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SHORT COMMUNICATION

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Using time-weighted average change from baseline of SARS-CoV-2 viral load to assess impact of hydroxychloroquine as postexposure prophylaxis and early treatment for COVID-19

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Abstract

Two randomized controlled trials demonstrated no clinical benefit of hydroxychloroquine (HCQ) for either postexposure prophylaxis or early treatment of SARS-CoV-2 infection. Using data from these studies, we calculated the time-weighted average change from baseline SARS-CoV-2 viral load and demonstrated that HCQ did not affect viral clearance.

KEYWORDS

COVID-19, early treatment, hydroxychloroquine, PEP, SARS-CoV-2

1 | BACKGROUND

First identified in December 2019, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), the etiologic agent of COVID-19, is now the third-leading cause of death in the United States and has killed more than 6 million worldwide as of July 2022.[1–3] Hydroxychloroquine (HCQ) was evaluated as postexposure prophylaxis (PEP) or early treatment for COVID-19, based on in vitro and observational data suggesting that HCQ shortened the duration of SARS-CoV-2 viral detection.[4,5] However, in several randomized controlled trials (RCTs), HCQ did not demonstrate clinical benefit.[6–8]

During the initial phase of the pandemic, we conducted two RCTs to evaluate HCQ as a therapy to prevent or treat SARS-CoV-2 infection. We found no preventive benefit from HCQ versus placebo-like control.[9] We also found no treatment benefit for COVID-19 disease resolution in high-risk and low-risk participants in a three-arm study evaluating HCQ and HCQ + azithromycin (AZ) against placebo equivalents.[10] In the Early Treatment study, viral clearance was slightly faster in the HCQ (but not HCQ + AZ) group compared to the control (median of 5 days vs. 7). This difference was not evident in a post hoc sensitivity analysis by adding baseline cycle threshold (Ct) values to the model and limiting the upper Ct threshold to 34 (i.e., excluding low viral loads).[10] However, the primary analysis did not fully address the fact that participants entered the trial at different times in the viral infection.

Studies of monoclonal antibodies have assessed SARS-CoV-2 viral clearance using time-weighted average (TWA) change from baseline, which takes into account the baseline viral load and represents change over a fixed time period.[11] This approach has previously been utilized in evaluating the impact of medications on other respiratory viruses, such as a respiratory syncytial virus.[12] It controls more precisely for baseline viral load, clarifying whether the intervention had an effect on viral detection.

We used TWA change to determine whether HCQ was associated with decreased SARS-CoV-2 RNA detection over 14 days compared to placebo equivalent among people with SARS-CoV-2 infection at baseline during the PEP study or with detectable SARS-CoV-2 in follow-up in the early treatment study.

2 | METHODS

Both the PEP and Early Treatment cohorts were conducted remotely and participants were enrolled (1) within 96 h of close contact with a person with laboratory-confirmed SARS-CoV-2 for the PEP study or (2) within 72 h of virologically confirmed SARS-CoV-2 diagnosis for the Early Treatment study. Participants from the PEP study were included if they had SARS-CoV-2 RNA detected by reverse transcriptase polymerase chain reaction (RT-PCR) at baseline; treatment participants were included if they had at least 1 day with SARS CoV-2 RNA detected by RT-PCR. As previously described, participants self-collected mid-nasal swabs daily from Days 1–14.[9,10] Online questionnaires were self-administered daily to record medication adherence and symptoms. RT-PCR testing for SARS-CoV-2 nucleocapsid genes N1 and N2 was performed from swabs and Ct, a semiquantitative measure of the quantity of SARS-CoV-2 RNA (viral load), was determined.[13] Participants received a cumulative dose of 3400 mg of HCQ over 14 days in the PEP study or 4400 mg over 10 days in the Early Treatment study. The trials are registered at clinicaltrials.gov (PEP, NCT04328961; Early Treatment, NCT04354428).

2.1 | Outcome

TWA change in the quantity of SARS-CoV-2 RNA was measured by Ct on each of the 13 days after initiation of HCQ or placebo. The reversed Ct value was calculated as 40 minus Ct (set to 0 if the virus was not detected). The area under the curve was calculated using the trapezoidal method with the baseline (Day 1) value subtracted from each observation. The resulting value was divided by the day span to calculate the TWA. Negative values of TWA, in cycles, indicate a decrease from baseline while positive values indicate an increase. The closer the value is to zero, the less change from baseline.

2.2 | Statistical methods

Adjusted HCQ effects on viral load clearance (measured by the TWA outcome) were estimated by using multivariable linear regression and pooling study-specific adjusted effects with random effects meta-analysis (z-based). The regression models were adjusted for age, sex, and symptom severity at the start of treatment, defined as the severity of the most severe symptom on Day 1 of Early Treatment. Additionally, we adjusted for the relationship to the index case in the PEP cohort (healthcare vs. household/social contact), days between contact and first HCQ or placebo dose in the PEP cohort, and days since symptom onset and risk cohort (high, as defined by established risk factors for severe COVID-19 vs. low) in the Early Treatment cohort. All adjustment variables were hypothesized to affect either the virologic burden of SARS-CoV-2, illness severity, or timing of HCQ use relative to the course of infection.[14,15] Due to the possible impacts of differential HCQ dosing in the PEP and Early Treatment study, analyses were performed separately for each study. We hypothesized that the greatest effect on viral load, if present, would be seen among people presenting after exposure or earlier in illness. Therefore, in a sensitivity analysis, we restricted the cohort to participants with <4 days to study medication initiation since contact in the PEP group or since symptom onset in the Early Treatment group.

3 | RESULTS

Eighty-three (10%) of 829 participants had SARS-CoV-2 RNA detected at baseline in the PEP study and 175 (76%) of 231 participants had at least 1 day with SARS-CoV-2 RNA detected in

TABLE 1 Participant characteristics by study and arm

	PEP placebo (*N* = 54)	PEP HCQ (*N* = 29)	PEP, overall (*N* = 83)	Early Tx placebo (*N* = 65)	Early Tx HCQ (*N* = 52)	Early Tx HCQ + AZ (*N* = 58)	Early Tx, overall (*N* = 175)
Age, mean ± SD (range)	36 ± 16 (18–71)	41 ± 16 (18–68)	38 ± 16 (18–71)	40 ± 14 (18–70)	35 ± 12 (19–68)	40 ± 14 (18–71)	39 ± 13 (18–71)
Female sex, *n* (%)	28 (52)	18 (62)	46 (55)	36 (55)	28 (54)	35 (60)	99 (57)
Days since symptom onset, mean ± SD (range)	-	-	-	6 ± 3 (1–12)	6 ± 2 (2–14)	6 ± 3 (2–13)	6 ± 3 (1–14)
High-risk cohort, *n* (%)	-	-	-	40 (62)	26 (50)	31 (53)	97 (55)
Days between contact and first dose survey, mean ± SD (range)	2 ± 2 (0–6)	2 ± 2 (0–7)	2 ± 2 (0–7)	-	-	-	-
Contact type = healthcare, *n* (%)	3 (6)	0 (0)	3 (4)	-	-	-	-
Symptoms at treatment start, *n* (%)							
Not at all	-	-	-	3 (5)	2 (4)	2 (3)	7 (4)
A little bit	-	-	-	6 (9)	10 (19)	9 (16)	25 (14)
Somewhat	-	-	-	17 (26)	9 (17)	10 (17)	36 (21)
Quite a bit	-	-	-	9 (14)	7 (13)	13 (22)	29 (17)
Very much	-	-	-	30 (46)	24 (46)	24 (41)	78 (45)
Symptoms at treatment start, *n* (%)							
None	29 (54)	14 (48)	43 (52)	-	-	-	-
Mild	18 (33)	12 (41)	30 (36)	-	-	-	-
Moderate	5 (9)	1 (3)	6 (7)	-	-	-	-
Severe	2 (4)	2 (7)	4 (5)	-	-	-	-

Abbreviations: AZ, azithromycin; HCQ, hydroxychloroquine; PEP, postexposure prophylaxis; Tx, Treatment.

the Early Treatment study and were included in the analysis. Overall demographics, risk factors, and other patient characteristics are described in Table 1. The TWA change in HCQ and placebo groups for the PEP and Early Treatment studies is shown in Figure 1A. In multivariate models incorporating a priori factors that could be associated with viral shedding (Figure 1B), there was no statistically significant effect of HCQ in SARS-CoV-2 TWA change as compared to placebo equivalent in the PEP study (difference = 1.1 Ct, 95% CI: −1.9 to 4.1, $p = 0.47$). Similarly, in the Early Treatment study, HCQ had no statistically significant effect on TWA change versus placebo-equivalent (difference = −0.7 Ct, 95% CI: −2.4 to 1.1, $p = 0.44$), nor did HCQ + AZ (difference = −1.1 Ct, 95% CI: −2.8 to 0.6, $p = 0.21$).

In the subset analysis of people with early exposure (PEP, $n = 64$) or who enrolled <96 h since symptom onset (Early Treatment, $n = 43$), there was no statistically significant difference in TWA change in the HCQ versus placebo equivalent for PEP (difference = 0.9, 95% CI: −2.7 to 4.5, $p = 0.64$) or in treatment with HCQ versus placebo equivalent (difference = −1.3, 95% CI: −6.1 to 3.6, $p = 0.60$) or HCQ + AZ (difference = 0.6, 95% CI: −3.4 to 4.5, $p = 0.77$).

4 | DISCUSSION

In an HCQ prevention trial and an HCQ early treatment trial, we found no difference in the TWA change in SARS-CoV-2 viral load between HCQ and control groups as measured from baseline viral load. Further, no benefit of HCQ and/or HCQ + AZ was seen among those who enrolled within the 72 h of COVID-19 symptoms. For interventional studies, the TWA change analysis allows for the incorporation of baseline viral load data as well as viral load kinetics, which may better measure the antiviral effect compared to the use of time for virologic clearance.

This analysis using TWA change is consistent with multiple studies that found no in vivo antiviral effect of HCQ on SARS-CoV-2 replication.[6,16] For instance, an RCT conducted on healthcare workers found that HCQ was not effective for PEP.[7] In a multicenter RCT, Mitjà et al.[6] observed no difference in a mean reduction in SARS-CoV-2 viral load in patients given HCQ vs. standard care for early treatment. In the NOR-Solidarity trial of hospitalized patients, similar decreases in SARS-CoV-2 oropharyngeal viral load were seen across groups treated with remdesivir, HCQ, or standard of care.[16]

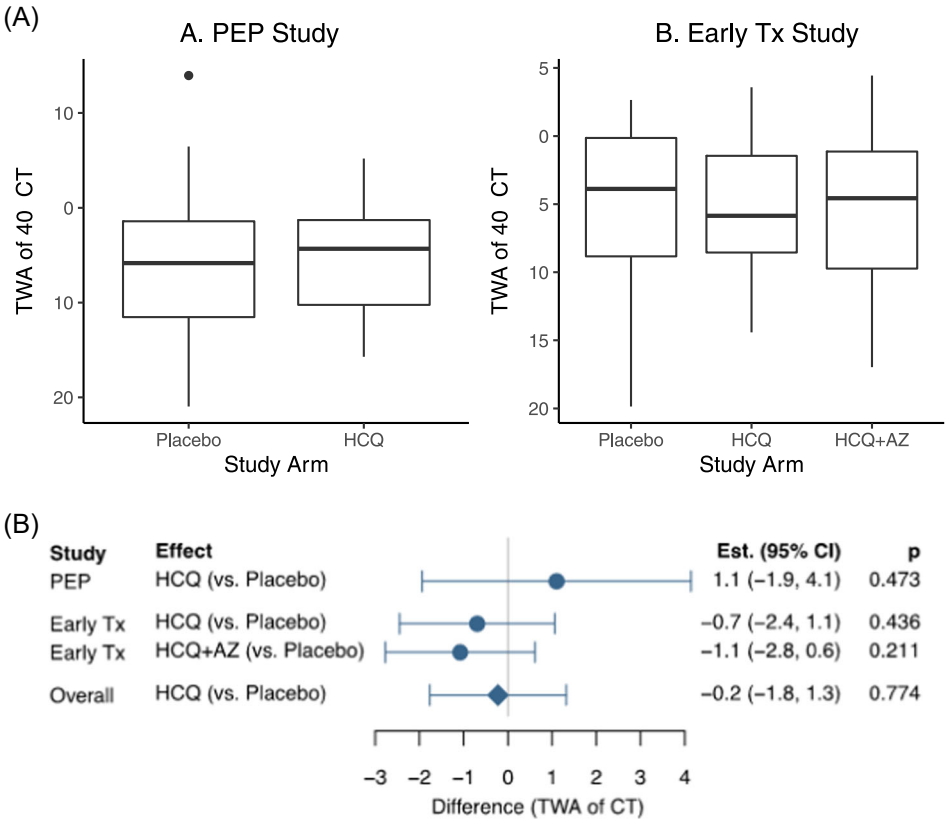

FIGURE 1 (A) Distribution of the time-weighted average (TWA) of the change in the reversed cycle threshold (Ct) from Day 1 in the postexposure prophylaxis (PEP) study (A.) and Early Treatment study (B.) by study arm. Box plots represent the median TWA and whiskers indicate 95% confidence interval (CI). Reverse Ct calculated as 40-Ct, as 40 represents the limit of detection. No statistically significant differences were seen. (B) Estimated adjusted hydroxychloroquine (HCQ) effects by study and overall. PEP Study adjusted for days between contact and first dose survey, contact type (healthcare vs. household/social), sex, age, and symptom severity; Early Treatment Study adjusted for days since symptom onset, cohort risk, age, sex, and symptom severity.

Strengths of this study include frequent virologic monitoring with daily self-collected nasal swabs with a high level of specimen receipt allowing for measurement of baseline viral load and evaluation of time to viral clearance. We included PEP study participants to evaluate those who had SARS-CoV-2 at enrollment and likely initiated HCQ early after disease acquisition, in addition to those diagnosed after symptom onset in the Early Treatment study. We included those who received a placebo to assess TWA change from baseline to provide a comparison with untreated infection and those who received HCQ + AZ to assess those who received any amount of HCQ. One limitation of these remote studies is that kit shipment time may have delayed medication receipt after enrollment in a few cases. Another limitation of these studies is that they did not include more recently circulating variants of concern, as they were conducted from March to August 2020 before the emergence of beta and delta variants.[17] Further, given that swabs were self-collected, we cannot rule out sampling errors in ascertaining yield, though this may have been a factor even in provider-collected samples.

Overall, with data obtained from two rigorously performed RCTs, we found no effect of HCQ, with or without AZ, on SARS-CoV-2 viral load clearance when used for either PEP or early treatment. Using TWA changes from the baseline of quantitative viral load is a meaningful metric of viral clearance since it accounts for the baseline viral load before the intervention. Future studies should control for differences in initial viral loads due to participants presenting at different stages of infection to reflect variability in viral kinetics.

5 | CONCLUSION

Using the TWA approach, which incorporates baseline viral load and subsequent changes, we confirmed that there is no significant effect of HCQ or HCQ + AZ in reducing SARS-CoV-2 viral load as a postexposure prophylaxis or early treatment. As previously demonstrated, HCQ is not an effective intervention for COVID-19.

AUTHOR CONTRIBUTIONS

Christine Johnston, Elizabeth R. Brown, and Ruanne V. Barnabas provided the conception and design of the study. Ruanne V. Barnabas, Jenell Stewart, Helen Y. Chu, Raphael J. Landovitz, Patricia J. Kissinger, Michael K. Paasche-Orlow, Anna Bershteyn, Kathleen M. Neuzil, and Alfred Luk collected study data. Keith R. Jerome and Alfred Luk performed laboratory analyses. Moni Neradilek and Elizabeth R. Brown provided statistical expertise, developed and performed the data analysis, and had full access to the study data. Anna Wald, Helen Y. Chu, Ruanne V. Barnabas, and Christine Johnston provided supervision. Raaka Kumbhakar and Christine Johnston wrote the first draft of the manuscript. All authors provided interpreted the data, critically revised the manuscript, and approved the submitted manuscript.

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CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Vivli at doi:10.25934/00006837.0 (PEP), reference number VIV0000683; and doi:10.25934/PR00007132.0 (Early Treatment), reference number VIV0000713.

ETHICS STATEMENT

All patients had consent obtained for research at the time of recruitment for previous studies, both of which were approved by the Western Institutional Review Board with reliance agreements with collaborating institutions.

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