Silva Borba MG, Almeida Val F, Sousa Sampaio V, et al. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial. JAMA Network Open. 2020;3(4.23):e208857.

BACKGROUND – THE STUDY QUESTION?		
Background	• There is no specific antiviral therapy recommended for COVID-19 • Recent publications have suggested a possible benefit of chloroquine (CQ), but in vitro studies indicate a high concentration of the drug would be needed for an antiviral effect against SARS-CoV-2	
Previous trials	• Two controversial studies have been conducted in France by the same investigators Gautret et al, in which patients received hydroxychloroquine (HCQ) with or without azithromycin [1,2]. The first was a preliminary report comparing outcomes of forty-two patients who received either HCQ 200 mg orally three times a day x 10 days (n= 20) or standard of care (n=16). Six of the HCQ patients were also given azithromycin 500mg on day 1 followed by 250 mg per day x 4 days. The authors state HCQ patients experienced higher rates of viral eradication than control group, and those on combination therapy achieved higher viral clearance than monotherapy. This study has received significant criticism along with a statement published by the International Society of Antimicrobial Chemotherapy Journal stating the study had not met its scientific standards for publication but that the peer review process had met standards [3]. • The second study was an observational report in which 80 patients were given HCQ (200 mg three times a day x 10 days) and azithromycin (500mg on the first day then 250mg daily for the next four days) with 6 days of follow up [2]. They concluded that for 79/80 patients, the combination of HCQ and azithromycin resulted in a "clinical improvement that appeared significant" when compared to the natural evolution in patients with a definite outcome. They reported a rapid fall of nasopharyngeal viral load tested by qPCR with 83% negative at day 7, and 93% at day 8. They also reported virus cultures from patient respiratory samples were negative in 97.5% patients at day 5. Although the sample size was slightly larger in this study, the follow-up period of 6 days was still substandard, there was no control group for comparison, and clinical outcomes were poorly described.	
Why this study?	• Prior to this study, no published reports of robust/randomized clinical studies on safety and/or efficacy of CQ and/or hydroxychloroquine (HCQ) for treatment of COVID-19, and none comparing different dosages of CQ/HCQ with a thorough safety assessment • Note: Chloroquine conversion 250mg Chloroquine Phosphate (CP-p) = 150 mg Chloroquine base (CP-b) • The Health Commission of Guangdong Province recommended the use of phosphate CQ-p tablets at a dose of 500 mg twice daily for 10 days (total dose, 10 g CQ-p) for the treatment of patients aged 18-65 years with mild, moderate, or severe pneumonia secondary to COVID-19 • Authors state that because the compassionate use of CQ or HCQ to treat COVID-19 has already been formally indicated for patients with severe disease "in many countries", it would be unethical to test proper efficacy owing to the lack of a placebo group as a comparator	
Null Hypothesis	• The working hypothesis of this trial was that the lethality rate in the high-dose group would be half that of the low-dose group by day 28	
GENERAL STUDY OVERVIEW		
	Summary	Critique
Funding	• Funded by Government of the Amazonas State, Farmanguinhos (Fiocruz), Superintendência da Zona Franca de Manaus, Coordination for the Improvement of Higher Education Personnel, Fundação de Amparo à Pesquisa do Estado do Amazonas, and federal funds	• Funders had no role in the design/conduct of the study nor the collection, analysis, or interpretation of the data

	facilitated by the Brazilian Senate	
Trial design	• Parallel, double-masked, randomized, phase IIb clinical trial	• No placebo control group without CQ treatment for comparison of lethality rate
Objectives	• To assess primarily safety, and secondarily efficacy, of two different CQ dosages (“high” and “low”) as adjunctive therapy (+ ceftriaxone and azithromycin) of hospitalized patients in Brazil with SARS-CoV-2	• Safety/lethality rate of CQ likely confounded by the concomitant use of both azithromycin and oseltamivir, which could have contributed to adverse cardiac outcomes
Enrollment	• Hospitalized adult patients with clinical suspicion of SARS-CoV-2 in Manaus, Brazilian Amazon • Enrollment started March 23, 2020 • According to hospital protocol, all patients with acute respiratory distress syndrome also received ceftriaxone 1g IV twice daily for 7 days + azithromycin 500 mg daily for 5 days (+/- oseltamivir 75 mg twice daily for 5 days when influenza was suspected)	• Conducted in single public hospital in Brazil • While the study aimed to compare safety of two CQ doses, all patients were also receiving concomitant azithromycin and 86.8% were receiving oseltamivir
METHODS		
Inclusion criteria	• Respiratory rate > 24 rpm and/or heart rate > 125 bpm (in absence of fever) and/or peripheral oxygen saturation < 90% in ambient air and/or shock (defined as mean arterial pressure < 65 mmHg, with need for vasopressors, or oliguria, or a lower level of consciousness) • Enrolled prior to laboratory confirmation of COVID-19	• Randomization prior to confirmed COVID-19 diagnosis reasonable as focus of the study was safety
Exclusion criteria	• Children < 18	• Patients with elevated QTc interval at baseline were not excluded
Interventions	• Eligible participants allocated at 1:1 ratio to receive either (1) high-dose CQ (600 mg twice daily for 10 days) or (2) low-dose CQ (450 mg twice daily on first day followed by 450 mg once daily for 4 days) • For the low-dose group, patients received placebo tablets from day 5 to 9	• All patients were also receiving concomitant azithromycin + ceftriaxone, per hospital protocol, and 86.8% were receiving oseltamivir • Note: Chloroquine conversion 250mg Chloroquine Phosphate (CP-p) = 150 mg Chloroquine base (CP-b) so patients in the high dose arm actually received a total of 20g CQ-p (12g CQ-b) which is significantly higher (double) the dose recommended from the Chinese guidelines
Primary Endpoints	• Reduction in lethality by at least 50% in the high-dose group compared to low-dose group by day 28	• Since the trial was terminated early, mortality outcomes only assessed until day 13
Secondary Endpoints	• Lethality on day 13 • Participant clinical status • Laboratory examinations • Electrocardiogram results on days 13 and 28	• Secondary endpoints were poorly described and did not appear to be assessed in a standardized manner • Preprint version of this article (published in MedRxIV) stated that mechanical ventilation or supplemental oxygen duration would be assessed as secondary outcome; also noted time from treatment to discharge

Statistical analyses	• Sample size calculated assuming a 20% lethality incidence in critically-ill patients and that high-dose CQ would reduce lethality by at least 50% compared with low-dose • For 80% power and a 5% α, 197 participants were needed per study arm (n = 394 total); adding 10% for losses, the final sample of 440 participants was obtained • Interim analyses were originally planned between the groups when the study reached 25%, 50%, and 75% of the total sample size. However, global lethality (without unmasking) was measured daily for security purposes, and the DSMB was informed accordingly	•
RESULTS		
Enrollment	• 81 total patients were enrolled: 41 (50.6%) to the high-dose group and 40 (49.4%) to the low-dose group • A preliminary analysis was performed on April 5, 2020, per DSMB recommendation, when 11 patients had died (7 [63.6%] in the high-dose group; 4 [36.4%] in the low-dose group) • 62/81 (76.5%) of patients had COVID-19 confirmed by RT-PCR, with 31 patients with confirmed infections in each group	• Very small sample size due to early termination of trial by DSMB based on mortality rate • The death rate was also quoted as the reason for early termination at the pre-print version from day 6 analysis with 11 patient deaths (Table 3 below). However, they misquoted the groups that the deaths occurred in and this was never explicitly stated outside of the tables. In this JAMA-published study, the numbers are reversed showing higher deaths in the high-dose group **Table 3**. Efficacy outcomes after enrollment, in the intention-to-treat population until Day 6*. • *Except viral clearance, which was performed on Day 4.
Baseline characteristics	• Mean age of patients was 51.1 years: mean age in the low-dose group was 47.4 years vs. 54.7 years in the high-dose group • The most common comorbidities were hypertension (45.5% overall; 53.6% high-dose vs. 37% low-dose group), diabetes (25.5% overall; 32.1% high-dose vs. 18.5% low-dose group), alcohol use disorder (27.5% overall), heart disease (9.1% overall; 17.9% high-dose vs. 0% low-dose), and asthma (7.4% overall; 10.7% high-dose vs. 3.8% low-dose group)	• Comorbidities, especially hypertension, diabetes, heart disease, and asthma tended to be more frequent in patients receiving high-dose CQ • Also, due to an error in enrollment practices, patients > 75 years old (5 patients total) were exclusively enrolled into high-dose arm (this was noted in the pre-print version of the article)
Monitoring	• Laboratory parameters and electrocardiograms (EKG) were performed at the clinician's discretion	• Unclear which patients had EKG monitoring and how frequent monitoring was

Variable	Total	CQ low dosage	CQ high dosage	*p*-value
Oxygen support need (%)	4/28 (14.3)	1/13 (7.7)	3/15 (20.0)	0.35
Invasive mechanical ventilation need (%)	6/39 (15.4)	2/19 (10.5)	4/20 (20.0)	0.41
ICU need, %	2/13 (15.4)	1/11 (9.1)	1/2 (50.0)	0.14
Need for inotropics (%)	1/34 (2.9)	1/19 (5.3)	0/15 (0.0)	0.37
Death (%)	11/81 (13.6)	7/40 (17.5)	4/41 (9.7)	0.35
Naso/oropharyngeal swab viral clearance (%)	1/26 (3.9)	1/12 (8.3)	0/14 (0.0)	0.27

Primary Outcome	• Lethality until day 13 was 39% (16/41) in the high-dose group and 15% in the low-dose group (6/40) • High-dose group was associated with lethality (odds ratio, 3.6; 95%CI, 1.2-10.6). Despite small sample size, in an exploratory multivariate analysis, the high-dose CQ was no longer associated with death when controlled by age (odds ratio, 2.8; 95%CI, 0.9-8.5) • Based on results in which a higher dosage of CQ showed opposite of the study's hypothesis, the DSMB recommended the immediate interruption of the high-dose group for all ages and that all patients be unmasked and reverted to the low-dose group	• Limitation of lethality assessment is that the high-dose group included more patients susceptible to cardiac complications (older, with heart disease), with or without CQ treatment • Study lacked placebo group control group
Secondary Outcomes	• Overall 11 of 73 patients (15.1%) had QTc interval corrected by the Fridericia method (QTcF) > 500 ms, with 8 of 57 patients (14.0%) with confirmed cases of COVID-19 • QTcF > 500 ms was more frequent in the high-dose group than the low-dose group (7/37 [18.9%] vs 4/36 [11.1%]). • Two of 37 patients (2.7%) in the high-dose group, both with confirmed COVID-19, experienced ventricular tachycardia before death, without torsade de pointes. • Respiratory secretion at day 4 was RT-PCR negative in only 6/27 (22.2%) of patients with either nasopharyngeal and/or oropharyngeal samples collected	• Supplement 2 provides some clinical details of 12 patients with QTcF prolongation and ventricular tachycardia, but unclear whether regular EKG monitoring was performed
Other Clinical events	• One patient developed rhabdomyolysis, which was attributed to CQ, and the drug was withdrawn • In 2 patients, myocarditis was suspected based on CKMB elevation since the first day of hospitalization, suggesting myocarditis related to SARS-CoV-2 itself	•

AUTHORS' CONCLUSIONS

- In this study, a high-dosage of CQ (12 g CQ-b; 20g CQ-p) given for 10 days concurrently with azithromycin and oseltamivir was not sufficiently safe to warrant continuation of that study group.
- Authors recommend that similar dosages no longer be used for the treatment of severe COVID-19, especially because treatment based on older patients with previous cardiac diseases who are receiving concomitant cardiotoxic drugs should be the rule.
- To better understand the role of CQ or HCQ in the treatment of COVID-19, authors recommend the following next steps: (1) randomized clinical trials evaluating its role as a prophylactic drug and (2) randomized clinical trials evaluating its efficacy against the progression of COVID-19 when administered to patients with mild or moderate disease
- Patients using CQ (irrespective of dosage) failed to present evidence of substantial viral clearance by day 4, even with the concomitant use of azithromycin

GENERALIZABILITY/CRITIQUE/DISCUSSION

- This trial, although randomized, was terminated early so the study arms were very small and appeared unbalanced with respect to age and underlying comorbidities—importantly, heart disease and age
- Secondary endpoints were poorly described and did not appear to be assessed in a standardized manner, especially QTc monitoring.
- **All patients were receiving CQ + azithromycin, and most (86.8%) were receiving oseltamivir which can also prolong the QTc interval. Therefore, it may be concluded from this trial that high-dose chloroquine (and by close association, hydroxychloroquine) in combination with azithromycin and possibly oseltamivir, is potentially associated with increased mortality among patients with severe, suspected COVID-19.**
- This trial does not answer the question as to whether CQ (at any dose) should be recommended for the treatment of COVID-19

References

1. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents. 2020 Mar 20:105949. doi: 10.1016/j.ijantimicag.2020.105949. [Epub ahead of print]
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