No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19 Infection by,Jean Michel Molina et al

| BACKGROUND – THE STUDY QUESTION? | |
|---|---|
| Background | Hydroxychloroquine (HCQ) and chloroquine (CQ) have FDA labeled indications as antimalarial agents and autoimmune diseases such as lupus and rheumatoid arthritis. Azithromycin is a macrolide used commonly for bacterial pneumonia.<br>It is thought that these agents exert their anti-viral activity by increasing endosomal pH required for virus / cell fusion with impairment of ACE2 receptor glycosylation and by direct immune modification by way of reduction of cytokine production specifically Il1 and Il6 and inhibition of toll like receptor signaling. [1 -2] |
| Previous trials | The *in vitro* antiviral activity of CQ was first identified in the late 1960's and anti- SARSCOV2 activity of both CQ and HCQ have recently been assessed in cell culture [3-5]. In-vitro results by Wang et al [3] and Yao et al [4] indicate HCQ as more potent than CQ whereas Liu et al. [5] found HCQ to be less potent. Popert [6] reported high tissue HCQ levels, with levels in lungs, spleen, kidney and eye reaching 200 to 700 times that of plasma. Laaksonen [7] reported HCQ doses of 6-6.5mg/kg per day generates safe serum levels of 1.4-1.5 μM in humans. Previous murine studies have demonstrated HCQ/CQ with broad antiviral activity, including human coronavirus OC43, enterovirus EV-A71, Zika virus and influenza A H5N1 [8].<br>However, when used in patients, CQ /HCQ have consistently failed to produce benefits in Dengue [13], Ebola [9-10], worsened clinical symptoms and delayed viral clearance of Chikungunya [12-13] and HIV [14] and failed to prevent influenza [11]. Among the possible factors for this discrepancy the main one is the dose needed to treat viral infections is several folds higher than needed for malaria [9]. Also, since, the pathogenesis of SARSVOV2 is still not fully elucidated, the immunomodulatory effects provoked by CQ/HCQ could potentially be harmful [15].<br>Human COVID 19 trials up until this data include: 3 studies conducted in China and two in France:<br>• The first by *Gao et al* [16] is an unpublished observational report of 100 patients in which investigators report CQ as superior to a control treatment by inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting seroconversion, and shortening the disease course. Severe adverse reactions to CQ were not disclosed.<br>• The second by Chen et al [17] was a small pilot study in which 30 patients with mild disease were randomized to either HCQ or placebo. No significant difference in virus clearance or clinical endpoints (absence of fever, radiological progression) were found.<br>• The third report by Chen et al [18] was a randomized parallel group trial, in which 62 patients with mild disease were given either HCQ or standard of care. Primary end points were time to virologic clearance and clinical symptoms and CT changes. Overall, HCQ had a modest effect on total time to clinical recovery vs standard of care ( fever resolution 2.2 days vs 3.2 days, cough 2 days vs 3.1 days, and potentially more effective in reducing progression from mild to severe disease (0% vs 12.9%), pneumonia exacerbation (6.5% vs 29%) but more adverse drug reactions (6.4% vs 0%).<br>• Two studies were conducted in France by the same investigators Gautret et al [19]. The first was a preliminary report comparing outcomes of forty-two patients who received either HCQ 200mg po 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 day1 followed by 250mg per day x 4days.The author's state HCQ patients experienced higher rates of viral eradication than control group, and those on combination therapy achieved higher viral clearance than monotherapy. Percentage negative NP swabs control vs HCQ vs HCQ + azithromycin post inclusion ( P values HCQ monotherapy vs combination): day 3: 6.3%, 35.7%, 83.3% (p=0.002), day 4 25%,50%,83.3%( p=0.05), day 5 (18.8%, 50%, 100% (p=0.002), day 6: 12.5%,57.1%, 100% (p< .001)<br>• The second, was an observational report in which eighty patients were given HCQ (200mg 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 [20]. Six of the patients were also those from the first study. The primary endpoints were assessed as (i) an aggressive clinical course requiring oxygen therapy or transfer to the ICU after at least three days of treatment, (ii) contagiousness as assessed by PCR and culture, and (iii) length of stay in the ID ward. They conclude for 79 of 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, as described in the literature. They reported a rapid fall of nasopharyngeal viral load tested by qPCR with 83% negative at day7, and 93% at day8. Virus cultures from patient respiratory samples were negative in 97.5% patients at day5.<br>• Both Gautret et al reports had many flaws including methodology, reporting bias, internal and external validity, lack of randomization and control group. Indeed, there was an Official Statement from International Society of Antimicrobial Chemotherapy Journal stating the study had not meet its scientific standards for publication [21-22]. | |
| Why this study? | Considerable interest in use of use if HCQ+/- Azithromycin. Conflicting results between previous findings and need to replicate /validate previous small-scale findings of Gautret at al [19] and previous studies from China [17]. This study was technically prospective pending the results of clinical trials | |
| Null Hypothesis | • Compared to baseline, HCQ and azithromycin make no difference in outcomes for COVID 19 | |
| GENERAL STUDY OVERVIEW | | |
| | Summary | Critique |
| Funding | • Not stated | • Not disclosed but it appears to have been conducted hence funded by APHP Saint Louis Hospital Paris, France. Potential investigator bias |
| Trial design | • Prospective, non-randomized, non-comparative open labeled, single center | • Lack of comparator arm and it is a small preliminary study pending ongoing trials |
| Objectives | • Determine if HCQ and Azithromycin can rapidly clear SARSCOV2 and provide clinical benefit. | • Lack of comparative arm<br>• Even if the study results replicate that observed in the Gautret et al study (which was their primary hypothesis) the pts criteria, sample size, baseline demographics, confounding factors were not matched and so would not have been sufficient to confirm theory |
| Enrollment | • Patients enrolled in 1 hospital in France | • Enrollment method not provided- possible bias |
| | | |
| Inclusion criteria | • None indicated | • Not provided |
| Exclusion criteria | • Non indicated | • Not provided |
| Interventions | • All patients given HCQ 600mg/day for 10 days and azithromycin 500mg day 1 then 250 mg day 2-5 | • Attempting to validate the preliminary findings of Gautret et al [19] by repeating the dosage and frequency in small cohort of patients. Severity of illness, viral load, confounding factors not clear or addressed for in either trial. Other trial used Loading dose of 400 mg x 2 then 200 mg po bid [17-18, 23] |
| Primary Endpoints | • NP qualitative PCR assay for virus clearance<br>• Clinical outcomes | • Not clearly defined specifically timing.<br>• Time to onset of infection, severity of illness, viral load, confounding factors not clear or addressed. |
| Secondary Endpoints | • HCQ trough levels at day 3- 7 after initiation | • Therapeutic drug levels not established for HCQ nor for COVID 19.<br>• The timing for “troughs” was not stated and left for assumption.<br>• The authors spell trough wrong and say “through”.<br>• Not clear if that’s a random level, a trough or a peak.<br>• Lack of references to what authors considered therapeutic, |

| | | |
|---|---|---|
| | | appropriate or comparable to other studies for HCQ |
| Statistical analyses | • Descriptive statistics: 95% confidence interval | • No comparative arm, just at 95% of the mean value |
| RESULTS | | |
| Enrollment | • 11 patients in APHP hospital in France | • Very small number |
| Baseline characteristics | • 11/11 SARSCOV 2 positive<br>• 1/11 fever and on nasal oxygen<br>• mean age 58.7 years (range 20-77)<br>• 8/11 comorbidities: 3/8 solid cancer; 2/8 obese, 2/8 hematological cancer, 1/8 HIV | • Time from onset of COVID 19 not provided<br>• Authors do not disclose if it was a NP swab, we are just assuming as that is how they followed the patients<br>• Viral load unknown<br>• Source /site of baseline PCR abstraction not provided |
| Monitoring | • During hospitalization | • Not explained how this was conducted such as signs and symptoms, Pneumonia progression, CT imaging, ADR /QTc monitoring and frequency |
| Primary Outcome | • 8/10 (80%, 95% CI 49-94) positive NP swabs at day 5 – 6 after treatment initiation<br>• 1/11 died, 2/11 transferred to ICU, 1/11 HCQ and azithromycin stopped at day 4 due to prolonged QT interval prolongation. | • Cause of death or transfer to ICU not explained- pts had co-morbidities.<br>• Due to the death of one patient the primary outcome of the study could not be established (since one patient stopped taking the drug at day 4 due to QTc prolongation, that patient was not included in the primary outcome).<br>• Risk and confounding factors for QTc prolongation were not addressed for the subjects who experienced it. |
| Secondary Outcomes | • Mean trough blood concentration of HCQ 678ng/ml (range 381-891) at days 3-7 after treatment initiation. | • Only provided HCQ levels were measured<br>• Therapeutic trough concentrations for HCQ or azithromycin have not been established for SARSCOV2. It is unclear where the investigators drew their HCQ reference range from.<br>• How plasma levels correlate with lung epithelial levels or immunomodulating effects for SARScov2 have not been established.<br>• Half-life of HCQ is ~ 40 days, whether patients had achieved Cmax or steady state is unlikely. A loading dose was not given.<br>• In-vitro EC50/EC90, CT, SI SARSCOV2 are variable and unclear how to extrapolate to human infections (48 hr Ec 50= 0.72 µM) Yao et al [4] and 1.13 µM Wang et al [3].<br>• Popert paper suggested serum levels 370 to 470 µg/l (1.4 to 1.5 µmol /l) during HCQ therapy are safe which equates to 370-470 ng/ml. They also state due to HCQ preferentially concentrates into lungs, spleen, kidney and leukocytes and eyes at 200-700 levels that of plasma [6]<br>• New PK/PD paper by Perinel et al quoted SARSCOV2 Plasma Therapeutic range as 1-2mg/l (1000 ng/ml to 2000 ng/ml) [23] unknown if adequate levels had been reached or if safe levels had been exceeded. |

| AUTHORS' CONCLUSIONS |
|---|
| • These virologic results stand in contrast with those reported by Gautret et al. and cast doubts about the strong antiviral efficacy of this combination. In addition demonstrate safety risk that was excluded by Gautret et al [19].<br>• These results matched Chen et al [17] i.e. HCQ failed to demonstrate virus clearance or clinical benefit and also matched outcomes seen in other viral infections (HIV, Dengue, Ebola, influenza) [1-2, 8-15]<br>• No evidence of a strong antiviral activity or clinical benefit for combination HCQ and azithromycin for treatment of hospitalized patients with severe COVID-19. |
| **GENERALIZABILITY/CRITIQUE/DISCUSSION** |
| • Very small-scale observational feasibility study, no control arm compared to outcomes to baseline and previous studies<br>• Viral load, onset of disease, severity of disease not disclosed and confounding factors not adjusted for.<br>• Optimal dose, duration and exposure are still unknown well as how they relate to therapeutic level monitoring, AUC, adverse reactions<br>• If the latest PK/PD study by Perinel et al is followed [23], adequate plasma concentration was not achieved.<br>• Cardiac toxicity with HCQ at 600mg/day was demonstrated early on in 9% of patients and 80% of patients that survived during the evaluation period had not eradicated the virus. One patient died 1/11 (9%) and 2 were transferred to ICU (18%) however confounding factors were not addressed.<br>• Larger prospective double blind randomized clinical trials are needed to validate these results and quality of standard of care needs to be evaluated across different publications. |

**References:**


1 Raghu Chivukula Massachusetts General Hospital Advances in Motion March 27 https://advances.massgeneral.org/research-and-innovation/article.aspx?id=1145&utm_medium=email&utm_source=marketo-providers&utm_campaign=FY20-endocrinologyadvances-28mar2020-%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20%20coronavirus&mkt_tok=eyJpIjoiTWpsaVptUmxPREZpWkdRMSIsInQiOiJmenJDZjZMWmxISGJmVG1xTEp4S1J6ZEl1eVV2NHdpd0FrQytyMFJ5TkFES0pvTGZoRUhRTDRLSmQ1c2xCV2sxTzBMNGVMbkcySWpCblZ2UmZ5b0xIRW5jd3VGbHJhbXZRd2RtcVQybXdvWlVXQ0oreEhYOXROQjF2Zkh1eHQ5WCJ9

2. Al-Bari AA. Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. Pharmacology research and perspectives. 2017;5:e00293

3. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell research 2020; 30:26971.

4. Yao et al https://doi.org/10.1093/cid/ciaa237

5. Liu et al. Cell Discovery (2020) 6:16 https://doi.org/10.1038/s41421-020-0156-0

6. Popert Chloroquine review. *Rheumatology*, Volume 15, Issue 3, August 1976, Pages 235–238,https://academic.oup.com/rheumatology/article-abstract/15/3/235/1780159?redirectedFrom=fulltext

7. Laaksonen A et al Dosage of Antimalarial Drugs for Children with Juvenile Rheumatoid Arthritis and Systemic Lupus Erythematosus: A Clinical Study with Determination of Serum Concentrations of Chloroquine and Hydroxychloroquine Journal Scandinavian Journal of Rheumatology Volume 3, 1974 - Issue 2 https://www.tandfonline.com/doi/abs/10.3109/03009747409115809

*8.* Touret et al Of chloroquine and COVID-19 Antiviral Research Volume 177, May 2020, 104762 *https://www.sciencedirect.com/science/article/pii/S0166354220301145?via%3Dihub*

*9. Andre Kalil treating COVID-19—Off-Label Drug Use, Compassionate Use, and Randomized Clinical Trials during Pandemics JAMA.* Published online March 24, 2020. https://jamanetwork.com/journals/jama/fullarticle/2763802

10. Paton NI, Lee L, Xu Y, et al. Chloroquine for influenza prevention: a randomized, double-blind, placebo-controlled trial. Lancet Infectious Diseases, 2011; 11:677-83 https://www.ncbi.nlm.nih.gov/pubmed/21550310

11. Wu et al, Chloroquine enhances replication of influenza A virus A/WSN/33 (H1N1) in dose-, time-, and MOI-dependent manners in human lung epithelial cells A549. J Med Virol. 2015 Jul; 87(7):1096-103. doi: 10.1002/jmv.24135. Epub 2015 Feb 25. https://www.ncbi.nlm.nih.gov/pubmed/25715935

12. Roques et al, Paradoxical Effect of Chloroquine Treatment in Enhancing Chikungunya Virus Infection Viruses. 2018 May; 10(5): 268.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5977261/
13. Tricou V, Minh NN, Van TP et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLos Neglected tropical diseases 2010; 4:e785
14. Paton NI, Goodall RL, Dunn DT, et al. Effects of hydroxychloroquine on immune activation and disease progression among HIV-infected patients not receiving antiretroviral therapy: a randomized controlled trial. JAMA. 2012; 308 (4):353-61. https://www.ncbi.nlm.nih.gov/pubmed/22820788
15. Maurizio Guastalegname et al Could Chloroquine /Hydroxychloroquine Be Harmful in Coronavirus Disease 2019 (COVID-19) Treatment? *Clinical Infectious Diseases*, ciaa321, https://doi.org/10.1093/cid/ciaa321
16. Gao et al Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020 Mar 16; 14(1):72-73. doi: 10.5582/bst.2020.01047 https://www.ncbi.nlm.nih.gov/pubmed/32074550
17. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19) http://www.zjujournals.com/med/article/2020/1008-9292/20200108.shtml DOI: 10.3785/j.issn.1008-9292.2020.03.03
18. Chen et al, Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial https://www.medrxiv.org/content/10.1101/2020.03.22.20040758v1
19. 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 (ahead of print). https://www.mediterranee-infection.com/wp-content/uploads/2020/03/Hydroxychloroquine_final_DOI_IJAA.pdf
20. Gautret P et al Hydroxychloroquine-Azithromycin and COVID-19. https://www.mediterranee-infection.com/wp-content/uploads/2020/03/COVID-IHU-2-1.pdf
21. https://www.isac.world/news-and-publications/official-isac-statement
22. https://retractionwatch.com/2020/04/06/hydroxychlorine-covid-19-study-did-not-meet-publishing-societys-expected-standard/
23. Perinel,S et al , Towards Optimization of Hydroxychloroquine Dosing in Intensive Care Unit COVID-19 Patients *Clinical Infectious Diseases*, ciaa394, https://doi.org/10.1093/cid/ciaa394https://academic.oup.com/cid/article/doi/10.1093/cid/ciaa394/5816960