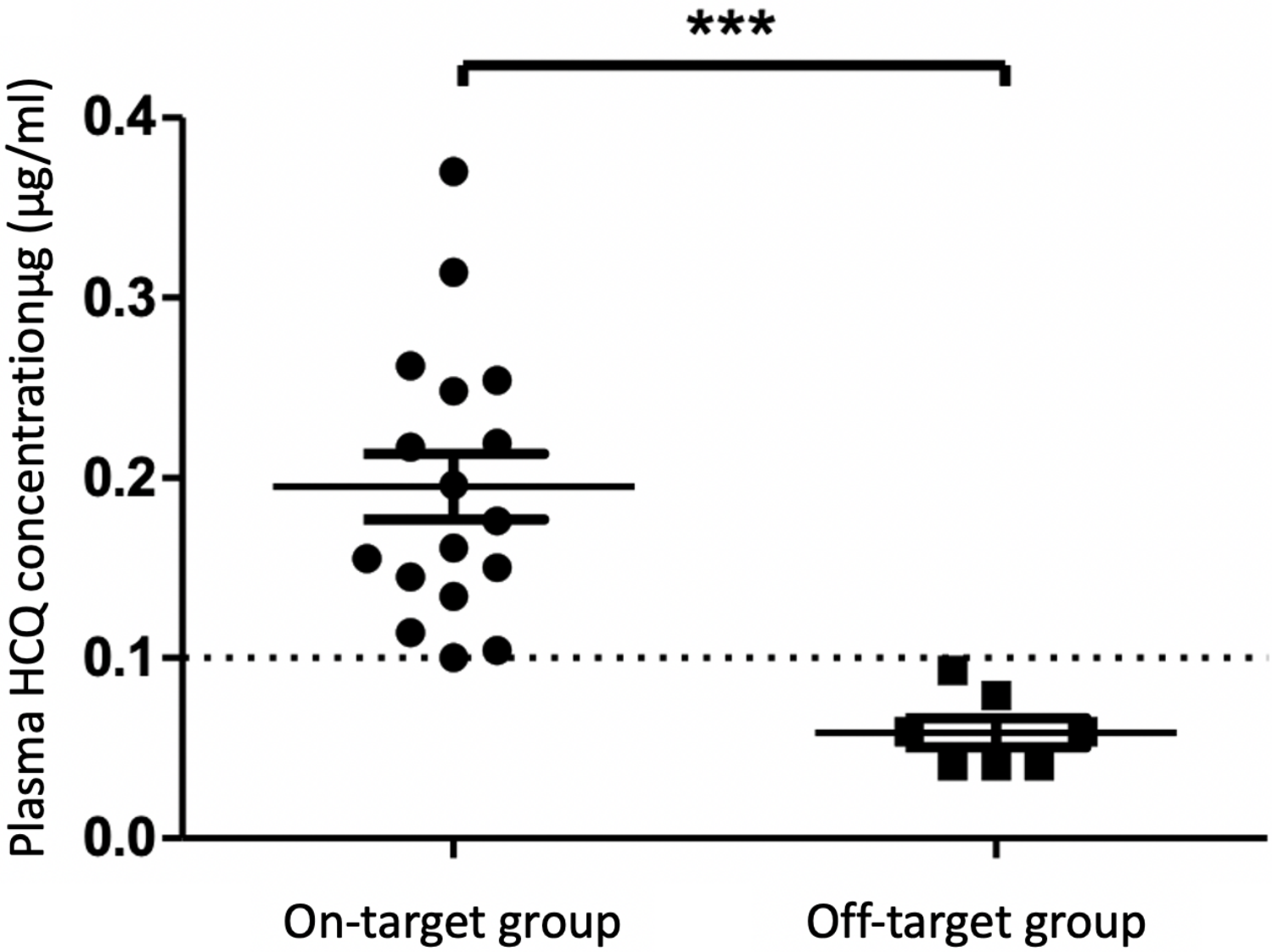

Figure 2: Viral load in nasopharyngeal swab at Day 15 in the two groups

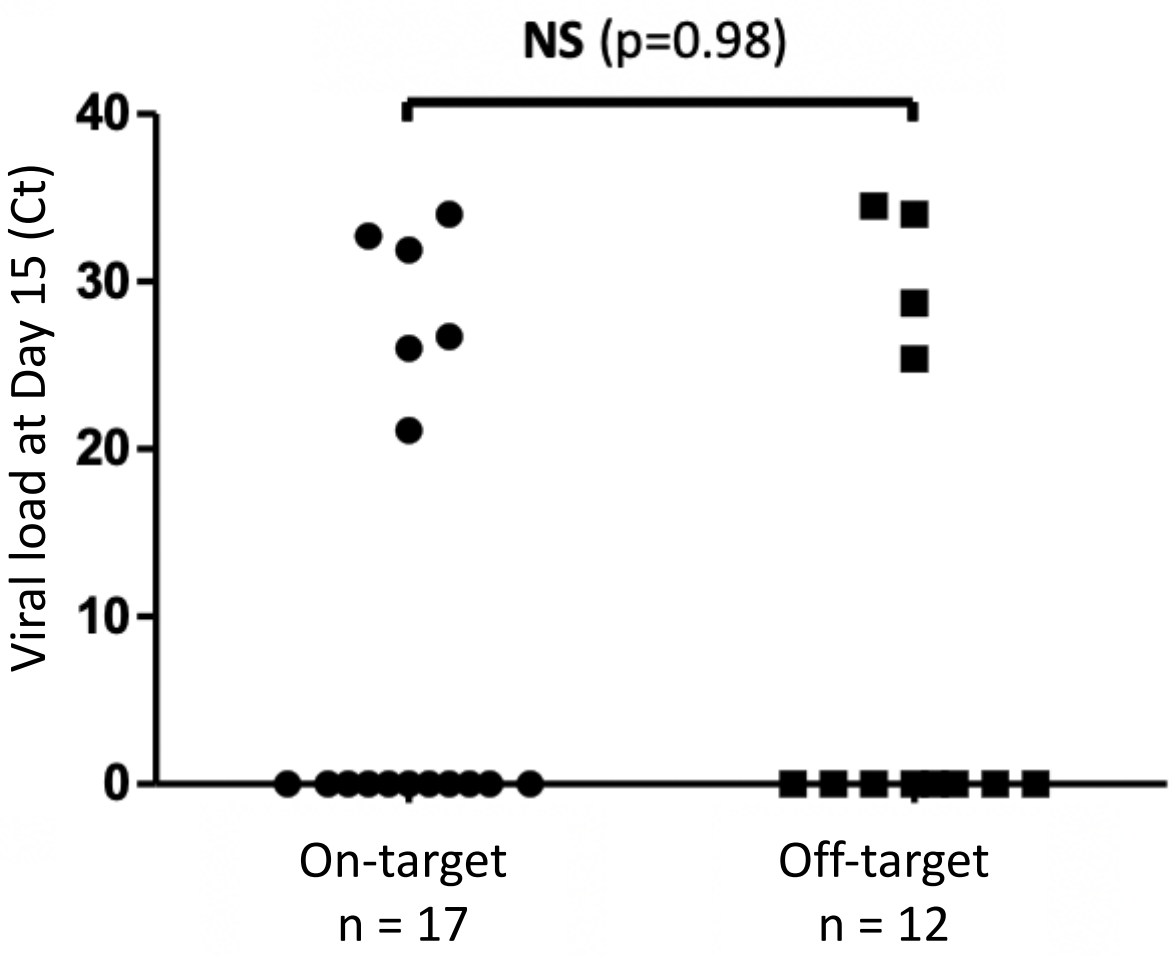

Table 1. Demographic and Clinical Findings

Characteristics		**On-target group n = 17**	**Off-target group n = 12**	***P*-value**
Sex	Men, n (%)	12 (71)	12 (100)	0.06
Age, mean ± SD, years		56 ± 15	62 ± 15	0.30
BMI, mean ± SD, kg/m^2		31 ± 5	29 ± 4	0.51
Co-morbidities, n (%)				
	Coronary disease	5 (29)	4 (33)	0.86
	Hypertension	10 (59)	9 (75)	0.61
	Chronic obstructive pulmonary disease	2 (12)	1 (8)	1
	Habitual smoker	4 (24)	3 (25)	1
	Active cancer	2 (12)	1 (8)	1
	Immunodepression	0	1 (8)	0.41
	Chronic kidney disease	1 (6)	0	1
	Diabetes	6 (35)	6 (50)	0.68
Pregnant women, n (%)		3 (18)	0	0.25
In ICU Admission				
SAPS II, mean ± SD		29 ± 11	38 ± 16	0.10
SOFA Score, mean ± SD[b]		4 ± 2	5 ± 4	0.46
PaO2/FiO_2 ratio, mean ± SD		167 ± 74	127 ± 52	0.12
Mechanical ventilation, n (%)		10 (59)	8 (67)	0.97
Covid-19 Infection history and treatment				
Respiratory symptoms at hospital admission, n (%)				
	Cough	13 (76)	8 (67)	0.87

	Dyspnea	17 (100)	11 (92)	0.41
Systemic symptoms at admission, n (%)				
	Fever	15 (88)	11 (92)	1
	Diarrhea	3 (18)	4 (33)	0.40
	Myalgia	11 (65)	7 (58)	0.97
	Anosmia, dysgeusia	5 (29)	6 (50)	0.46
Know sick contact. n (%)		8 (47)	3 (25)	0.41
Travel to a country where Covid-19 is endemic previous 3 months. n (%)		0	0	
Mean ± SD duration of symptoms before hospital admission (days)		5 ± 2	7 ± 4	0.15
Mean ± SD duration between treatment initiation and ICU admission, days		0 ± 1	0 ± 1	0.13
Mean ± SD duration between symptom onset and ICU admission (days)		7 ± 2	8 ± 4	0.32
Mean ± SD viral load at Day 1, Ct		25 ± 12	30 ± 4	0.43
15 days follow-up				
4-6 days plasma concentration hydroxychloroquine on-target treatment, n (%)		14 (82)	0	***
Negative PCR, n (%)		11 (65)	8 (67)	0.77
Mean ± SD viral load change between Day 1 to Day 15, Ct		- 15 ± 16	- 20 ± 18	0.45
Mean ± SD duration to PCR negative under treatment, days		7 ± 6	6 ± 5	0.71

Mean ± SD duration to negative PCR since symptoms onset	13 ± 6	15 ± 7	0.43
15 days mortality	0	2 (17)	0.16
Still in ICU at 15 days	9 (53)	9 (75)	0.41
Still in hospital at 15 days	11 (65)	7 (64)	0.95
Length of mechanical ventilation, mean ± SD, days	7 ± 7	8 ± 7	0.92
Length of vasopressor administration, mean ± SD, days	3 ± 5	3 ± 3	0.95

Abbreviations: BMI, Body Mass Index; SAPS II, Simplified Acute Physiology Score II; SOFA, Sepsis-related Organ Failure Assessment; PaO_2/FiO_2 ratio, ratio of partial of arterial oxygen partial to the fraction of inspired oxygen; HC, Hydroxychloroquine; PCR, Polymerase Chain Reaction; ICU, Intensive Care Unit; Ct, Cycle threshold; SD, Standard Derivation.

[a] Data are expressed as N (%) of participants unless otherwise indicated.

[b] The SAPS II ranges from 0 to 163, with higher scores indicating higher risk of mortality. A patient with a score of 30 has an estimated mortality risk of 10%.

*** $P < 0.001$