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**1 Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-**  
**2 label non-randomized clinical trial**

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25

## 26 *Abstract*

### 27 Background

28 Chloroquine and hydroxychloroquine have been found to be efficient on SARS-CoV-2, and  
29 reported to be efficient in Chinese COV-19 patients. We evaluate the role of  
30 hydroxychloroquine on respiratory viral loads.

### 31 Patients and methods

32 French Confirmed COVID-19 patients were included in a single arm protocol from early  
33 March to March 16<sup>th</sup>, to receive 600mg of hydroxychloroquine daily and their viral load in  
34 nasopharyngeal swabs was tested daily in a hospital setting. Depending on their clinical  
35 presentation, azithromycin was added to the treatment. Untreated patients from another center  
36 and cases refusing the protocol were included as negative controls. Presence and absence of  
37 virus at Day6-post inclusion was considered the end point.

### 38 Results

39 Six patients were asymptomatic, 22 had upper respiratory tract infection symptoms and eight  
40 had lower respiratory tract infection symptoms.

41 Twenty cases were treated in this study and showed a significant reduction of the viral  
42 carriage at D6-post inclusion compared to controls, and much lower average carrying duration  
43 than reported of untreated patients in the literature. Azithromycin added to  
44 hydroxychloroquine was significantly more efficient for virus elimination.

### 45 Conclusion

46 Despite its small sample size our survey shows that hydroxychloroquine treatment is  
47 significantly associated with viral load reduction/disappearance in COVID-19 patients and its  
48 effect is reinforced by azithromycin.

49

50 *Key words:* 2019-nCoV; SARS-CoV-2; COVID-19; hydroxychloroquine; azithromycin;  
51 clinical trial

52

## 1. Introduction

In late December 2019, an outbreak of an emerging disease (COVID-19) due to a novel coronavirus (named SARS-CoV-2 latter) started in Wuhan, China and rapidly spread in China and outside [1,2]. The WHO declared the epidemic of COVID-19 as a pandemic on March 12<sup>th</sup> 2020 [3]. According to a recent Chinese stud, about 80% of patients present with mild disease and the overall case-fatality rate is about 2.3% but reaches 8.0% in patients aged 70 to 79 years and 14.8% in those aged  $\geq 80$  years [4]. However, there is probably an important number of asymptomatic carriers in the population, and thus the mortality rate is probably overestimated. France is now facing the COVID-19 wave with more than 4500 cases, as of March 14<sup>th</sup> 2020 [5]. Thus, there is an urgent need for an effective treatment to treat symptomatic patients but also to decrease the duration of virus carriage in order to limit the transmission in the community. Among candidate drugs to treat COVID-19, repositioning of old drugs for use as antiviral treatment is an interesting strategy because knowledge on safety profile, side effects, posology and drug interactions are well known [6,7].

A recent paper reported an inhibitor effect of remdesivir (a new antiviral drug) and chloroquine (an old antimalarial drug) on the growth of SARS-CoV-2 *in vitro*, [8] and an early clinical trial conducted in COVID-19 Chinese patients, showed that chloroquine had a significant effect, both in terms of clinical outcome and viral clearance, when comparing to controls groups [9,10]. Chinese experts recommend that patients diagnosed as mild, moderate and severe cases of COVID-19 pneumonia and without contraindications to chloroquine, be treated with 500 mg chloroquine twice a day for ten days [11].

Hydroxychloroquine (an analogue of chloroquine) has been demonstrated to have an anti-SARS-CoV activity *in vitro* [12]. Hydroxychloroquine clinical safety profile is better than that of chloroquine (during long-term use) and allows higher daily dose [13] and has fewer

concerns about drug-drug interactions [14]. Our team has a very comprehensive experience in successfully treating patients with chronic diseases due to intracellular bacteria (Q fever due to *Coxiella burnetii* and Whipple's disease due to *Tropheryma whipplei*) with long-term hydroxychloroquine treatment (600 mg/day for 12 to 18 months) since more than 20 years. [15,16] We therefore started to conduct a clinical trial aiming at assessing the effect of hydroxychloroquine on SARS-CoV-2-infected patients after approval by the French Ministry of Health. In this report we describe our early results, focusing on virological data in patients receiving hydroxychloroquine as compared to a control group.

## 2. Study population and Methods

### *Setting*

This ongoing study is coordinated by The Méditerranée Infection University Hospital Institute in Marseille. Patients who were proposed a treatment with hydroxychloroquine were recruited and managed in Marseille centre. Controls without hydroxychloroquine treatment were recruited in Marseille, Nice, Avignon and Briançon centers, all located in South France.

### *Patients*

Hospitalized patients with confirmed COVID-19 were included in this study if they fulfilled two primary criteria: i) age >12 years; ii) PCR documented SARS-CoV-2 carriage in nasopharyngeal sample at admission whatever their clinical status.

Patients were excluded if they had a known allergy to hydroxychloroquine or chloroquine or had another known contraindication to treatment with the study drug, including retinopathy, G6PD deficiency and QT prolongation. Breastfeeding and pregnant patients were excluded based on their declaration and pregnancy test results when required.

## *Informed consent*

Before being included in the study, patients meeting inclusion criteria had to give their consent to participate to the study. Written informed signed consent was obtained from adult participants ( $\geq 18$  years) or from parents or legal guardians for minors ( $<18$  years). An information document that clearly indicates the risks and the benefits associated with the participation to the study was given to each patient. Patients received information about their clinical status during care regardless of whether they participate in the study or not. Regarding patient identification, a study number was assigned sequentially to included participants, according to the range of patient numbers allocated to each study centre. The study was conducted in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines of good clinical practice, the Helsinki Declaration, and applicable standard operating procedures.

The protocol, appendices and any other relevant documentation were submitted to the French National Agency for Drug Safety (ANSM) (2020-000890-25) and to the French Ethic Committee (CPP Ile de France) (20.02.28.99113) for reviewing and approved on 5<sup>th</sup> and 6<sup>th</sup> March, 2020, respectively. This trial is registered with EU Clinical Trials Register, number 2020-000890-25.

## *Procedure*

Patients were seen at baseline for enrolment, initial data collection and treatment at day-0, and again for daily follow-up during 14 days. Each day, patients received a standardized clinical examination and when possible, a nasopharyngeal sample was collected. All clinical data were collected using standardized questionnaires. All patients in Marseille center were proposed oral hydroxychloroquine sulfate 200 mg, three times per day during ten days (in this preliminary phase ,we did not enrolled children in the treatment group based in data indicating that children develop mild symptoms of COVID-19 [4]). Patients who refused the treatment or had an



exclusion criteria, served as controls in Marseille centre. Patients in other centers did not receive hydroxychloroquine and served as controls. Symptomatic treatment and antibiotics as a measure to prevent bacterial super-infection was provided by investigators based on clinical judgment. Hydroxychloroquine was provided by the National Pharmacy of France on nominative demand.

### *Clinical classification*

Patients were grouped into three categories: asymptomatic, upper respiratory tract infection (URTI) when presenting with rhinitis, pharyngitis, or isolated low-grade fever and myalgia, and lower respiratory tract infections (LRTI) when presenting with symptoms of pneumonia or bronchitis.

### *PCR assay*

SARS-CoV-2 RNA was assessed by real-time reverse transcription-PCR [17].

### *Hydroxychloroquine dosage*

Native hydroxychloroquine has been dosed from patients' serum samples by UHPLC-UV using a previously described protocol [18]. The peak of the chromatogram at 1.05 min of retention corresponds to hydroxychloroquine metabolite. The serum concentration of this metabolite is deduced from UV absorption, as for hydroxychloroquine concentration. Considering both concentrations provides an estimation of initial serum hydroxychloroquine concentration.

### *Culture*

For all patients, 500 µL of the liquid collected from the nasopharyngeal swab were passed through 0.22-µm pore sized centrifugal filter (Merck millipore, Darmstadt, Germany), then were inoculated in wells of 96-well culture microplates, of which 4 wells contained Vero E6 cells (ATCC CRL-1586) in Minimum Essential Medium culture medium with 4% fetal calf serum and 1% glutamine. After centrifugation at 4,000 g, microplates were incubated at 37°C. Plates were observed daily for evidence of cytopathogenic effect. Presumptive detection of virus in supernatant was done using SU5000 SEM (Hitachi) then confirmed by specific RT-PCR.

### *Outcome*

The primary endpoint was virological clearance at day-6 post-inclusion. Secondary outcomes were virological clearance overtime during the study period, clinical follow-up (body temperature, respiratory rate, long of stay at hospital and mortality), and occurrence of side-effects.

### *Statistics*

Assuming a 50% efficacy of hydroxychloroquine in reducing the viral load at day 7, a 85% power, a type I error rate of 5% and 10% loss to follow-up, we calculated that a total of 48 COVID-19 patients (ie, 24 cases in the hydroxychloroquine group and 24 in the control group) would be required for the analysis (Fleiss with CC). Statistical differences were evaluated by Pearson's chi-square or Fisher's exact tests as categorical variables, as appropriate. Means of quantitative data were compared using Student's t-test. Analyses were performed in Stata version 14.2.

## **3. Results** (detailed results are available in supplementary Table 1)

## *Demographics and clinical presentation*

We enrolled 36 out of 42 patients meeting the inclusion criteria in this study that had at least six days of follow-up at the time of the present analysis. A total of 26 patients received hydroxychloroquine and 16 were control patients. Six hydroxychloroquine-treated patients were lost in follow-up during the survey because of early cessation of treatment. Reasons are as follows: three patients were transferred to intensive care unit, including one transferred on day2 post-inclusion who was PCR-positive on day1, one transferred on day3 post-inclusion who was PCR-positive on days1-2 and one transferred on day4 post-inclusion who was PCR-positive on day1 and day3; one patient died on day3 post inclusion and was PCR-negative on day2; one patient decided to leave the hospital on day3 post-inclusion and was PCR-negative on days1-2; finally, one patient stopped the treatment on day3 post-inclusion because of nausea and was PCR-positive on days1-2-3. The results presented here are therefore those of 36 patients (20 hydroxychloroquine-treated patients and 16 control patients). None of the control patients was lost in follow-up. Basic demographics and clinical status are presented in Table 1. Overall, 15 patients were male (41.7%), with a mean age of 45.1 years. The proportion of asymptomatic patients was 16.7%, that of patients with URTI symptoms was 61.1% and that of patients with LRTI symptoms was 22.2%). All patients with LRTI symptoms, had confirmed pneumonia by CTScan. Hydroxychloroquine-treated patients were older than control patients (51.2 years vs. 37.3 years). No significant difference was observed between hydroxychloroquine-treated patients and control patients with regard to gender, clinical status and duration of symptoms prior to inclusion (Table 1). Among hydroxychloroquine-treated patients six patients received azithromycin (500mg on day1 followed by 250mg per day, the next four days) to prevent bacterial super-infection under daily electrocardiogram control. Clinical follow-up and occurrence of side-effects will be described in a further paper at the end of the trial.

195 *Hydroxychloroquine dosage*

196 Mean hydroxychloroquine serum concentration was  $0.46 \mu\text{g/ml} \pm 0.2$  (N=20).

197 *Effect of hydroxychloroquine on viral load*

198 The proportion of patients that had negative PCR results in nasopharyngeal samples  
199 significantly differed between treated patients and controls at days 3-4-5 and 6 post-inclusion  
200 (Table 2). At day6 post-inclusion, 70% of hydroxychloroquine-treated patients were  
201 virologically cured comparing with 12.5% in the control group ( $p= 0.001$ ).

202 When comparing the effect of hydroxychloroquine treatment as a single drug and the effect of  
203 hydroxychloroquine and azithromyc in combination, the proportion of patients that had  
204 negative PCR results in nasopharyngeal samples was significantly different between the two  
205 groups at days 3-4-5 and 6 post-inclusion (Table 3). At day6 post-inclusion, 100% of patients  
206 treated with hydroxychloroquine and azithromycin combination were virologically cured  
207 comparing with 57.1% in patients treated with hydroxychloroquine only, and 12.5% in the  
208 control group ( $p<0.001$ ). These results are summarized in Figures 1 and 2. Drug effect was  
209 significantly higher in patients with symptoms of URTI and LRTI, as compared to  
210 asymptomatic patients with  $p<0.05$  (data not show).

211 Of note, one patient who was still PCR-positive at day6-post inclusion under  
212 hydroxychloroquine treatment only, received azithromycin in addition to hydroxychloroquine  
213 at day8-post inclusion and cured her infection at day-9 post infection. In contrast, one of the  
214 patients under hydroxychloroquine and azithromycin combination who tested negative at  
215 day6 post-inclusion was tested positive at low titer at day8 post-inclusion.

216 *Cultures*

217 We could isolate SARS-CoV-2 in 19 out of 25 clinical samples from patients.

#### 4. Discussion

For ethical reasons and because our first results are so significant and evident we decide to share our findings with the medical community, given the urgent need for an effective drug against SARS-CoV-2 in the current pandemic context.

We show here that hydroxychloroquine is efficient in clearing viral nasopharyngeal carriage of SARS-CoV-2 in COVID-19 patients in only three to six days, in most patients. A significant difference was observed between hydroxychloroquine-treated patients and controls starting even on day3 post-inclusion. These results are of great importance because a recent paper has shown that the mean duration of viral shedding in patients suffering from COVID-19 in China was 20 days (even 37 days for the longest duration) [19]

Very recently, a Chinese team published results of a study demonstrating that chloroquine and hydroxychloroquine inhibit SARS-CoV-2 *in vitro* with hydroxychloroquine ( $EC_{50}=0.72\mu M$ ) found to be more potent than chloroquine ( $EC_{50}=5.47\mu M$ ) [14]. These *in vitro* results corroborate our clinical results. The target values indicated in this paper [14] were reached in our experiments. The safer dose-dependent toxicity profile of hydroxychloroquine in humans, compared to that of chloroquine [13] allows using clinical doses of hydroxychloroquine that will be over its  $EC_{50}$  observed *in vitro* [14].

Our preliminary results also suggest a synergistic effect of the combination of hydroxychloroquine and azithromycin. Azithromycin has been shown to be active *in vitro* against Zika and Ebola viruses [20-22] and to prevent severe respiratory tract infections when administrated to patients suffering viral infection [23]. This finding should be further explored to know whether a combination is more effective especially in severe cases. Speculated potential risk of severe QT prolongation induced by the association of the two drugs has not

been established yet but should be considered. As for each treatment, the cost benefits of the risk should be evaluated individually. Further studies on this combination are needed, since such combination may both act as an antiviral therapy against SARS-CoV-2 and prevent bacterial super-infections.

The cause of failure for hydroxychloroquine treatment should be investigated by testing the isolated SARS-CoV-2 strains of the non-respondents and analyzing their genome, and by analyzing the host factors that may be associated with the metabolism of hydroxychloroquine. The existence of hydroxychloroquine failure in two patients (mother and son) is more suggestive of the last mechanism of resistance.

Such results are promising and open the possibility of an international strategy to decision-makers to fight this emerging viral infection in real-time even if other strategies and research including vaccine development could be also effective, but only in the future. We therefore recommend that COVID-19 patients be treated with hydroxychloroquine and azithromycin to cure their infection and to limit the transmission of the virus to other people in order to curb the spread of COVID-19 in the world. Further works are also warranted to determine if these compounds could be useful as chemoprophylaxis to prevent the transmission of the virus, especially for healthcare workers. Our study has some limitations including a small sample size, limited long-term outcome follow-up, and dropout of six patients from the study, however in the current context, we believe that our results should be shared with the scientific community.

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267

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273 **Competing Interests:** N/A

274 **Ethical Approval:** French Ethic Committee (CPP Ile de France) (20.02.28.99113)

275

## References

- [1] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents*. 2020 Feb 17:105924. doi: 10.1016/j.ijantimicag.2020.105924. [Epub ahead of print]
- [2] Wang LS, Wang YR, Ye DW, Liu QQ. A review of the 2019 Novel Coronavirus (COVID-19) based on current evidence". *Int J Antimicrob Agents*. 2020 [Epub ahead of print]
- [3] WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. [<https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020>]
- [4] Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020 Feb 24. doi: 10.1001/jama.2020.2648. [Epub ahead of print]
- [5] Santé Publique France. Infection au nouveau Coronavirus (SARS-CoV-2), COVID-19, France et Monde [<https://www.santepubliquefrance.fr/maladies-et-traumatismes/maladies-et-infections-respiratoires/infection-a-coronavirus/articles/infection-au-nouveau-coronavirus-sars-cov-2-covid-19-france-et-monde>]
- [6] Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. *Int J Antimicrob Agents*. 2020 Feb 15:105923. doi: 10.1016/j.ijantimicag.2020.105923. [Epub ahead of print]



- 298 [7] Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and  
 299 hydroxychloroquine as available weapons to fight COVID-19. *Int J Antimicrob Agents*. 2020  
 300 [Epub ahead of print]
- 301 [8] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine  
 302 effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*  
 303 2020;10-0282.
- 304 [9] Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent  
 305 efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*  
 306 2020 Feb 19. doi: 10.5582/bst.2020.01047. [Epub ahead of print]
- 307 [10] Chinese Clinical Trial Registry. <http://www.chictr.org.cn/>  
 308 searchproj.aspx?title=%E6%B0%AF%E5%96%B9&offi  
 309 cialname=&subjectid=&secondaryid=&applier=&study  
 310 leader=&ethicalcommitteesanction=&sponsor=&studya  
 311 ilment=&studyailmentcode=&studytype=0&studystage=  
 312 0&studydesign=0&minstudyexecutetime=&maxstudyexe  
 313 cutetime=&recruitmentstatus=0&gender=0&agreetosign  
 314 =&secsponsor=&regno=&regstatus=0&country=&prov  
 315 ince=&city=&institution=&institutionlevel=&measure=  
 316 &intercode=&sourceofspends=&createyear=0&isupload  
 317 rf=&whetherpublic=&btngo=btn&verifycode=&page=1.
- 318 [11] Multicenter collaboration group of Department of Science and Technology of  
 319 Guangdong Province and Health Commission of Guangdong Province for chloroquine in the  
 320 treatment of novel coronavirus pneumonia. Expert consensus on chloroquine phosphate for

- 321 the treatment of novel coronavirus pneumonia]. *Zhonghua Jie He He Hu Xi Za Zhi*. 2020 Mar  
 322 12;43(3):185-188. doi: 10.3760/cma.j.issn.1001-0939.2020.03.009.
- 323 [12] Biot C, Daher W, Chavain N, Fandeur T, Khalife J, Dive D, et al. Design and synthesis  
 324 of hydroxyferroquine derivatives with antimalarial and antiviral activities. *J Med Chem*  
 325 2006;49:2845-2849.
- 326 [13] Marmor MF, Kellner U, Lai TY, Melles RB, Mieler WF; American Academy of  
 327 Ophthalmology. Recommendations on Screening for Chloroquine and Hydroxychloroquine  
 328 Retinopathy (2016 Revision). *Ophthalmology*. 2016 Jun;123(6):1386-94. doi:  
 329 10.1016/j.optha.2016.01.058. Epub 2016 Mar 16.
- 330 [14] Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In Vitro Antiviral Activity and  
 331 Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe  
 332 Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020 Mar 9. pii:  
 333 ciaa237. doi: 10.1093/cid/ciaa237. [Epub ahead of print]
- 334 [15] Raoult D, Houpihan P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment  
 335 of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or  
 336 hydroxychloroquine. *Arch Intern Med*. 1999 Jan 25;159(2):167-73.
- 337 [16] Lagier JC, Raoult D. Whipple's disease and *Tropheryma whipplei* infections: when to  
 338 suspect them and how to diagnose and treat them. *Curr Opin Infect Dis*. 2018 Dec;31(6):463-  
 339 470. doi: 10.1097/QCO.0000000000000489. [x] Expert consensus on chloroquine phosphate  
 340 for the treatment of novel coronavirus pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi*. 2020  
 341 Mar 12;43(3):185-188. doi: 10.3760/cma.j.issn.1001-0939.2020.03.009.
- 342 [17] Amrane S, Tissot-Dupont H, Doudier B, Eldin C, Hocquart M, Mailhe M et al. Rapid  
 343 viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the

- 344 infectious diseases referral hospital in Marseille, France, - January 31st to March 1st, 2020: A  
 345 respiratory virus snapshot. *Travel Med Infect Dis.* 2020 [Epub ahead of print].
- 346 [18] Armstrong N, Richez M, Raoult D, Chabriere E. Simultaneous UHPLC-UV analysis of  
 347 hydroxychloroquine, minocycline and doxycycline from serum samples for the therapeutic drug  
 348 monitoring of Q fever and Whipple's disease. *J. Chromatogr. B Analyt. Technol. Biomed. Life*  
 349 *Sci.* 2017; 1060, 166-172.
- 350 [19] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for  
 351 mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study.  
 352 *Lancet.* 2020 Mar 11. pii: S0140-6736(20)30566-3. doi: 10.1016/S0140-6736(20)30566-3.  
 353 [Epub ahead of print]
- 354 [20] Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, Sandoval-Espinosa C, et al.  
 355 Zika virus cell tropism in the developing human brain and inhibition by azithromycin. *Proc*  
 356 *Natl Acad Sci U S A.* 2016 Dec 13;113(50):14408-14413. Epub 2016 Nov 29.
- 357 [21] Madrid PB, Panchal RG, Warren TK, Shurtleff AC, Endsley AN, Green CE, Kolokoltsov  
 358 A, et al. Evaluation of Ebola Virus Inhibitors for Drug Repurposing. *ACS Infect Dis.* 2015 Jul  
 359 10;1(7):317-26. doi: 10.1021/acsinfecdis.5b00030. Epub 2015 May 11.
- 360 [22] Bosseboeuf E, Aubry M, Nhan T, de Pina, JJ, Rolain JM, Raoult D, et al. Azithromycin  
 361 inhibits the replication of Zika virus. *J Antivirals Antiretrovirals.* 2018 10(1):6-11. doi:  
 362 10.4172/1948-5964.1000173.
- 363 [23] Bacharier LB, Guilbert TW, Mauger DT, Boehmer S, Beigelman A, Fitzpatrick AM, et  
 364 al. Early administration of azithromycin and prevention of severe lower respiratory tract  
 365 illnesses in preschool children with a history of such illnesses: A randomized clinical trial.  
 366 *JAMA.* 2015 Nov 17;314(19):2034-2044. doi: 10.1001/jama.2015.13896.

367   **Titles for figures**

368   **Figure 1.** Percentage of patients with PCR-positive nasopharyngeal samples from inclusion to  
369   day6 post-inclusion in COVID-19 patients treated with hydroxychloroquine and in COVID-  
370   19 control patients.

371   **Figure 2.** Percentage of patients with PCR-positive nasopharyngeal samples from inclusion to  
372   day6 post-inclusion in COVID-19 patients treated with hydroxychloroquine only, in COVID-  
373   19 patients treated with hydroxychloroquine and azithomycin combination, and in COVID-19  
374   control patients.

Table 1 Characteristics of the study population.

	Age (years)			Male gender		Clinical status				Time between onset of symptoms and inclusion (days)		
	Mean $\pm$ SD	t	p-value	n (%)	p-value	Asymptomatic	URTI	LRTI	p-value	Mean $\pm$ SD	t	p-value
Hydroxychloroquine treated patients (N=20)	51.2 $\pm$ 18.7	-1.95	0.06	9 (45.0)	0.65	2 (10.0)	12 (60.0)	6 (30.0)	0.30	4.1 $\pm$ 2.6	-0.15	0.88
Control patients (N=16)	37.3 $\pm$ 24.0			6 (37.5)		4 (25.0)	10 (62.5)	2 (12.5)		3.9 $\pm$ 2.8		
All patients (36)	45.1 $\pm$ 22.0			15 (41.7)		6 (16.7)	22 (61.1)	8 (22.2)		4.0 $\pm$ 2.6		

URTI: upper tract respiratory infection, LRTI: lower tract respiratory infection

Table 2. Proportion of patients with virological cure (negative nasopharyngeal PCR) by day, in COVID-19 patients treated with hydroxychloroquine and in COVID-19 control patients.

	Day3 post inclusion			Day4 post inclusion			Day5 post inclusion			Day6 post inclusion		
	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p- value	Number of negative patients/total number of patients	%	p- value	Number of negative patients/total number of patients	%	p- value
Hydroxychloroquine treated patients (N=20)	10/20	50.0	0.005	12/20	60.0	0.04	13/20	65.0	0.006	14/20	70.0	0.001
Control patients (N=16)	1/16	6.3		4/16	25.0		3/16	18.8		2/16	12.5	

<sup>a</sup>control patients from centers other than Marseille did not underwent daily sampling, but were sampled every other day in most cases, they were considered positive for PCR when actually positive the day(s) before and the day(s) after the day(s) with missing data.

Table 3. Proportion of patients with virological cure (negative nasopharyngeal PCR) by day, in COVID-19 patients treated with hydroxychloroquine only, in COVID-19 patients treated with hydroxychloroquine and azithromycin combination, and in COVID-19 control patients.

	Day3 post inclusion			Day4 post inclusion			Day5 post inclusion			Day6 post inclusion		
	Number of negative patients/total number of patients	%	p-value	Number of negative patients/total number of patients	%	p- value	Number of negative patients/total number of patients	%	p- value	Number of negative patients/total number of patients	%	p- value
Control patients	1/16	6.3	0.002	4/16	25.0	0.05	3/16	18.8	0.002	2/16	12.5	<0.001
Hydroxychloroquine treatment only	5/14	35.7		7/14	50.0		7/14	50.0		8/14	57.1	
Hydroxychloroquine and azithromycin combined treatment	5/6	83.3		5/6	83.3		6/6	100		6/6	100	

Supplementary Table 1.

Patient	Age (years)	Sex	Clinical status	Time between onset of symptoms and inclusion (days)	Hydroxychloroquine treatment	Hydroxychloroquine serum concentration µg/ml (day of dosage)	Azithromycin treatment	D0	D1	D2	D3	D4	D5	D6
1	10	M	Asymptomatic	-	No	-	No	31	NEG	NEG	NEG	NEG	NEG	NEG
2	12	F	Asymptomatic	-	No	-	No	26	ND	33	34	NEG	34	NEG
3	14	F	Asymptomatic	-	No	-	No	26	31	23	22	27	NEG	26
4	10	M	Asymptomatic	-	No	-	No	24	NEG	33	33	NEG	NEG	32
5	20	M	URTI	4	No	-	No	24	24	24	27	NEG	31	29
6	65	F	URTI	2	No	-	No	POS	ND	POS	ND	POS	ND	POS
7	46	M	URTI	Unknown	No	-	No	28	ND	ND	ND	26	ND	30
8	69	M	LRTI	2	No	-	No	POS	ND	POS	ND	POS	POS	POS
9	62	F	LRTI	10	No	-	No	POS	ND	POS	ND	POS	ND	POS
10	66	F	URTI	0	No	-	No	POS	ND	POS	ND	ND	ND	POS
11	75	F	URTI	3	No	-	No	POS	ND	POS	ND	POS	ND	ND
12	23	F	URTI	5	No	-	No	ND	ND	POS	ND	POS	ND	ND
13	45	F	URTI	Unknown	No	-	No	POS	ND	POS	ND	POS	ND	POS
14	16	M	URTI	2	No	-	No	POS	ND	POS	ND	ND	POS	ND
15	42	F	URTI	5	No	-	No	ND	ND	ND	POS	ND	POS	ND
16	23	F	URTI	6	No	-	No	POS	ND	ND	ND	ND	POS	ND
17	44	F	URTI	6	Yes	0.519 (D6)	No	30	ND	29	26	32	26	31
18	54	M	Asymptomatic	-	Yes	0.462 (D6)	No	29	NEG	NEG	NEG	NEG	NEG	NEG
19	25	M	URTI	3	Yes	0.419 (D6)	No	23	25	28	25	NEG	NEG	NEG
20	59	F	Asymptomatic	-	Yes	0.288 (D4)	No	30	NEG	NEG	NEG	NEG	NEG	NEG
21	49	F	URTI	1	Yes	0.621 (D6)	No	34	27	19	16	34	24	22
22	24	F	URTI	10	Yes	0.723 (D6)	No	28	NEG	32	34	NEG	NEG	NEG
23	81	F	LRTI	2	Yes	0.591 (D6)	No	22	21	30	NEG	32	28	NEG
24	85	F	LRTI	1	Yes	0.619 (D6)	No	17	21	23	21	26	24	24
25	40	M	URTI	3	Yes	0.418 (D6)	No	22	ND	28	21	15	20	17
26	53	M	URTI	5	Yes	0.515 (D6)	No	27	28	32	31	NEG	NEG	NEG
27	63	F	URTI	8	Yes	0.319 (D4)	No	34	NEG	30	NEG	NEG	NEG	NEG
28	42	F	URTI	1	Yes	0.453 (D6)	No	19	16	17	17	19	20	31
29	87	F	URTI	5	Yes	0.557 (D6)	No	25	30	NEG	NEG	NEG	ND	ND
30	33	M	URTI	2	Yes	0.194 (D2)	No	15	23	26	26	NF	32	32
31	53	F	LRTI	7	Yes	1.076 (D6)	Yes	28	31	34	NEG	34	NEG	NEG
32	48	M	URTI	2	Yes	0.57 (D6)	Yes	23	29	29	NEG	NEG	NEG	NEG
33	50	F	LRTI	5	Yes	0.827 (D6)	Yes	30	27	NEG	NEG	NEG	NEG	NEG
34	20	M	URTI	2	Yes	0.381 (D6)	Yes	27	31	29	NEG	NEG	NEG	NEG
35	54	M	LRTI	6	Yes	0.366 (D4)	Yes	24	ND	ND	29	NEG	NEG	NEG
36	60	M	LRTI	4	Yes	0.319 (D4)	Yes	29	31	31	NEG	NEG	NEG	NEG

URTI: upper tract respiratory infection, LRTI: lower tract respiratory infection, POS: positive PCR, NEG: negative PCR (CT value  $\geq 35$ ), ND: PCR not done





