

A pragmatic randomized controlled trial reports the efficacy of hydroxychloroquine on coronavirus disease 2019 viral kinetics

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Abstract

We randomized 53 patients hospitalized with coronavirus disease 2019 (COVID-19) to hydroxychloroquine therapy (at a dose of 400 mg twice daily for seven days) in addition to standard care or standard care alone (ClinicalTrials.gov Identifier, NCT04316377). All severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive patients 18 years of age or older were eligible for study inclusion if they had moderately severe COVID-19 at admission. Treatment with hydroxychloroquine did not result in a significantly greater rate of decline of SARS-CoV-2 oropharyngeal viral load compared to standard care alone during the first five days. Our results suggest no important antiviral effect of hydroxychloroquine in humans infected with SARS-CoV-2.

Background

Hydroxychloroquine is a registered therapeutic against malaria and several autoimmune conditions, and has in vitro inhibitory effects on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in non-toxic concentrations.¹ In a recent report, hydroxychloroquine given more than two weeks after first symptoms had no effect on the clearance of SARS-CoV-2 in various respiratory tract specimens of afebrile patients hospitalized with coronavirus 2019 (COVID-19) partly pre-treated with antiviral drugs.² Viral clearance is however an incomplete characterization of viral kinetics, and the impact of hydroxychloroquine therapy on SARS-CoV-2 viral kinetics in subjects hospitalized with COVID-19 remains to be elucidated. Hydroxychloroquine is postulated to affect viral replication, and it is reasonable to assume that an effective antiviral drug will affect respiratory tract viral titers and thereby improve symptoms and host inflammatory responses, including the cytokine and chemokine expression that is likely responsible for many of the clinical symptoms of COVID-19.³ Accordingly, in the two-arm, open label, pragmatic randomized controlled trial Norwegian Coronavirus Disease 2019 (NO COVID-19) Study (ClinicalTrials.gov Identifier, NCT04316377), we assessed the efficacy and safety of hydroxychloroquine therapy on SARS-CoV-2 oropharyngeal viral kinetics in patients hospitalized with moderately severe COVID-19.

Results

From March 25, 2020, through May 25, 2020, 27 patients were randomized to hydroxychloroquine sulphate in addition to standard care and 26 patients to standard care alone (**Figure 1**). Details regarding baseline demographics, clinical variables on hospital admission and safety during the trial can be found in **Table**. Time from onset of symptoms to randomization was 8 (interquartile range [IQR] 7 to 12) days in our study. Median age was 62 (IQR 50 to 73), 35 patients (66.0%) were male and 2 patients (3.8%) were current smokers. Median body temperature was 38.2 (IQR 37.5 to 38.7) °C and 20 (37.7%) patients required supplemental oxygen on admission. We found no substantial differences in numbers and proportion of adverse events of special interest, serious adverse events or suspected unexpected serious adverse reactions between hydroxychloroquine plus standard care versus standard care. Fifty-one participants were included in the intention-to-treat analysis; 117 samples of the 133 RT-qPCR-results were

above the limit of detection ($2.11 \log_{10}$ RNA copies/mL). The rate of reduction in SARS-CoV-2 viral load was 0.24 (95% CI 0.03 to 0.46) \log_{10} RNA copies/mL/24h in the hydroxychloroquine group and 0.14 (95% CI -0.10 to 0.37) \log_{10} RNA copies/mL/24h in the standard care group (reduction rate difference between the groups 0.11 [95% CI -0.43 to 0.21] \log_{10} RNA copies/mL/24h; **Figure 2**).

Discussion

In patients with moderately severe COVID-19 in need of hospital admission, treatment with hydroxychloroquine sulphate initiated median 8 days after first symptoms did not result in a significantly greater rate of decline of SARS-CoV-2 oropharyngeal viral load compared to standard care alone during the next five days. Measures of rate of decline in virus replication as primary end points to evaluate antiviral drug efficacy are crucial.³ Rapid reductions in active viral replication may be essential to prevent tissue damage and to further clinical recovery, as well as reduce risk of viral complications and mortality. By exploring viral load as a continuous outcome, a more sensitive statistical index compared to dichotomy,⁴ the neutral result of hydroxychloroquine versus standard care in our study strongly suggests no major effect of hydroxychloroquine on the principal pathology in COVID-19. This model is supported by no apparent effect of hydroxychloroquine on SARS-CoV-2 negative conversion rate in Chinese patients with mild to moderate COVID-19.² The patients in the aforementioned report were however younger, afebrile and partly pre-treated with antiviral drugs. The duration from onset of symptoms was additionally notably longer compared to the current investigation, which with median 8 days from start of symptoms to start of therapy closely mimics the typical clinical course of COVID-19 characterized by clinical deterioration and need for hospitalization admission one week after illness onset.⁵ Accordingly, our trial extends the results of previous investigations to more acutely ill and febrile patients in need of hospital admission. In light of the COVID-19 pandemic, there is an unmet need of pharmacological interventions aimed at reducing morbidity and mortality. Three medical therapies were early in the COVID-19 pandemic considered to be strong candidates in this regard; lopinavir-ritonavir, remdesivir, and hydroxychloroquine. Treatment with lopinavir-ritonavir has so far failed to demonstrate any benefit in COVID-19 beyond standard care.⁶ The results for remdesivir appear more promising, but evidence is still conflicting.^{7,8} Retrospective data examining the clinical effect of hydroxychloroquine in COVID-19 are also diverging,^{9,10} and properly conducted and adequately powered randomized trials with peer-reviewed reports are accordingly still needed to assess the therapeutic value of hydroxychloroquine on clinical outcomes in patients with COVID-19.

Our current investigation has several limitations. The study was non-blinded without placebo treatment and we recognize that the lack of blinding may have influenced the standard care treatment and decision making by the treating physician, ultimately affecting our results. However, the study outcome was SARS-CoV-2 viral load, and study personnel performing the RT-qPCR and statistical analyses were blinded concerning group allocation. We assume that changes in viral load in the upper respiratory tract is a valid measure for ongoing viral replication, but we did not perform analyses differentiating viable from non-viable virus. Oropharyngeal samples were obtained for the viral analyses, contrary to the common

practice of nasopharyngeal sampling for the diagnosis of upper airway respiratory viruses. Nasopharyngeal sampling is however associated with significant discomfort for the patient, possibly to a degree leading to study discontinuation. A recent report by Wölfel et al.¹¹ found no significant differences in viral loads when comparing naso- and oropharyngeal sampling for SARS-CoV-2. Wang et al.⁷ found comparable viral loads in upper and lower respiratory tract samples, suggesting that SARS-CoV-2 viral kinetics can be studied in the upper respiratory tract. Stringent scientific support for this assumption is however still lacking. Electrocardiograms were not routinely taken during trial conduction, barring us from assessing the effect of hydroxychloroquine therapy on corrected QT interval. Finally, due to early study cessation, sample size was less than planned with resulting lower study power.

In conclusion, therapy with hydroxychloroquine did not impact SARS-CoV-2 viral kinetics in patients admitted to hospital with moderately severe COVID-19. Our results suggest no important antiviral effect of hydroxychloroquine in humans infected with SARS-CoV-2.

Methods

Trial design

The NO COVID-19 Study is a single center, two-arm, open label, group-sequential, pragmatic randomized controlled trial of hydroxychloroquine sulphate in adults hospitalized with COVID-19. Patients were randomly assigned to receive hydroxychloroquine sulphate (at a dose of 400 mg twice daily for seven days) in addition to standard care or standard care alone¹². Because of rapidly decreasing incidence of COVID-19 in Norway, the trial was prematurely stopped by the trial sponsor on May 25, 2020. The study protocol was approved by the Regional Committees for Medical Research Ethics (REC 121446) and the Norwegian Medicines Agency. The study was performed according to standard rules for Good Clinical Practice, with statistical methods and stopping rules described in the protocol and statistical analysis plan (see **Supplementary Material**).

Patients

All reverse transcriptase polymerase chain reaction (RT-qPCR) SARS-CoV-2 positive patients 18 years of age or older were eligible for study inclusion if they had moderately severe COVID-19 at admission (National Early Warning Score 2 [NEWS2]¹³ of 6 or less). For patients who tested positive for SARS-CoV-2 before admission, SARS-CoV-2 status was verified with the external laboratory. Exclusion criteria included (1) the need of admission to intensive care unit on hospital admission, (2) history of psoriasis, (3) reduced hearing/tinnitus, (4) visual impairment, (5) known adverse reaction to hydroxychloroquine sulphate, (6) pregnancy, or (7) prolonged corrected QT interval (>450 ms). All study participants provided written informed consent before study inclusion.

Data on coexisting conditions were acquired from the hospital electronic patient records. Coronary artery disease was defined as history of myocardial infarction, coronary artery bypass grafting, or percutaneous coronary intervention. Diabetes was defined as history of diabetes mellitus type 1 or type 2, and the use

of antidiabetic medication. Hypertension was defined as history of hypertension and the use of antihypertensive medication. Obstructive pulmonary disease was defined as history of chronic obstructive pulmonary disease or asthma. Obesity was defined as body mass index of 30 kg/m² or above. Current smoking was defined as daily consumption of cigarettes.

Primary outcome

The primary outcome was rate of decline in SARS-CoV-2 viral load in the oropharynx from baseline through the first 96 hours after randomization, using a single batch of swabs and a standardized sampling procedure to saturate them. Oropharyngeal swab samples were taken from patients at inclusion, at 48 hours and at 96 hours, by a selected group of study physicians. For analysis, total nucleic acids were extracted from 300 µl of each specimen using the Maxwell® RSC Viral total Nucleic Acid Purification Kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions and eluted in 50 µl nuclease-free water. SARS-CoV-2 detection was performed in duplicate by RT-qPCR on 5 µl nucleic acid eluate in a total reaction volume of 25 µl on a QuantStudio™ 7 Flex Real-Time PCR System (Thermofisher Scientific, Waltham, Massachusetts, USA), according to the protocol published in January 2020 by Corman et al.¹⁴ that targets the viral E-gene of sarbecoviruses. For each patient, all samples in the time series were analyzed in the same extraction and PCR set-up. Single batches of all reagents for extraction and PCR were used for all samples in the study. SARS-CoV-2 RNA quantitation was calculated using a serial dilution of a synthetic Wuhan coronavirus 2019 E gene RNA control comprising the viral region to be amplified, provided by the European Virus Archive Global (EVAg). Viral loads are expressed in log₁₀ RNA copies/mL transport medium. The limits of detection (LoD) and quantitation (LoQ) of the assay are of 2.11 and 2.55 log₁₀ RNA copies/mL, respectively. For data analyses, results below LoD (SARS-CoV-2 RNA not detected) were set to 0 log₁₀ RNA copies/mL, and results below LoQ were set to the mean between LoD and LoQ values (i.e. 2.36 log₁₀ RNA copies/mL). A qPCR assay targeting human β-globin was performed on all samples where no viral RNA was detected for assessment of sample adequacy.¹⁵ For the intention-to-treat population (n = 51), all actual samples were positive for human β-globin analysis, indicating adequate sample quality. Further details regarding study sampling and analysis can be found in the **Supplementary Methods**.

Statistical analyses

The primary outcome was analyzed using a generalized linear mixed model, with subject-specific random intercept and slope. The complete statistical analysis plan can be found in **Supplementary Methods**. The statistical analyses were performed with STATA 16 (StataCorp LP, College Station, TX).

Declarations

Funding

No external funding was received.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Tables

Table. Demographics, baseline characteristics and safety during the trial.

	All (n = 53)	Hydroxychloroquine plus standard care (n = 27)	Standard care (n = 26)
Age, years	62 (50, 73)	56 (41, 72)	69 (51, 74)
Male sex, n (%)	35 (66.0%)	19 (70.4%)	16 (61.5%)
Body mass index, kg/m ²	26.4 (23.9, 30.5)	25.6 (23.9, 29.4)	27.6 (24.2, 33.0)
Current smoker, n (%)	2 (3.8%)	1 (3.7%)	1 (3.8%)
Time from symptom onset to randomization, days	8 (7, 12)	8 (7, 13)	8 (6, 11)
Coexisting conditions			
Hypertension, n (%)	17 (32.1%)	6 (22.2%)	11 (42.3%)
Diabetes mellitus, n (%)	9 (17.0%)	4 (14.8%)	5 (19.2%)
Coronary heart disease, n (%)	5 (9.4%)	3 (11.1%)	2 (7.7%)
Obstructive pulmonary disease, n (%)	14 (26.4%)	5 (18.5%)	9 (34.6%)
Obesity, n (%)	16 (30.8%)	5 (19.2%)	11 (42.3%)
≥ 1 coexisting condition, n (%)	33 (62.3%)	14 (51.9%)	19 (73.1%)
On admission			
Systolic blood pressure, mmHg	134 (124, 144)	129 (120, 142)	137 (130, 145)
Diastolic blood pressure, mmHg	75 (71, 85)	75 (70, 87)	74 (71, 79)
Heart rate, beats per minute	86 (80, 98)	88 (76, 98)	86 (80, 100)
Respiratory rate, breaths per minute	24 (20, 32)	22 (20, 30)	26 (20, 32)
Oxygen saturation, %	95 (93, 96)	95 (94, 96)	95 (92, 96)
NEWS2	5 (2, 6)	4 (2, 6)	5 (3, 7)
Body temperature, °C	38.2 (37.5, 38.7)	38.2 (37.3, 38.7)	38.2 (37.5, 38.6)
Body temperature > 37.8°C, n (%)	35 (66.0%)	17 (63.0%)	18 (69.2%)
Supplemental oxygen, n (%)	20 (37.7%)	8 (29.6%)	12 (46.2%)
Safety			
Adverse events ^a	237	125	112
Serious adverse events ^b	11 (20.8%)	5 (18.5%)	6 (23.1%)
Suspected unexpected serious adverse reactions	1 ^c (1.9%)	0 (0.0%)	1 ^c (3.8%)

NEWS2, National Early Warning Score 2. Obesity was defined as body mass index of 30 kg/m² or above. All values are presented as median with interquartile range for continuous variables or absolute numbers with percentages for categorical variables. One patient in hydroxychloroquine plus standard care had missing data for body mass index, and values are calculated based on available information.

^a Adverse events of special interest were assessed daily and included visual disturbances, gastrointestinal discomfort, diarrhea, headache, nausea or dizziness.

^b No patient had more than one serious adverse event. Serious adverse events included acute respiratory distress syndrome (n = 1), pneumonia (n = 2), respiratory failure (n = 7) and urinary tract infection (n = 1).

^c Urinary tract infection.

Figures

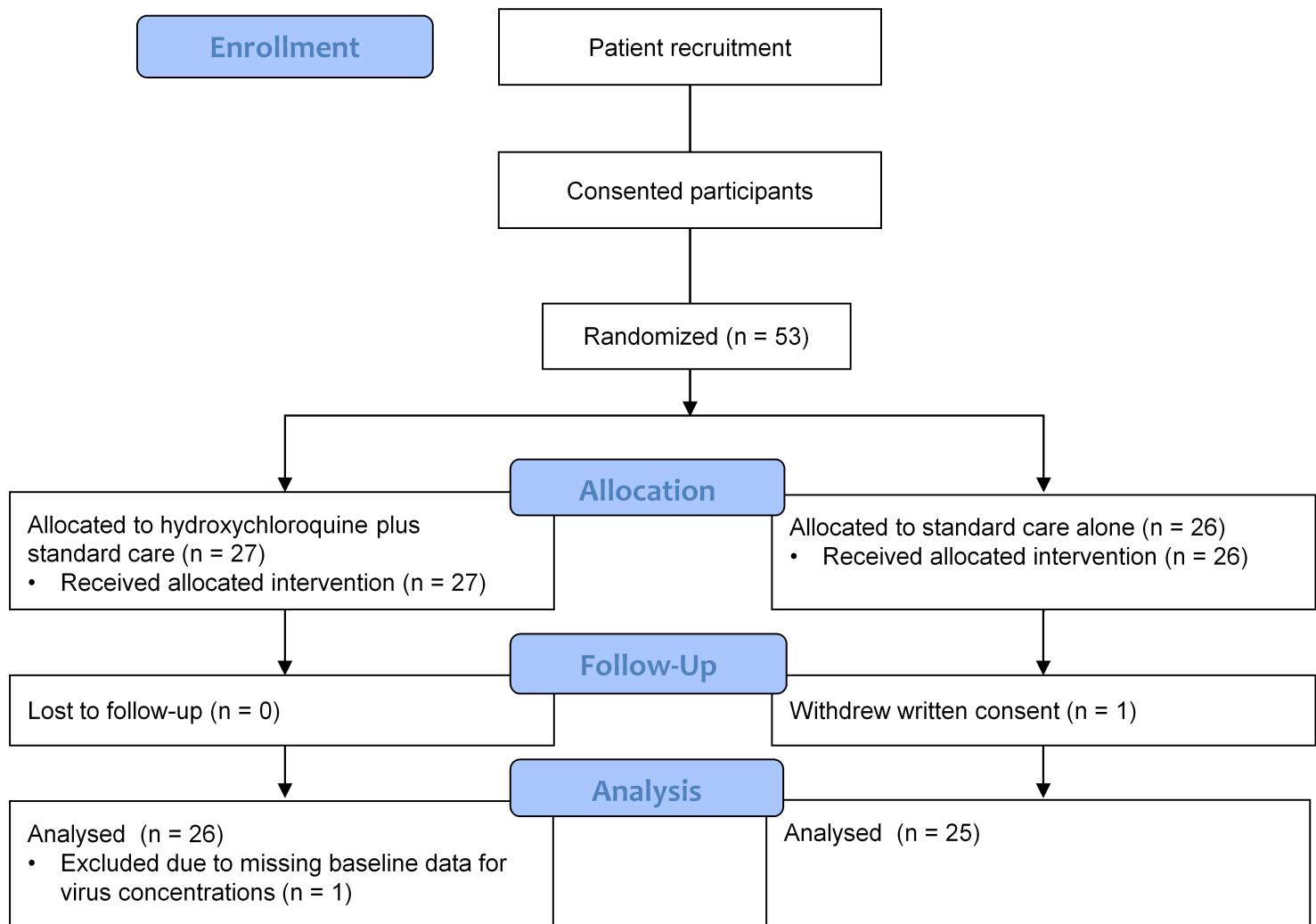


Figure 1

CONSORT diagram

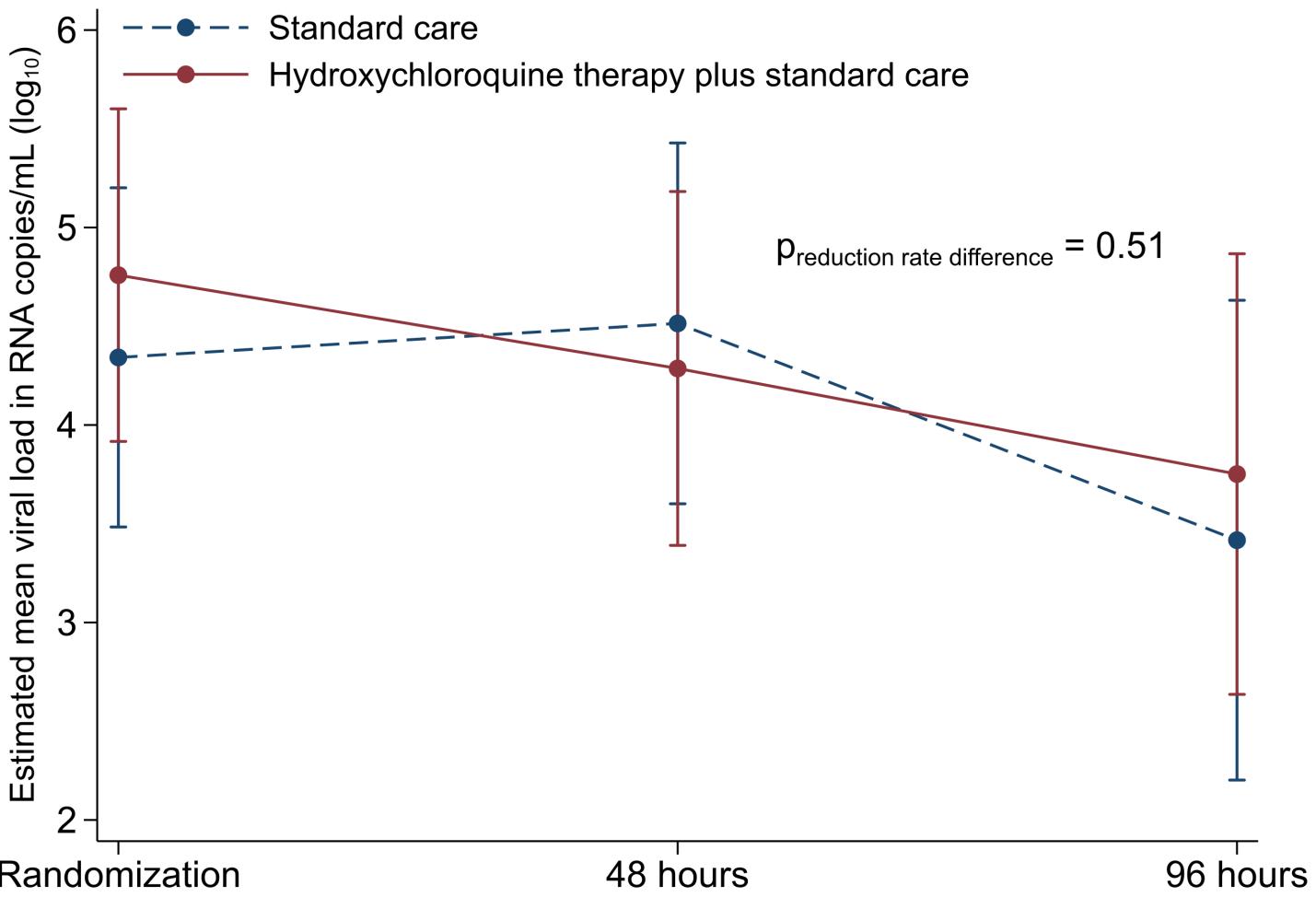


Figure 2

Oropharyngeal viral load (log₁₀) in hydroxychloroquine plus standard care versus standard care in intention-to-treat population (n = 51). One patient in the hydroxychloroquine plus standard care had missing baseline data for viral concentrations and one patient in standard care withdrew consent before viral load assessment at 48 hours. Data are accordingly shown for 26 patients assigned to hydroxychloroquine plus standard care and 25 patients assigned to standard care. Estimated mean difference between groups was 0.27 (95% CI -0.92 to 1.47) log₁₀ RNA copies/mL at randomisation, 0.06 (95% CI -1.15 to 1.26) log₁₀ RNA copies/mL at 48 hours, and -0.16 (95% CI 1.67 to 1.36) log₁₀ RNA copies/mL at 96 hours.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 20200712NOCOVID19supplementncommmerged.pdf