Viral clearance in patients with COVID-19: associated factors and the role of antiviral treatment

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Abstract

The role of hydroxychloroquine (HCQ) in lowering the viral load of patients with COVID-19 is controversial. In our Institute, we treated more than 30,000 people with COVID-19 in 2020 and 2021, using the same diagnostic tools and the same treatment dosages. In this retrospective comparative study of data collected over this period, we aimed to compare the viral clearance in the nasopharynx as determined by qPCR in patients who were treated with 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). Of the 1 276 patients selected from our database, 776 were treated with HCQ and 500 were not. Viral clearance in the treatment group was reached significantly earlier than in the non-treatment group, at days 5, 10 and 30. These differences remain significant after adjustments for confounding factors. In conclusion, although age, initial viral load, and time to treatment do influence the viral load in patients with COVID-19, hydroxychloroquine associated with azithromycin still independently significantly lowered viral load more rapidly than other treatments, including azithromycin alone.

Introduction

Since the discovery of the AIDS virus 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 viral hepatitis. It is commonly accepted that a decrease in the viral load attests to an improvement in the infection or even to a cure (1), making it possible to use it to judge the therapeutic effectiveness of new antiviral drugs. Indeed, this method is widely used as a gold standard marker in randomised clinical trials (2). In addition, viral load monitoring has been applied to monitoring the effectiveness of treatments for acute viral infections such as cytomegalovirus (CMV) (3) and Epstein-Barr virus (EBV) (4). Monitoring viral load through quantitative PCR has been recommended as a way of monitoring therapeutic efficacy (5). For example, the effectiveness of EDP-938, a non-fusion replication inhibitor of respiratory syncytial virus (RSV), was evaluated in a randomised controlled trial involving volunteers who were intra-nasally inoculated with the RSV-A Memphis 37b strain. This trial concluded that all EDP-938 regimens were better than placebo in terms of lowering of the viral load (6). More recently, several randomised 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 viral load in a double-blind Phase IIb clinical trial (9). In a very preliminary paper, we reported that 26 patients with COVID-19 treated with HCQ with or without azithromycin had a significant shorter virus shedding period compared to 16 untreated patients with COVID-19 (10). This paper was severely criticised, 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 (11). 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 (12). A number of 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 (13–15). Other confounding factors include the timescale between the onset of symptoms and admission (16), being immunocompromised, and the initial viral load (17). Armed with this new knowledge, we aim here to re-analyse our database, investigating the role of HQC on the viral shedding of patients with COVID-19.

Patient data and methods

Ethics and regulation:

This is a retrospective, observational cohort study. All patients diagnosed with COVID-19 by PCR identification of SARS-CoV-2 were invited to attend either the outpatient clinic or our hospital for evaluation and treatment. Data which emerged from epidemiological interviews and clinical and biological assessments were recorded in the hospital information system (HIS). For the purposes of this study, data from patients hospitalised between 3 March 2020 and 13 March 2021 were extracted from the HIS. To comply with the European General Data Protection Regulation, 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 reference method MR 004 was filed prior to the onset of this study and was the subject of a declaration in the GDPR/APHM Register No.2020-152.

Data collection: We first selected all patients hospitalised in our Institute within the one-year study period. To avoid bias or the use of inappropriate data, we excluded those who were immunocompromised (3), those mis-diagnosed as having COVID-19 (3), those treated with ivermectin alone (48), one minor patient (under the age of 18), 273 patients who were hospitalised 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 PCR test was more than 15 days (**Figure 1**). We then retained those for whom a positive PCR was obtained between D0-1 and D0+1. For patients who were treated with HCQ and/or AZT, "D0" was defined as the date treatment started (first treatment received). For patients who were not treated with HCQ or AZT, "D0" was defined as the date of admission to the IHU. Finally, we included those who had a second (positive or negative) PCR test between D0+1 and D0+10. All qPCR tests were performed in the same laboratory. When the CT of the qPCR was over 35 cycles, it was considered to be negative timescale between the onset (18). 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 GDPR. We identified four treatment groups: those who were treated with the HCQ regimen; those that 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 were followed for 30 days after the onset of treatment (from DO to D0+30). Patients who did not became 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 non-parametric Kaplan-Meier survival analysis. We then used the multivariable Cox Proportional-Hazards model 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 PCR SARS-CoV-2 (CT) viral load, 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 became PCR-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 PCR test. The time-cumulative incidence of patients

with a negative viral load according to treatment group was estimated by non-parametric competing risk analysis. We then used the multivariable Fine-Gray sub-distribution hazard model (21) 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 SAS 9.4 statistical software (SAS Institute, Cary, NC).

Results:

Inclusion and exclusion: Of the 2 799 patients hospitalised 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 (1 294) and those who had a PCR within the first ten days of care (1 276) (**Figure 1**). Of them, 747 were PCR negative within 30 days of follow-up and 529 were censored at the date of their last positive PCR during follow-up (**Table 1**).

Comparison of treatment with HCQ versus no HCQ: The population analysed included 776 people who received hydroxychloroquine (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 favour to 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 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 [1.01-1.38], p =.037), suggesting that the HCQ treatment had a significant impact upon 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 increases (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 PCR increases by one unit, the probability of negativization increases by 12% (Hazard ratio=1.12) i.e. the lower the initial viral load, the greater the probability of a negative result. Finally, an increased likelihood of negativization was observed when the time to treatment was longer (longer time associated with a lower viral load at the time of treatment).

When treatment with both HCQ and AZT was compared to treatment with AZT 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**).

Discussion

Our team has previously published a very controversial study which involved a small group of patients hospitalised for COVID-19 and treated with either HCQ alone or with HCQ plus AZT. This research was supposed to include a control group, as it was not randomised (10). By chance, we had also carried out diagnostic work in different hospital centres which were not included in the research on the evolution of viral load according to these treatments, which served as controls. This work has been highly cited, triggering either strongly hostile or strongly supportive positions. Few teams have been able to carry out comparable studies. In order to carry out such studies, it is necessary to have access to data on patients who have been

treated over several days and for whom their regular viral loads have been assessed until negativization, as recommended for hospital discharge. In addition, some studies only report late viral loads without considering or integrating the date of negativization of the viral load, which was the objective of our first study. This first study was confirmed by a study on 3 737 patients showing through principal component analysis that the treatment was associated with a decrease in viral load (12). In the current study, we wanted to evaluate viral clearance in patients who could actually be analysed according to the different treatments they received, including treatment with and without HCQ, and treatment with the combination of HCQ plus AZT. 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 AZT, than in other patients, all of whom were treated with an antiviral and/or anti-inflammatory drug. This study avoids a certain number of biases. The treatment dosage was always the same, whereas in other trials these dosages are either lower and probably inactive (19), or in the toxic zone of the drug (20). Furthermore, patients were all treated in the same place by the same team and, therefore, the standard of care was the same for all patients, even if it evolved over time. There is, therefore, no heterogeneity which can arise in multicentre studies. Since the beginning of the outbreak, the cut-off point for defining PCR negativity is the same, at 35 cycles on our apparatus, a cut-off which has been confirmed by several thousand *in vitro* viral cultures. From 35 cycles onwards, there is no longer any live virus in the inoculated samples [20]. Taken as a whole, we found that HCQ treatment significantly increased the probability of viral clearance by 20% independently 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 multivariable Fine-Gray sub-distribution hazard model (21) with a similar 20% risk difference. The median time to negative PCR was decreased by 2 days (6 vs 8 days), which may have important consequences at 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. The weakness of this study is that, being a single-centre study, the efficacy of treatment is partly due to being managed by a particular team who have treated more than 30,000 people, and the general quality of that management and the experience of the practitioners has probably played a role in both compliance by and the evolution of the patients treated. This means that the results here can be generalised only to similar patients, and a similar organisation of care, and cannot be extrapolated in their entirety to other centres or in multicentre studies.

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