



Randomized double-blinded placebo-controlled trial of hydroxychloroquine with or without azithromycin for virologic cure of non-severe Covid-19

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ABSTRACT

Background: Hydroxychloroquine (HC) ± azithromycin (AZ) is widely used for Covid-19. The Qatar Prospective RCT of Expediting Coronavirus Tapering (Q-PROTECT) aimed to assess virologic cure rates of HC±AZ in cases of low-acuity Covid-19.

Methods: Q-PROTECT employed a prospective, placebo-controlled design with blinded randomization to three parallel arms: placebo, oral HC (600 mg daily for one week), or oral HC plus oral AZ (500 mg day one, 250 mg daily on days two through five). At enrollment, non-hospitalized participants had mild or no symptoms and were within a day of Covid-19 positivity by polymerase chain reaction (PCR). After six days, intent-to-treat (ITT) analysis of the primary endpoint of virologic cure was assessed using binomial exact 95% confidence intervals (CIs) and χ^2 testing. (ClinicalTrials.gov NCT04349592, trial status closed to new participants.)

Findings: The study enrolled 456 participants (152 in each of three groups: HC+AZ, HC, placebo) between 13 April and 1 August 2020. HC+AZ, HC, and placebo groups had 6 (3.9%), 7 (4.6%), and 9 (5.9%) participants go off study medications before completing the medication course ($p = 0.716$). Day six PCR results were available for all 152 HC+AZ participants, 149/152 (98.0%) HC participants, and 147/152 (96.7%) placebo participants. Day six ITT analysis found no difference ($p = 0.821$) in groups' proportions achieving virologic cure: HC+AZ 16/152 (10.5%), HC 19/149 (12.8%), placebo 18/147 (12.2%). Day 14 assessment also showed no association ($p = 0.072$) between study group and viral cure: HC+AZ 30/149 (20.1%), HC 42/146 (28.8%), placebo 45/143 (31.5%). There were no serious adverse events.

Interpretation: HC±AZ does not facilitate virologic cure in patients with mild or asymptomatic Covid-19.

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1. Introduction

In March 2020, the World Health Organization (WHO) acknowledged the pandemic status of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and its associated disease Covid-19. As of August 2020, there is neither a Covid-19 vaccine nor definitive therapy.

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Research in context

Evidence before this study

Despite the worldwide scope of Covid-19 disease, as of August 2020 therapy is based upon inconclusive evidence. A variety of approaches have been studied, but there is no vaccine and optimal treatment remains elusive.

A search of PubMed-indexed Covid-19 evidence published (or available as accepted pre-publication manuscript) between 2019 and 1 August 2020 was executed. The evidence identified the antimalarial hydroxychloroquine (HC), often administered with the macrolide azithromycin (AZ), as one of the more commonly discussed Covid-19 therapies. In March 2020 these repurposed antimicrobials began to garner substantial worldwide attention to a degree that was arguably out of proportion to the quality of supporting data.

For Covid-19 patients with disease sufficiently severe to require hospitalization, HC's lack of efficacy is strongly suggested by an evolving literature that includes high-quality, large-scale randomized controlled trials (RCTs) in the United Kingdom (RECOVERY) and worldwide (Solidarity). However, as of end-August 2020 there are no double-blinded RCTs assessing HC±AZ facilitation of virologic cure in asymptomatic or mildly ill participants with laboratory-confirmed Covid-19.

Added value of this study

This is the first randomized, double-blind, placebo-controlled trial to report virologic outcomes of HC±AZ therapy in patients with asymptomatic or mild laboratory-confirmed Covid-19. Q-PROTECT adds to the existing evidence base in its firm demonstration, using objective laboratory measures, that neither HC nor HC+AZ has any role in eliminating or reducing SARS-CoV-2 in non-hospitalized cases. The study findings add to current evidence casting increasing doubt as to utility of HC±AZ anywhere in the Covid-19 acuity spectrum. Q-PROTECT fills a void in the evidence base in its addition of relatively precise RCT-based estimates of HC±AZ-effected virologic cure in ambulatory patients.

Implications of all the available evidence

HC, with or without AZ, is highly unlikely to result in meaningful benefit in patients with mild or asymptomatic Covid-19.

of SARS-CoV-2 on real-time reverse transcriptase polymerase chain reaction (PCR). The primary aim was to determine whether, as compared to placebo, use of HC or HC+AZ was associated with higher rates of viral clearance after six days of therapy. There were two secondary study aims: assessment of day 14 virologic cure, and assessment of semi-quantitative change in viral load from baseline to day six.

2. Methods

2.1. Study design

Q-PROTECT was a parallel 1:1:1 allocation ratio RCT, with blinding of participants, study staff, treating clinicians, and analysts. The study occurred at two units of Qatar's national healthcare system, Hamad Medical Corporation (HMC). The first was the Emergency Department (ED) at HMC's tertiary hospital, Doha's Hamad General Hospital (HGH). The second unit was a 3500-bed quarantine facility 20 miles north of Doha, at Umm Qarn. Participants were enrolled at HGH ED or at Umm Qarn, and patient care and monitoring occurred at Umm Qarn.

The HMC ethics board reviewed and approved the trial protocol [5], which was registered at ClinicalTrials.gov (NCT04349592). The study followed CONSORT guidelines for trials.

2.2. Participants

The study's planned population consisted of SARS-CoV-2 PCR-positive males and females with mild or no symptoms. In practical application, as described in [Appendix 1](#) Q-PROTECT sampling was composed of young, expatriate males.

The selection of the lower end of the acuity spectrum was driven by the need to comply with institutional ethics board requirements, which were in turn dictated by Q-PROTECT's inclusion of a placebo arm. At the time of study design, Qatar's national treatment criteria required antiviral therapy (e.g. HC, oseltamivir) in patients meeting any of the following criteria: hospitalization, tachypnoea (respirations >29/minute), or hypoxemia (pulse oximetry on room air <93%); treatment was also recommended for any patient with chest X-ray abnormality who had risk factors of older age (>60), immunocompromise, or co-morbidity (e.g. diabetes or hypertension). These preceding factors defining requirement for antiviral treatment also constituted exclusion criteria for Q-PROTECT.

Other inclusion and exclusion criteria were related either to logistics or to risks of study medications. Eligibility was restricted to adults (age at least 18) with positive SARS-CoV-2 PCR who were quarantined at Umm Qarn due to inability to self-quarantine.

Exclusion criteria based on documented or patient-reported past medical history were: retinal or macular disease; psoriasis; hepatic or renal disease; porphyria; glucose-6-phosphate-dehydrogenase (G6PD) deficiency; QT-interval prolongation; or hypersensitivity to HC or AZ.

Breastfeeding patients were ineligible. Pregnancy (as assessed by patient report) also constituted grounds for exclusion.

Medication-related exclusions based on drug safety were current therapy with tamoxifen, antimalarials, or dapsone. Exclusions for potential confounding of results were made in the case of recent (within one week) therapy with either of the study drugs or with any antivirals (e.g. oseltamivir).

Other exclusion criteria were dictated by initial laboratory and electrocardiography (ECG) results. Participants were excluded if laboratory assessment (within 24 h before study screening) revealed low levels of potassium or magnesium, or elevated creatinine or transaminases. ECG-based exclusion criteria related to QT prolongation risk followed American College of Cardiology (ACC) guidelines for HC+AZ therapy [6].

One therapeutic approach that was ultimately dropped from the UK's RECOVERY (in June 2020) and the WHO's Solidarity (in July 2020) is the repurposed antimalarial hydroxychloroquine (HC). Initial enthusiasm for HC, administered with or without the macrolide azithromycin (AZ), was fueled by a March 2020 report from a Marseille open-label (non-RCT) study [1] and a mid-April follow-up from the same group [2]. Covid-19 HC treatment attained a level of notoriety that was arguably disproportionate to the strength of supporting evidence.

In assessing the evidence in April 2020 – evidence that included a study from another French group [3] that failed to replicate Marseille's favorable results – reviewers concluded there was insufficient evidence to support HC as a standard of Covid-19 care [4]. Given the lack of certainty, in April 2020 a multidisciplinary group from the Qatari national healthcare system designed the Qatar Prospective RCT of Expediting Coronavirus Tapering (Q-PROTECT).

Pre-symptomatic transmission warrants focus as a target to protect a population from SARS-CoV-2. Q-PROTECT focuses on non-hospitalized low-acuity, emphasizing a dichotomous endpoint: presence

Participants were enrolled at two locations. During a run-in period, six participants were enrolled during an initial HGH ED visit and followed up at the Umm Qarn quarantine site. The study approach then changed such that all cases were both enrolled and followed at Umm Qarn. Further information on study enrollment site is provided in [Appendix 1](#).

Potential participants were screened based on positive initial SARS-CoV-2 PCR testing that was ordered as part of routine care. Once PCR test results returned positive, study staff approached treating physicians for approval to discuss Q-PROTECT with patients. Written informed consent was obtained.

2.3. Randomization and masking

Once participants were enrolled, they were each given a pair of study medication bottles. The bottles' medication contents were unknown to participants and staff. One bottle contained 21 tablets of either HC 200 mg (Sanofi-Aventis, Spain) or a similar-appearing placebo tablet; this bottle's label included an instruction to take the contents every eight hours for seven days. The other bottle contained six capsules of either AZ 250 mg (Pfizer, Italy) or a similar-appearing placebo. The second bottle's label included an instruction to take two capsules on the day of enrollment and one capsule each morning for the next four days.

Further details on Q-PROTECT randomization, allocation concealment, and triple-blinding procedures are provided in [Appendix 1](#).

2.4. Procedures

The study's interventions were provision of self-administered study medications and execution of swabs for virologic testing. Further details on PCR testing days and sample storage are provided in [Appendix 1](#).

Swabs were combination nasopharyngeal and oropharyngeal (Copan Diagnostics, Brescia, Italy). Swabs were executed by study-staff physicians, all of whom received training (in Covid-19 swab sample collection) from Qatar's national Communicable Disease Center. Samples were transported in universal transport medium to PCR testing equipment (see [Appendix 1](#) for details on PCR testing).

For all PCR testing, the primary endpoint of day six virologic cure (as well as the secondary endpoint of day 14 cure) was defined as being met if the machine's cycle threshold (Ct) interpretation algorithm reported a result of negative. For participants who did not achieve day six virologic cure, the semi-quantitative secondary endpoint of Ct increase (*i.e.* drop in viral load) was assessed. Restriction of semi-quantitative endpoint assessment to non-cured participants was necessary due to the non-reporting of a Ct for cured participants (PCR assay did not extend beyond Ct of 40). The semi-quantitative endpoint assessment was based on median Ct value for all assessed targets (ranging from one for the Bioneer, to three for the Thermo Fisher TaqPath).

Monitoring procedures included daily ECGs for the first week, and daily in-person visits and physical examinations for the first two weeks. There were phone reassessments on days 15–20. A final in-person visit was executed on day 21 for most patients; this was changed to phone follow-up visit when the study protocol was modified to drop the day 21 swab execution (see [Appendix 1](#)).

The study was not focused on, nor was it powered to assess, clinical endpoints (including therapeutic risks). Symptom tracking focused on patient-reported fever and respiratory complaints (rhinitis, pharyngitis, cough, or chest pain). Adverse effects tracking was assessed with open-ended questioning and monitoring for events such as death, hospitalization, pneumonia development, or QT prolongation.

The study approach for QT monitoring was based on ACC recommendations [6] that recommend considering discontinuing therapy if

QT is prolonged more than 30–60 msec. Q-PROTECT's initial protocol called for withdrawing participants for QT prolongation exceeding 30 msec, but the protocol was modified to increase the cut-off to 60 msec (see [Appendix 1](#)).

2.5. Primary and secondary outcomes

The study's primary outcome was achievement of virologic cure (PCR-negative status) as assessed on day six. The secondary outcomes were day 14 virologic cure and, for cases not achieving the primary outcome, virologic semi-quantitative analysis of Ct decreases from day one to day six. Additional exploratory endpoints are described in [Appendix 2](#).

2.6. Statistical analysis

Sample-size calculations were estimated using the freeware STPLAN (Version 4.5, Department of Biomathematics, University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA). Calculations were based on a best-estimate baseline (control group) virologic cure rate of 50%, as assessed at day six. Estimates were generated in March 2020 based on clinical experience in Qatar and on assessment of the relevant non-severe Covid-19 cases in the extant evidence base [7]. The minimum clinically important absolute effect difference was defined to be 10%. Given these assumptions, the study was designed to accrue 152 participants per group (total $n = 456$) to achieve 80–90% power (depending on drop-out rate). Details of sample-size and power calculations are provided in [Appendix 1](#).

Study planning dictated intent-to-treat (ITT) analysis. Per-protocol analysis was also executed, but only for exploratory assessments. All analyses other than sample-size calculations were performed using Stata (version 16.1 MP, StataCorp, College Station, Texas, USA).

Interim analyses were conducted after accrual of 100 participants and 200 participants. Pre-specified O'Brien-Fleming levels were defined for the interim α (0.001 and 0.015) and final α (0.047) and this information was provided to Q-PROTECT's Data Safety Monitoring Board (DSMB). Details on interim analysis planning are provided in [Appendix 1](#).

Q-PROTECT's primary dichotomous endpoint, virologic cure at day six, was assessed for each of the three study groups. This endpoint was reported as a proportion with 95% binomial exact confidence interval (CI). Comparison of the proportions was executed using χ^2 testing. Pairwise absolute differences in between-groups proportions were calculated as the absolute risk difference with 95% CI.

Secondary endpoint analysis, conducted for participants who did not achieve the primary endpoint of virologic cure, assessed Ct changes from day one to day six. Ct increase (corresponding to decreased viral load) was assessed for normality (with Shapiro-Wilk testing identifying the data as non-normal). Ct was described using the median with its interquartile range (IQR) at baseline, and its 95% CI for day six and day 14. Kruskal-Wallis testing was used to assess for group-related differences in magnitude of Ct increase.

The trial is registered at ClinicalTrials.gov (NCT04349592).

2.7. Role of the funding source

Q-PROTECT was wholly funded and resourced by the study institution (the government healthcare entity of the State of Qatar). The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

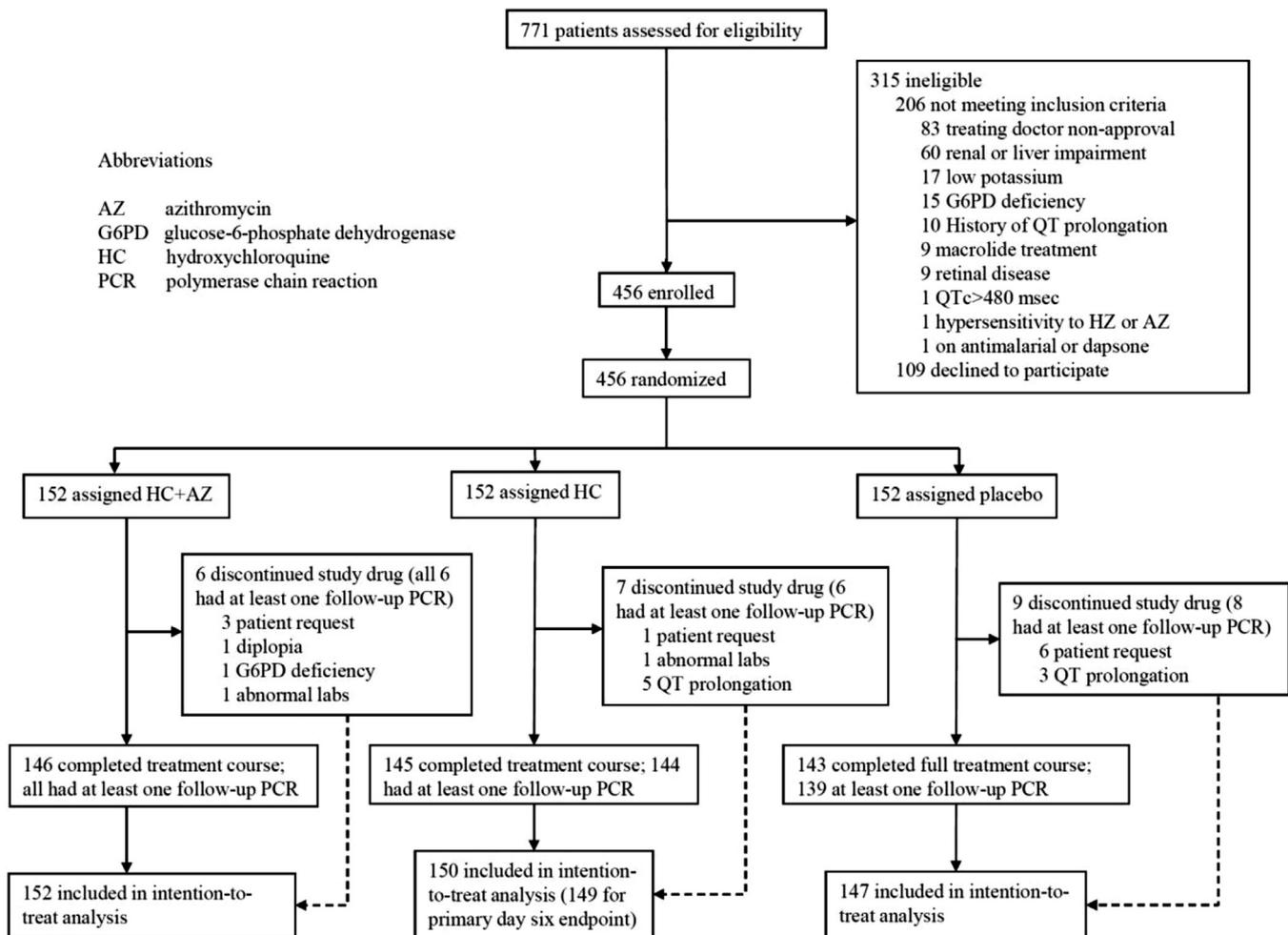


Fig. 1. Trial profile.

3. Results

Q-PROTECT commenced enrollment on 13 April 2020. Accrual of the $n = 456$ participants concluded on 1 August 2020 at the point of the trial's reaching its initially targeted accrual goal. Two interim analyses were conducted as planned, with results reviewed by the institutional ethics board and the DSMB; at each interim analysis the decision was made to proceed with Q-PROTECT accrual. At least one follow-up PCR (on day six or day 14) was available for all 152 HC+AZ participants, 150 HC participants (one of whom had only a day 14 PCR available, thus 149 HC participants were assessed for the primary endpoint), and 147 placebo participants. The participant trial profile is depicted in Fig. 1.

Baseline data showing demographic and clinical characteristics for each group are shown in Table 1.

The primary outcome, virologic cure at day six, was assessed on an ITT basis and thus included participants who had discontinued study medications if those participants had day six PCR results. Day six virologic cure data were available for 152/152 HC+AZ participants, 149/152 (98.0%) HC participants, and 147/152 (96.7%) placebo participants. Results for the primary outcome are shown in Fig. 2 and Table 2. The between-groups differences in proportions achieving day six virologic cure were: placebo minus HC+AZ 1.7% (95% CI -5.5 to 8.9%), HC minus placebo 0.5% (95% CI -7.0 to 8.0%), HC minus HC+AZ 2.2% (95% CI -5.0 to 9.5%). The preceding results and those in Fig. 2 present absolute risk; differences in relative risk are presented in Appendix 2.

Table 2 also shows results for the secondary outcomes of day 14 virologic cure and change in Ct from baseline (day one) to day six. Day 14 virologic cure data were available for 149/152 (98.0%) HC+AZ participants, 146/152 (96.1%) HC participants, and 143/152 (94.1%) placebo participants. Fig. 3 depicts the day 14 virologic cure secondary endpoint achievement.

The study defined an additional secondary endpoint of Ct change from baseline to day six, to be calculated for participants not achieving virologic cure. In the HC+AZ group, after subtracting the 16 participants with day six virologic cure from the initial group ($n = 152$) there were 136 participants for comparison of day one and day six Ct; both baseline and day six Ct data were available for all 136 participants. In the HC group, subtraction of the 19 virologically cured participants left 133 remaining; both baseline and day six Ct data were available for 129/133 (97.0%). Subtraction of the 18 virologically cured participants from the placebo group left 134 remaining; both baseline and day six Ct data were available for 129/134 (96.3%).

There were no deaths or serious adverse events. There was no association ($p = 0.708$) between study group and development of pneumonia, which was diagnosed in seven participants (1.5%): three (2.0%) in the HC+AZ group, one (0.7%) in the HC group, and three (2.0%) in the placebo group. Pneumonia accounted for seven of the 11 all-cause hospitalizations, which were not associated ($p = 1.00$) with study group. (Further information on hospitalizations is provided in Appendix 2.)

No patient had palpitations, syncope, or other symptoms indicative of cardiac dysrhythmia. Torsade de pointes was not identified on any ECG. Further results regarding QT are provided in Appendix 2.

Table 1
Baseline characteristics of the intent-to-treat population.

	Hydroxychloroquine + Azithromycin n = 152	Hydroxychloroquine n = 152	Placebo n = 152
Male sex	150 (98.7%)	149 (98.0%)	150 (98.7%)
Age (years)	42 (38–48)	40 (31–47)	41 (31–47)
Nationality			
Bangladesh	31 (20.4%)	32 (21.1%)	29 (19.1%)
Egypt	0 (0%)	1 (0.7%)	0 (0%)
Ghana	5 (3.3%)	1 (0.7%)	1 (0.7%)
India	54 (35.5%)	63 (41.5%)	47 (30.9%)
Indonesia	0 (0%)	0 (0%)	1 (0.7%)
Kenya	0 (0%)	1 (0.7%)	1 (0.7%)
Nepal	45 (29.6%)	38 (25.0%)	47 (30.9%)
Pakistan	0 (0%)	1 (0.7%)	3 (2.0%)
Philippines	7 (4.6%)	6 (4.0%)	6 (4.0%)
Romania	0 (0%)	0 (0%)	1 (0.7%)
Somalia	0 (0%)	1 (0.7)	0 (0%)
Sri Lanka	10 (6.6%)	6 (4.0%)	14 (9.2%)
Sudan	0 (0%)	1 (0.7%)	1 (0.7%)
Uganda	0 (0%)	1 (0.7%)	1 (0.7%)
Baseline Ct*	22.0 (18.4–28.0)	23.4 (20.0–28.4)	22.2 (19.2–26.4)
Symptoms at enrollment			
Patient-reported fever	46 (30.3%)	51 (33.6%)	52 (34.2%)
Respiratory symptoms**	37 (24.3%)	36 (23.7%)	34 (22.4%)

Data are n (%) or median (IQR).

* Ct=cycle threshold (median for all markers assessed); baseline Ct not available for two participants in hydroxychloroquine group and two participants in placebo group, thus data available for 452 of 456 randomized patients.

** Respiratory symptoms: Chest pain, cough, pharyngitis, or rhinitis.

Table 2
Primary and secondary outcomes.

	Hydroxychloroquine + Azithromycin n = 152	Hydroxychloroquine n = 152	Placebo n = 152	p
Virologic cure at day six*	16/152 (10.5%, 6.1–16.5%)	19/149 (12.8%, 7.9–19.2%)	18/147 (12.2%, 7.4–18.7%)	0.821
Increase in Ct from day one to day six*	7.2 (3.9–11.5; 6.1–8.8)	7.5 (3.4–11.5; 5.7–8.8)	8.0 (4.1–11.7; 7.3–9.0)	0.634
Virologic cure at day 14**	30/149 (20.1%, 14.0–27.5%)	42/146 (28.8%, 21.6–36.8%)	45/143 (31.5%, 24.0–39.8%)	0.072

Data are n/N (%; 95% CI) or median (IQR; 95% CI for median). Ct=cycle threshold (lower values correspond to higher viral load).

* Data not assessed for all randomized participants.

** Data assessed for 394 of 403 cases not reaching day six virologic cure endpoint.

A total of 22 participants (4.8% of 456) withdrew from the study-medication portion of Q-PROTECT after receiving at least one day of study medication. The placebo group accounted for nine withdrawals (5.9% of 152), the HC+AZ group seven (4.6% of 152), and the HC group six (4.0% of 152). There was no association ($p = 0.716$ by Pearson χ^2) between study group and participant withdrawal.

The most common reason for study withdrawal was the participant requesting withdrawal without giving a symptom-based reason; this occurred in 10 cases (three in HC+AC group, one in HC group, six in placebo group). Eight participants (five in HC group, three in placebo group) were withdrawn due to asymptomatic QT

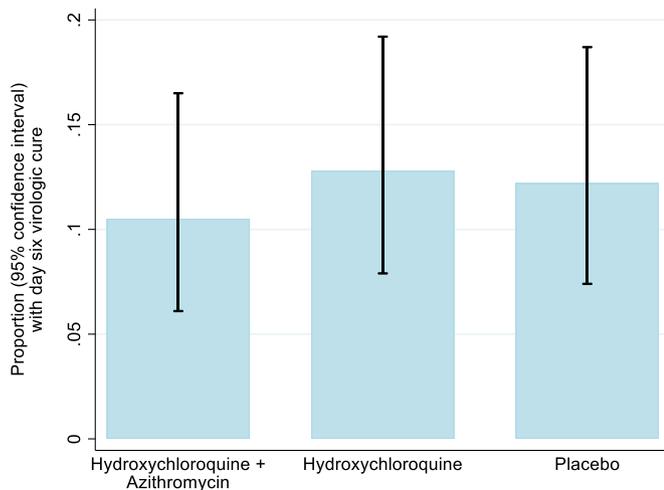


Fig. 2. Primary outcome of virologic cure at day six.

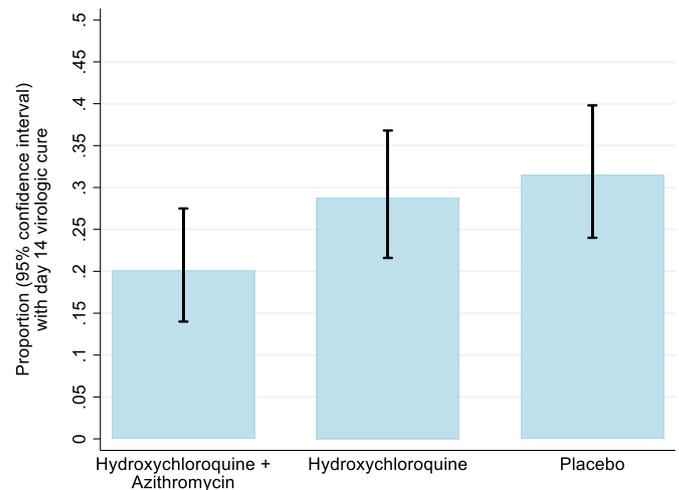


Fig. 3. Secondary outcome of virologic cure at day 14.

prolongation (see [Appendix 2](#)). Three withdrawals (two in the HC+AZ group and one in the HC group) were prompted by identification of lab abnormalities within 24 h of study enrollment. One participant in the HC+AZ group was withdrawn for (transient) diplopia.

Sensitivity analyses suggested that Q-PROTECT's primary and secondary endpoint findings were not influenced by the relatively few missing data. Similar lack of influence from withdrawals was suggested by per-protocol analyses. These analyses, as well as further exploratory analyses, are reported in [Appendix 2](#).

4. Discussion

Q-PROTECT's main finding was a failure of HC±AZ to have any salutary effect in mild or asymptomatic SARS-CoV-2 infection. In a group of relatively young, healthy participants (virtually all males) enrolled within 24 h of testing positive for the virus, HC±AZ neither improved virologic cure rates nor reduced viral burden. The therapeutic failure of HC±AZ was clear at both day six and day 14.

A promising March 2020 report from Marseille [1] focused interest on HC±AZ to speed viral clearance and effect clinical improvement. Within a few weeks, though, neither of these benefits were found by another French group assessing a small ($n = 11$) series of hospitalized patients [3]. The ensuing months have seen substantial criticism of both the initial Marseille study and the overall evidence base regarding use of HC±AZ for Covid-19 [8].

HC's performance with respect to post-exposure Covid-19 prophylaxis was assessed by Boulware and colleagues in an RCT instituting therapy within four days of high-risk exposure [9]. Even with relatively high doses (1400 mg on day one, followed by 800 mg daily for days two through five) HC did not reduce rates of SARS-CoV-2 viral detection or (in the relatively large proportion of patients for whom there was no viral testing) development of Covid-19 symptoms.

With regard to HC treatment of mostly mild or asymptomatic cases, the Marseille group reported their ongoing open-label treatment results in May 2020; their non-controlled series (numbering 1061) continued to be favorable, with 92% having good clinical outcome and virologic cure [10]. A few months later, a July 2020 open-label RCT from Catalonia arrived at a different conclusion: in non-hospitalized cases HC improved neither symptoms nor viral load [11]. The same month, a double-blind North American RCT of adult outpatients with early Covid-19 diagnosis identified no clinical benefit with HC [12].

For hospitalized cases with mainly mild or moderate disease, two Chinese open-label trials reported in May 2020 that HC failed to speed clinical improvement or virologic cure [13,14]. The same month, though, an open-label study of intubated patients in Wuhan found that HC reduced mortality via attenuation of cytokine storm [15].

Three other May 2020 observational cohort studies, two from the USA [16,17] and the other from France [18], failed to identify HC benefit. Furthermore, the analysis from New York [17] suggested that the combination of HC+AZ was associated with increased risk of death from cardiac arrest. Editorial commentary began to emphasize need for careful consideration of risks and benefits when considering Covid-19 treatment with QT-prolonging drugs [19].

By June 2020, the balance of evidence supported a case against treatment of Covid-19 cases with HC±AZ. An international registry-based study of hospitalized Covid-19 cases identified increased risk of harm (due to ventricular dysrhythmia) in patients receiving HC or HC+AZ [20]. The RECOVERY investigators announced withdrawal of the HC arm from their large-scale adaptive RCT [21]. June also saw the USA's Food and Drug Administration (FDA) warning of unfavorable risk:benefit ratio with use of HC outside the hospital setting [22].

In July 2020, there emerged more conflicting evidence on HC use in hospitalized Covid-19 cases. Preliminary analysis by WHO's Solidarity investigators prompted HC's removal from their RCT [23], and an open-label Brazilian RCT concurred in finding no HC clinical benefits [24]. However, two other reports, one from the USA and one from Marseille, left open the possibility of a role for HC+AZ in Covid-19.

From the USA, Arshad and colleagues' July 2020 observational cohort study reported that HC (and similarly, the combination HC+AZ) improved mortality in patients hospitalized with Covid-19 [25]. The Michigan group found that AZ alone did not improve outcome, and there was no statistically significant benefit to adding AZ to HC [25]. Favourable findings from the USA were echoed by the Marseille group, whose HC+AZ study population (now numbering 3119) spanned the acuity spectrum. The non-randomized Marseille series, which now included patients ($n = 618$) not treated with HC+AZ, found that HC+AZ cases had improved clinical outcomes and shorter duration of viral shedding [26].

As of 1 August 2020, the date of closure of enrollment in Q-PROTECT, existing data on HC±AZ use in hospitalized Covid-19 cases seems weighted toward the negative. However, as an early August commentary by Cohen states, the question of HC's potential utility in Covid-19 has not been definitively answered [27]. There is a void in the existing evidence base that is filled by the current study.

In its blinded RCT design, Q-PROTECT differs from all but two of the preceding studies (both of which were published by the same group of North American investigators) [9,12]. The preponderance of the HC Covid-19 evidence – even the largest, high-quality trials such as RECOVERY and Solidarity – comes from observational or open-label designs and addresses treatment in hospitalized patients.

It is noteworthy that one of the two double-blinded Covid-19 HC RCTs addressed post-exposure prophylaxis only [9]. The other [12] was limited by performance of SARS-CoV-2 testing in only 58% of participants. While both of the blinded trials undoubtedly advanced the state of Covid-19 knowledge, neither assessed the concrete endpoint of virologic cure in a PCR-positive Covid-19 population. Q-PROTECT is the first double-blinded RCT that assesses in virtually all of its participants, an objective virologic endpoint in cases (those with mild or no symptoms) in whom virologic clearance is critical to pandemic control.

It is not the case that virologic cure is more important than clinical outcomes, but Q-PROTECT's laboratory-assessed endpoints fill a gap in the blinded-RCT Covid-19 evidence base. Specifically, a blinded RCT could inform national decisions on utility of HC±AZ to expedite viral clearance and thus reduce transmission. When Q-PROTECT commenced in mid-April 2020, approximately 50,000 people in Qatar had been tested for Covid-19. There had been over 3000 positive SARS-CoV-2 results and seven deaths, with 252 new cases in the 24 h prior to study commencement. As enrollment closed in August 2020, the Covid-19 epidemic in Qatar was a few months past its peak but the daily PCR-positive n was still over 200. Approximately a half-million Covid-19 tests had been done in the country between March and August, with over 100,000 positive results and a death rate of roughly 1 in 1000. Q-PROTECT set out to determine whether HC±AZ could expedite viral clearance and thus likely reduce transmission.

When considered in context of the existing evidence, the current study contributes data that can help fill the final gaps in knowledge about HC±AZ utility in Covid-19. Q-PROTECT's main strengths are inherent in its blinded RCT design. There was no indication of flaws in either randomization or blinding, and the effect estimates are unbiased and reasonably precise (with acceptable CIs). The point estimates for the primary endpoint of day six virologic cure were nearly equal for HC and placebo (with HC+AZ's cure proportions lower). The point estimates for both secondary endpoints were actually more favourable for placebo than for either HC or HC+AZ. There is thus no indication that accruing a larger sample would change Q-PROTECT's results.

There are a number of study limitations that restrict the conclusions drawn from Q-PROTECT. Perhaps the most important is in the emphasis on a non-clinical endpoint rather than patient-centered outcomes (e.g. symptoms, immunity). The intent was to shed light on a public health outcome – transmissibility – via a surrogate of virologic testing. The assumption that PCR negativity on naso- and oropharyngeal swab samples is linked to lesser likelihood of Covid-19 transmission is rational, but unproven and potentially nuanced. It is likely, for example, that Ct is an oversimplified surrogate for transmission risk, and that factors such as respiratory symptoms (e.g. sneezing) may be important contributors [28].

Just as negative PCR may not always mean zero transmission risk, a positive PCR could simply reflect detection of inactive (non-infectious) viral remnants. There remains a small (but non-zero) chance that Q-PROTECT's non-identification of post-treatment PCR detection differences could obscure a clinically important infectivity difference. The study is limited by the failure to address the possibility that there could be inter-group differences in infectivity of whatever viral particles were present after treatment.

Even if the use of PCR is accepted as an indicator of transmission risk, there remain unanswered questions that translate into Q-PROTECT limitations. Selection of another Ct endpoint (e.g. Ct >30) may accurately classify patients at very low risk of transmission; if this is the case then the endpoint of negative PCR (i.e. Ct >40 on the equipment used in this study) would be too stringent. No *post hoc* analysis was executed on different Ct cut-offs.

Other study flaws constituted threats to both internal and external validity. The main internal validity problems included dropouts and other losses to follow-up. The most substantial external validity threats related to the medication regimen and the study population.

A potentially significant internal validity issue was failure to confirm medication compliance (e.g. by having staff administer medications or by assaying drug levels). Since unreported non-compliance with study therapy would likely be associated with an active-drug regimen (e.g. from gastrointestinal side effects), it is possible that differential medication compliance biased Q-PROTECT toward a null finding.

Even if internal validity questions are resolved, there were a number of study limitations that affect external validity. Among the most important are related to Q-PROTECT's study population and the study's specific medication regimen.

Q-PROTECT's participants were nearly all male, and relatively young. Viral clearance rates are likely similar in females and males, but older patients may clear Covid-19 more slowly [29]. Differential viral clearance in various races or ethnicities has not been well characterized. Q-PROTECT results are applicable only to patients similar to those enrolled in the current study.

The current study results applicability is also restricted in terms of medication regimen. While AZ use for asymptomatic or mildly symptomatic Covid-19 cases tends to follow consistent dosing (500 mg on day one, 250 mg on days two through five), HC dosing varies widely across the Covid-19 evidence base. The Q-PROTECT regimen was selected in March 2020, to match the approach reported successful in Marseille [1]. However, some studies have utilized higher HC doses in the initial days of therapy, and many studies use different daily maintenance doses or durations of therapy. For example, as compared to the one-week Q-PROTECT regimen of 200 mg HC three times daily, Arshad and colleagues [25] and Mitja and colleagues [11] both utilized a day one 800 mg dose followed by 200 mg twice daily for less than a week. Tang and colleagues [13], while also focusing on mild or moderate disease, administered a higher initial dose (1200 mg daily for three days) and a higher maintenance dose (800 mg) for a longer time frame (two to three weeks). In their post-exposure prophylaxis study, Boulware and colleagues [9] also used a relatively high initial HC dose (1400 mg on the day of exposure).

In the Covid-19 evidence base, HC dosing levels do not invariably correlate with efficacy findings. This absence of definitive correlation does not exclude potential importance of dosing regimen. Expert reviewers have remarked that initial therapy with at least 800 mg may be necessary for viral clearance [30].

Physiologically based pharmacokinetic (PBPK) modeling has also utilized a day-one dosage of 800 mg, but overall PBPK recommendations are not substantially inconsistent with the dosing approach used in Q-PROTECT. Yao and colleagues [31] used PBPK models to assess multiple regimens of HC SARS-CoV-2 in an *in vitro* (Vero cell) model. Their recommendation for a first-day loading dose of 800 mg followed by four daily doses of 400 mg was aimed at balancing safety and efficacy, but they did not assess a day-one loading dose of less than 800 mg. The overall approach suggested by PBPK modeling was not markedly different from Q-PROTECT's regimen: the current study provided a smaller day-one dose (600 mg rather than 800 mg) but a larger subsequent daily dose (600 mg rather than 400 mg). It is possible that a dosing regimen different from that of Q-PROTECT could produce different results.

If it is the case that early exposure to HC is needed in order for the drug to effectively inhibit viral replication, then dosing issues may be overshadowed by the fact that the medication was given too late in the course of illness. This hypothesis, while not able to be tested in the current dataset (Q-PROTECT is underpowered for the assessment), seems an unlikely major confounder. Medication was instituted rapidly after PCR – within hours, and never more than 24 h – in patients with disease that was either mild or asymptomatic. However, it must be acknowledged that the negative findings of Q-PROTECT do not necessarily rule out HC benefit if the drug is given earlier in the course of infection.

Any benefit from HC must be weighed against drug-associated adverse effects. As used for Covid-19, HC's most common side effects are gastrointestinal and rarely severe [13 11]. Serious adverse events (defined as mortality or major non-transient morbidity) did not occur in Q-PROTECT and were also rare or absent in other HC RCTs [9,11,13,25]. However, all reports acknowledge the most serious adverse effect of HC±AZ as QT prolongation with associated risk of dangerous dysrhythmias such as torsades de pointes (TdP).

Q-PROTECT was not powered to assess rare events such as TdP. Neither TdP nor any ventricular dysrhythmia was seen. However, in considering large-scale use of HC±AZ, even rare risks have important population-level implications [20]. Q-PROTECT's adverse-effect results should not be construed as confirming safety of HC±AZ. Exploration of rare but significant adverse effects remains the province of larger studies that are more focused on QT assessment.

The study's daily ECGs were judged to provide an acceptable safety margin for detection of significantly prolonged QT or concerning dysrhythmia. However, there remained important QT-related study limitations. The study methodology did not guarantee that for each participant, ECGs would be regularly timed (a few hours after medication dosing) [6], performed by the same machine, and undergo cross-validation (since machine algorithms can over- or underestimate QT) [32]. Study participants' QT monitoring in the quarantine environment was characterized by use of different ECG machines, irregularly timed ECG execution, delayed availability of hardcopy ECG tracings, and lack of cross-checking of machine-reported QT. The study data are therefore not suitable for analysis of QT prolongation associated with HC±AZ. Other investigators using appropriately rigorous methodology have already quantified QT prolongation by HC and AZ, confirming the fact that whatever benefits HC±AZ may bring, come with attendant risk [33].

The lessons of Q-PROTECT must be considered in light of the trial strengths and weaknesses, the medication risks and benefits, and the existing evidence base. Taking all of these factors into account, the investigators conclude that HC±AZ shows no sign of usefulness in

the population studied, and that there is low likelihood of undiscovered drug benefits outweighing therapeutic risks.

Data sharing

Deidentified Q-PROTECT study data (in spreadsheet form, with included data definitions) will be made available for sharing with publication, using Mendeley Data. Data sharing requests are participant to approval by the Q-PROTECT principal investigator. The Mendeley DOI and other information can be obtained by emailing: Sarah.Thomas19@Imperial.ac.uk.

Author contributions

Authors have made the following contributions consistent with ICJME recommendations for authorship:

All authors made meaningful contributions to manuscript preparation, review, and editing. In addition, the authors made contributions as follows:

- Stephen H. Thomas conceived the study, co-wrote the draft manuscript, and maintains overall responsibility for Q-PROTECT conduct and reporting.
- Ali S. Omrani made critical contributions/modifications to the study design and execution plans, and co-wrote the initial manuscript draft.
- Sameer A. Pathan led the execution stage of the study.
- Sarah A. Thomas planned the randomization and allocation concealment approaches, performed the literature search and initial-draft manuscript writing relevant to cell biology of putative drug action mechanisms, executed data-sharing processes, and assisted with revision of the manuscript during the editorial review process.
- Peter V. Coyle led the virology testing planning and execution and made key manuscript contributions in the arena of laboratory medicine.
- Naema Al Mawlawi and Reham Al Kahlout managed the lab specimens, executed all PCR analysis, and arranged virologic reporting data
- Tim R. E. Harris provided substantial manuscript editing, and coordinated physician resourcing for study participant accrual and data processing.
- Caroline E. Thomas led planning of, and manuscript writing describing, data entry spreadsheet/source-table design, preparing of participants' data entry forms; she also executed clinical trials registration and assisted with revision of the manuscript during the editorial review process.
- Isma Qureshi and Zain Bhutta tracked study accrual, dispatched study staff to quarantine centers, managed data tabulation and recording, and executed initial screening to search for eligible participants.
- Hussam Al Soub and Muna Al Maslamani advised on study trial arms design, guided ethics-board communications regarding placebo utilization, and directed collaboration with national Covid-19-management authorities.
- Mohammed Abu Khattab provided infectious disease and public-health expertise during manuscript preparation, and arranged training for study staff.
- Mulham M. Saleh and Andrew Jeremijenko coordinated and oversaw accrual activities at Qatar's quarantine centers.
- Aftab Azad led interdepartmental collaboration in study planning and coordinated discussions with the national authorities responsible for access to both participants and study medications.
- Joann Daghfal assisted with participant identification, statistical planning, data analysis, and interpretation.
- Ashraf El Malik advised on pharmacology, executed design and preparation of placebos, and led the processes related to study-medication preparation and administration.

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Declaration of Competing Interest

The authors have no financial or personal relationships with other people or organizations that could represent a conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.eclinm.2020.100645](https://doi.org/10.1016/j.eclinm.2020.100645).

Appendix 1. Methodology details

The Q-PROTECT study protocol is posted at: <https://www.hamad.qa/EN/Hospitals-and-services/Communicable-Disease-Center/Education-and-Research/Pages/QPROTECT-Protocol.aspx>

Study site and participants

The Q-PROTECT study population was sharply defined by the nature of participant sourcing. Residents of Qatar who tested positive for SARS-CoV-2, but who were asymptomatic or minimally symptomatic, were allowed home quarantine if this could be safely executed. In practice, this translated into home quarantine for long-term residents or families. Those who lived in shared domiciles (e.g. dormitories, hotels) were required to serve quarantine in facilities for which HMC provided healthcare, but for which administration (including access control and security) came under the purview of the Ministry of the Interior (MOI).

Gaining access to the quarantine facilities was logistically and administratively challenging. Facilities were well outside of Doha, entailing extended travel times for study physicians. Furthermore, since security arrangements for quarantine facilities were administered by the MOI there were extensive clearances required for each facility. This situation led to a decision to accrue participants at a single site, Umm Qarn (located within an hour of HGH).

Even within Umm Qarn, Q-PROTECT's study physicians (nearly all were male) were not able to gain access to the female section of the quarantine housing. Thus Q-PROTECT enrolled virtually all males, and the nationality cross-section reflected the nationality of expatriates rather than those native to Qatar or surrounding countries (who were most likely to live in single-family dwellings).

Tables A1,A2,A3,A4

Randomization and masking

Study bottle pairs were dispensed in sequential order. There was no disruption to the sequential dispensing order.

Randomization of the study's projected n of 456 was executed by computer in a location (Imperial College London) remote from the study site. Randomization was executed in a restricted (blocked)

Table A1
Power and sample-size calculations.

	Hypothesized % viral clearance	n per group (80% power)	n per group (90% power)
Best-estimate baseline endpoint achievement (50%); smaller effect size (10%)			
Placebo	50%	116	152
Hydroxychloroquine	60%	116	152
Hydroxychloroquine + Azithromycin	70%	116	152
Best estimate baseline endpoint achievement (50%); larger effect size (20%)			
Placebo	50%	25	33
Hydroxychloroquine	70%	25	33
Hydroxychloroquine + Azithromycin	90%	25	33
Very low endpoint achievement, smaller effect size			
Placebo	10%	77	101
Hydroxychloroquine	20%	77	101
Hydroxychloroquine + Azithromycin	30%	77	101
Very low endpoint achievement, larger effect size			
Placebo	10%	25	33
Hydroxychloroquine	30%	25	33
Hydroxychloroquine + Azithromycin	50%	25	33
Low endpoint achievement, smaller effect size			
Placebo	20%	101	133
Hydroxychloroquine	30%	101	133
Hydroxychloroquine + Azithromycin	40%	101	133
Low endpoint achievement, larger effect size			
Placebo	20%	29	38
Hydroxychloroquine	40%	29	38
Hydroxychloroquine + Azithromycin	60%	29	38
Fair endpoint achievement, smaller effect size			
Placebo	30%	116	152
Hydroxychloroquine	40%	116	152
Hydroxychloroquine + Azithromycin	50%	116	152
Fair endpoint achievement, larger effect size			
Placebo	30%	30	40
Hydroxychloroquine	50%	30	40
Hydroxychloroquine + Azithromycin	70%	30	40
Better-than expected endpoint achievement, smaller effect size			
Placebo	60%	101	133
Hydroxychloroquine	70%	101	133
Hydroxychloroquine + Azithromycin	80%	101	133
Better-than expected endpoint achievement, larger effect size			
Placebo	60%	21	27
Hydroxychloroquine	80%	21	27
Hydroxychloroquine + Azithromycin	90%	21	27

Table A2
Primary (day six) outcome: relative measures.

	Risk ratio (95% confidence interval)	Risk difference (95% confidence interval)
Hydroxychloroquine (12.8% cure rate) vs. placebo (12.2% cure rate)	1.04 (0.57–1.90)	0.01 (–0.07–0.08)
Hydroxychloroquine + Azithromycin (10.5% cure rate) vs. placebo (12.2% cure rate)	0.86 (0.46–1.62)	–0.02 (–0.09–0.05)
Hydroxychloroquine (12.8% cure rate) vs. Hydroxychloroquine + Azithromycin (10.5% cure rate)	1.21 (0.65–2.26)	0.02 (–0.05–0.09)

Table A3.
Symptom development or clearance.

	Hydroxychloroquine + Azithromycin n = 152*	Hydroxychloroquine n = 152*	Placebo n = 152*
Asymptomatic day one and:			
Symptomatic day seven	8 of 78 (10.3%, 4.5–19.2%)	9 of 78 (11.5%, 5.4–20.1%)	13 of 85 (15.3%, 8.4–24.7%)
Symptomatic day 14	3 of 79 (3.8%, 0.8–10.7%)	3 of 77 (3.9%, 0.8–11.0%)	4 of 84 (4.8%, 1.3–11.7%)
Symptomatic day 21	4 of 78 (5.1%, 1.4–12.6%)	2 of 77 (2.6%, 0.3–9.1%)	2 of 85 (2.4%, 0.3–8.2%)
Symptomatic day one and:			
Asymptomatic day seven	56 of 70 (80.0%, 68.7–88.6%)	55 of 69 (79.7%, 68.3–88.4%)	52 of 59 (88.1%, 77.1–95.1%)
Asymptomatic day 14	66 of 69 (95.7%, 87.8–99.1%)	64 of 69 (92.8%, 83.9–97.6%)	58 of 60 (96.7%, 88.5–99.6%)
Asymptomatic day 21	67 of 69 (97.1%, 89.9–99.6%)	68 of 69 (98.6%, 92.2–100.0%)	56 of 60 (93.3%, 83.8–98.2%)

manner to equalize group sizes at the pre-planned interim analysis points ($n = 100$ and $n = 200$) as well as the final study n of 456. Randomization was communicated directly from the UK to the HMC pharmacy, and the randomization scheme was seen only by the pharmacist who prepared the study bottles. The author (SAT) executing

randomization and communicating randomization to the pharmacy had no involvement in the analysis; her other investigation roles were researching and executing manuscript work relevant to cell biology, and executing Q-PROTECT's data-sharing plan after study completion.

Table A4.
QT prolongation.

	Hydroxychloroquine + Azithromycin <i>n</i> = 152	Hydroxychloroquine <i>n</i> = 152	Placebo <i>n</i> = 148*	<i>p</i>
QT prolongation > 30 msec	37 (24.3%; 17.8–32.0%)	31 (20.4%; 14.3–27.7%)	13 (8.8%; 4.8–14.6%)	0.001
QT prolongation > 60 msec	5 (3.3%; 1.1–7.5%)	4 (2.6%; 0.7–6.6%)	2 (1.4%; 0.2–4.8%)	0.641
Maximum QT	418 (403–434; 411–422)	415 (403–434; 412–420)	406 (394–427; 404–414)	0.002
Maximum QT prolongation for cases with + QT prolongation	23 (15–31; 20–24)	20 (13–29; 16–23)	13 (9–22; 11–15)	<0.001

Data are *n* (%; 95% binomial exact confidence interval) or median (IQR; 95% confidence interval for median).

* Data not available for all randomized patients.

Central allocation concealment was used. The randomization scheme was transmitted to the study institution's central pharmacy, where it was translated to identical-appearing sequentially numbered drug-bottle sets. Other routes of allocation concealment addressed three facets of appearances of bottles and study medications. First, the bottle pairs were identical (other than the different study numbers on the labels). Second, study physicians and nurses were not observing when the participants opened the bottle and took the medication. Third, the AZ placebo capsules were visually identical to the AZ capsules; the HC placebo tablets, while not identical to the HC tablets, had the same color as the HC tablets and were of similar size and shape.

Q-PROTECT was triple-blinded. Study staff (physicians and nurses who enrolled participants, executed virologic sampling, and assessed and recorded participant's clinical follow-up data) were unaware of study medication identity and did not see the contents of the study bottles. Study participants were unaware of the specific contents of their medication bottles. Those responsible for analysing data (and plotting graphs) executed analyses using anonymized group identification codes.

The potential for study unmasking was assessed definitively, with a questionnaire to study participants as to which medication they thought was in a particular bottle pair. Of the 456 study participants, data were available on study medication guess for 450 (98.7% of 456). Study dropouts constituted five of the cases for whom no study-medication guess was available; the sixth was transferred to a military facility (at which follow-up information was limited).

Overall, participants' study-medication guesses were biased towards the HC+AZ guess (which accounted for 2/3rd^s of guesses (300 of 450)). Guesses were biased against the double-placebo formulation; only 8% (36 of 450) guesses were of placebo-placebo group. Successful blinding was suggested by a lack of association between group identity and study-medication guess ($p = 0.132$).

Study staff and analysts were asked to indicate whether they had learned of the study-bottle codes. All answers were negative.

PCR testing, timing and handling of swab samples

Most universal transport median (UTM) aliquots were extracted on the QIASymphony platform (Qiagen, Germantown, Maryland USA) and tested with the Thermo Fisher TaqPath COVID-19 RT-PCR Kit (Thermo Fisher, Waltham, Massachusetts USA); this kit targeted the S, N and ORF-1a/b genes. Resource limitations dictated occasional use of three alternative PCR testing methods. Some UTM samples were loaded directly onto a Roche Cobas 6800 and assayed with the Cobas SARS-CoV-2 Test (Roche, Basel, Switzerland) targeting the ORF-1a/b and E gene regions. Other samples were run on a Thermo Fisher ABI 7500 or a Bioneer ExiPrep 96 (Bioneer Accupower, Daejeon, South Korea) that assessed RNA-dependent RNA polymerase (RdRp).

The initial study protocol called for swabs to be taken and tested on the first day of study enrollment and every second day up to day 10, with additional swabs on days 14 and 21. During study planning, it was ascertained that there would be insufficient PCR resources (e.g. reagents) to allow for contemporaneous PCR testing of all collected swabs (i.e. on days 1, 2, 4, 6, 8, 10, 14, and 21, for all participants).

Therefore, even before study commencement, a decision was made to execute testing for the initial Q-PROTECT report only on the swabs from days one, six, and day 14; other sampling days' swabs would be stored for later testing when circumstances allowed.

As the epidemic continued to tax virology resources, laboratory storage space became an issue. By the time Q-PROTECT's enrollment reached 300, the HMC virology laboratory had run out of storage space for untested swabs. Thus, the protocol was amended to include PCR swabs only on days one, six and 14.

Sample-size and power calculations

The sample-size calculation approach used was the *k*-sample binomial model. Each of three ($k = 3$) groups was planned to have the same sample size with χ^2 testing on the $2 \times k$ table.

During (March 2020) sample-size calculations the Q-PROTECT planners concluded there was substantial uncertainty regarding likely virologic cure rates in both the control and experimental study arms. Therefore, sample-size determination accommodated varying levels of control and experimental-arm virologic cure rates. The table is shown below.

The study was planned to accrue sufficient participants to allow for scenarios requiring larger patient *n*. The overall *n* was set at 456 (152 per group), which would provide 90% power at the two "worst-case" scenarios of 10% absolute separation between three study groups (virologic cure rates of 30%, 40%, and 50%). The 90% power level was used to determine target sample size, since with some drop-outs there would still be sufficient *n* to allow for at least 80% power.

Because of the low precision of estimates of virologic cure in the three study arms, the study planning called for two interim analysis, at 100 and 200 cases. The interim analysis after 100 cases was selected to provide 90% power (or between 80 and 90% power accounting for drop-outs) for scenarios with larger effect sizes. A second interim analysis was set at a compromise level of approximately 200 cases ($n = 198$); this would allow an additional assessment in case the baseline assumptions were incorrect in such fashion that $n = 100$ was insufficient but $n = 456$ represented an unnecessarily inefficient accrual target. A decision was made to not have a third (or fourth) interim analysis, due to the effects of such additional analyses which would render the interim analyses' frequentist *p* values impractically low.

With the two interim analyses and the final analysis, the total number of "looks" at the data (*R*) was set at three, with O'Brien-Fleming boundaries at 3.438, 2.431, and 1.985. The resulting *p* values for two interim analyses (at $n = 100$ and $n = 200$) and the final analysis (at $n = 456$) were: 1st interim $p = 0.001$, 2nd interim $p = 0.015$, final analysis $p = 0.047$. The data safety monitoring board (DSMB) was given these calculations prior to study commencement to inform adjudications as to possible Q-PROTECT termination for rejection of H_0 . There was no *a priori* plan for executing futility analysis.

QT. (QTc) monitoring

QT monitoring was based on ACC guidelines published in March 2020 [6]. For low-risk patients such as those in this study (i.e. Tisdale score <7), the ACC recommends either no follow-up ECG (if resources

are constrained) or a single follow-up ECG after 3 days of therapy. ACC recommends considering medication discontinuation if QT increases by “>30–60 msec”.

In order to take a conservative approach, the approved Q-PROTECT protocol called for daily ECGs (rather than the optional ECG at day three). The QT was reported by the ECG machine (*i.e.* not hand-calculated). Two ECG machines were used during the study: a Philips PageWriter TC70 Cardiograph (Koninklijke Philips NV, Amsterdam) and a Mortara ELI 280 (Welch Allyn, New York).

The initial Q-PROTECT protocol dictated that participants be withdrawn for QT prolongation of at least 30 msec. At a point after the interim analysis, it was discovered that study staff were not following the protocol; they were instead using the ACC’s “30–60 msec” cut-off, retaining participants whose QT was prolonged to a degree that exceeded 30 msec but did not reach 60 msec. Study accrual was paused and after consultation with the institutional ethics board and the study’s DSMB, the Q-PROTECT protocol was changed to utilize a 60-msec cut-off to exclude patients for QT prolongation.

Appendix 2. Extended results

Sensitivity and per-protocol analyses

The key sensitivity analysis addressed the fact that the primary endpoint of day six PCR was not assessed in all study participants. All of the HC+AZ participants had day six PCR, but the primary endpoint was not assessed in three HC participants and five placebo participants.

Two sensitivity analyses were performed. In the first, it was presumed that all HC participants missing day six PCR were actually cured, and all placebo participants missing day six PCR failed to achieve virologic cure. In this analysis, the primary endpoint remained non-significant ($p = 0.566$).

A second sensitivity analysis was performed presuming the opposite case to that just described: all HC participants missing day six PCR were classified as non-cured and all placebo participants missing day six PCR were classified as cured. In this analysis, the primary endpoint remained non-significant ($p = 0.323$).

The next sensitivity analyses addressed the fact that the secondary endpoint of day 14 PCR was not assessed in all study participants. In the HC+AZ group, three participants did not have day 14 PCR; the corresponding numbers lacking day 14 PCR in the HC and placebo groups were six and nine, respectively.

The ITT analysis of the day 14 virologic cure endpoint approached, but did not reach, significance ($p = 0.072$). Sensitivity analysis was executed to assess whether the missing data for participants not undergoing day 14 PCR could have influenced the results. The finding of non-significance remained ($p = 0.066$) when all placebo cases missing day 14 virology data were categorized as virologically cured and all HC+AZ cases missing data 14 virology data were categorized as not achieving virologic cure.

The next set of analyses were the per-protocol analyses. These calculations repeated the assessments of the primary endpoint and both secondary endpoints, restricted to only those participants who completed the entire seven-day course of study medication. This approach translated into removal of 22 participants from the initial ITT analyses.

For the primary endpoint, the per-protocol analysis replicated the main analysis result of lack of association ($p = 0.819$) between study group and proportion of participants achieving day six virologic cure. The corresponding proportions in the per-protocol analysis were 9.6% in the HC+AZ group, 11.9% in the HC group, and 10.8% in the placebo group.

Per-protocol analysis of the secondary endpoint of day 14 virologic cure replicated the findings of the day six primary endpoint analysis. There was no association ($p = 0.090$) between study group

and virologic cure (18.9% in HC+AZ group, 27.9 in HC group, and 29.4% in placebo group).

The final per-protocol analysis addressed the secondary endpoint of change in Ct from day one to day six. Per-protocol analysis of this change in Ct showed no association ($p = 0.595$) between study group and Ct increase.

Relative changes in primary endpoint

For the primary endpoint, relative risk was measured using multiplicative (risk ratio, null value of one) and arithmetic (risk difference, null value of zero) approaches. Each of the two treatment groups was assessed against placebo, then the treatment groups were assessed against each other. The relevant results are shown in the table below.

Semi-quantitative virologic outcome at day 14

Both baseline and day 14 Ct data were available in the following numbers of participants who failed to achieve day 14 virologic cure: 119 (97.5%) of 122 non-cured HC+AZ participants, 104 (94.5%) of 110 non-cured HC participants, and 98 (91.6%) of 107 non-cured placebo participants.

The HC+AZ participants’ median Ct rise (with IQR and 95% CI) from day one to day 14 was 11.9 (IQR 6.6–16.4; 95% CI 10.1–13.0). The HC participants’ median Ct rise from day one to day 14 was 12.3 (8.9–15.7; 10.5–13.1). Placebo participants’ median Ct rise from day one to day 14 was 11.9 (8.1–16.4; 11.0–13.5). There was no association ($p = 0.779$) between study group and change in Ct from day one to day 14. This lack of significance remained ($p = 0.912$) in per-protocol analysis.

Clinical outcomes: symptom appearance or resolution

Key outcome information (*e.g.* vital status) was reliably available for 100% of cases – no one left the country, and any illness or death would be identifiable in the national system’s electronic records. This section reports results of symptom assessment: development of new symptoms in those who were initially asymptomatic (on day 1), or resolution of symptoms in those who were initially symptomatic.

Symptom assessment was tabulated at baseline (day one) and on days seven, 14, and 21. If symptom assessment was not executed on the prespecified study day the variable was coded as missing, even if there were symptom assessments before or after the prespecified assessment day.

There were 432 participants (94.7% of 456) who had all three symptom assessment follow-ups at the prespecified time frames of days seven, 14, and 21. Symptom assessment was executed at the day seven follow-up in 439 participants (96.3% of 456), and the symptom assessment was executed in 438 participants (96.1%) on each of the days 14 and 21 follow-ups.

The most common reason for failure to execute follow-up was a participant’s dropping out of the study at participant request; this explanation accounted for eight missing follow-ups at each of the three prespecified time frames. The second most common reason for absence of symptom follow-up was hospitalization (with loss of research team access to patients); this occurred in six participants at day seven, eight participants at day 14, and six participants at day 21. In one case, a participant’s transfer to a military hospital resulted in loss of follow-up (for symptoms) at all three assessment points. Loss of follow-up due to patient unavailability for contact (*e.g.* patient in quarantine facility but not able to be questioned about symptoms) occurred in two participants on day seven, one participant on day 14, and three participants on day 21.

There was no association between study group and number of missing follow-up assessments ($p = 0.802$). For participants who were initially symptomatic, there was no association between study

group and asymptomatic status at days seven ($p = 0.377$), 14 ($p = 0.716$), or 21 ($p = 0.299$). For participants who were initially asymptomatic, there was no association between study group and progression to symptomatic status at days seven ($p = 0.596$), 14 ($p = 1.000$), or 21 ($p = 0.665$).

While these symptom-focused analyses were pre-planned, Q-PROTECT was powered to assess virologic endpoints, not changes in clinical symptoms. The study's low precision for symptom development or resolution endpoints is demonstrated by wide 95% CIs for the effect estimates shown in the table below.

Data are n of cases (%; 95% binomial exact confidence interval). *Data not available for all randomized patients.

Clinical outcomes: hospitalization

A total of 11 participants required hospitalization for any cause. The HC+AZ and placebo groups each had four participants hospitalized (for each group, 2.6% of 152). The HC group had three participants hospitalized (2.0% of 152). There was no association between group and proportion of hospitalized cases ($p = 1.000$ by Fisher's exact test).

Of the 11 participants requiring hospitalization, seven required inpatient care for pneumonia. The HC and placebo groups each had three participants hospitalized for pneumonia (for each group, 2.0% of 152). The HC+AZ group had one patient hospitalized for pneumonia (0.7% of 152). There was no association between group and proportion of cases hospitalized for pneumonia ($p = .708$ by Fisher's exact test)0.

QT (QTc) interval

Due to equipment inconsistencies and other methodological limitations, Q-PROTECT presents QT prolongation results as exploratory analysis. By all endpoints assessed, the HC+AZ and HC groups were associated with statistically similar effects on QT interval, with both of these groups manifesting significantly greater QT effects than those of placebo.

Of the 456 participants, 452 had at least two ECG results available for QT prolongation assessment; four participants in the placebo group did not have a second ECG. On the 452 participants in whom QT prolongation could be assessed, three QT-related endpoints were ascertained. First, each group's proportions of cases with QT prolongation of >30 msec were calculated. Next, each group's proportions of cases with QT prolongation of >60 msec were calculated. Third, each study participant's maximum QT interval (at any time during the study) was assessed; the groups' median values for maximum QT were calculated. For the 30-msec and 60-msec QT prolongation endpoints, proportions were compared across groups; for the continuous-variable endpoint of maximum QT prolongation (found to be non-normally distributed), medians and IQRs (as well as medians' 95% CIs) are reported in the table below.

The final QT-related endpoint assessed was the magnitude of the maximum QT-prolongation. This endpoint was assessed in cases in which two criteria were met: there were at least two ECGs, and the maximum QT prolongation was a positive integer.

The maximum QT prolongation was a negative integer if the initial ECG QT interval was the longest QT interval recorded. This finding of a "negative QT prolongation" was significantly ($p < 0.001$) more likely to be seen in the placebo group (44 of 148, 29.7%) than in either the HC+AZ group (16 of 152, 10.5%) or the HC group (11 of 152, 7.2%), in which the rates of finding of a negative maximum QT prolongation were statistically similar ($p = 0.313$).

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