TITLE PAGE

Full-length title: Early treatment of 1061 COVID-19 patients with hydroxychloroquine and azithromycin, Marseille, France

Short title: Treatment of COVID-19

Authors list: Matthieu Million[1,2]*, Jean-Christophe Lagier[1,2]*, Philippe Gautret[1,3]*, Philippe Colson[1,2], Pierre-Edouard Fournier[1,3], Sophie Amrane[1,2], Marie Hocquart[1], Morgane Mailhe[1], Vera Esteves-Vieira[1], Barbara Doudier[1], Camille Aubry[1], Florian Correard[4,5], Audrey Giraud-Gatineau[1,3,6,7], Yanis Roussel[1,2], Cyril Berenger[1,3], Nadim Cassir[1,2], Piseth Seng[1,2], Christine Zandotti[1], Catherine Dhiver[1], Isabelle Ravaux[1], Christelle Tomei[1], Carole Eldin[1,3], Hervé Tissot-Dupont[1], Stéphane Honoré[4,5], Andreas Stein[1,2], Alexis Jacquier[8], Jean-Claude Deharo[9], Eric Chabrière[1,2], Anthony Levasseur[1,2], Florence Fenollar[1,3], Jean-Marc Rolain[1,2], Yolande Obadia[1], Philippe Brouqui[1,2], Michel Drancourt[1,2], Bernard La Scola[1,2], Philippe Parola[1,3], Didier Raoult[1,2]

***** Equal contributors

Affiliations:

[1] IHU-Méditerranée Infection, Marseille, France

[2] Aix Marseille Univ., IRD, AP-HM, MEPHI, Marseille, France

[3] Aix Marseille Univ., IRD, AP-HM, SSA, VITROME, Marseille, France

[4] Aix Marseille Univ., Laboratoire de Pharmacie Clinique, Marseille, France

[5] AP-HM, hôpital Timone, service Pharmacie, Marseille, France

[6] Centre d'Epidémiologie et de Santé Publique des Armées (CESPA), Marseille, France

[7] AP-HM, Marseille, France

[8] Department of Radiology and Cardiovascular Imaging, Aix-Marseille Univ., UMR 7339, CNRS, CRMBM-CEMEREM (Centre de Résonance Magnétique Biologique et Médicale-Centre d'Exploration Métaboliques par Résonance Magnétique), Marseille, France

[9] AP-HM, Aix Marseille Univ., hôpital Timone, Cardiologie, Rythmologie, Marseille, France

Corresponding author: Didier Raoult, IHU - Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France. Tel.: +33 413 732 401, Fax: +33 413 732 402; email: didier.raoult@gmail.com

Key words: SARS-CoV-2; COVID-19; hydroxychloroquine; azithromycin; ethics; Hippocratic oath

Word counts: abstract, 200; text, 3,757

Figure: 1; **Tables**: 4

References: 39

Supplementary appendices: Figures: 2, **Tables:** 4

ABSTRACT

BACKGROUND: Hydroxychloroquine (HCQ) and azithromycin (AZ) are promising drugs against COVID-19.

METHODS: We conducted an uncontrolled non-comparative observational study in a cohort of 1061 unpublished infected patients treated with HCQ+AZ combination for at least three days. Endpoints were death, worsening and viral shedding persistence.

RESULTS: Good clinical outcome and virological cure were obtained in 973 patients within 10 days (91.7%). Prolonged viral carriage was observed in 47 patients (4.4%) and was associated to a higher viral load at diagnosis ($p < 10^{-2}$) but viral culture was negative at day 10. All but one were PCR-cleared at day 15. A poor clinical outcome was observed for 46 patients (4.3%) and 8 died (0.75%) (74-95 years old). Mortality was lower than in patients treated with other regimens in all Marseille public hospitals ($p< 10^{-2}$). Five patients are still hospitalized (98.7% of patients cured so far). Poor clinical outcome was associated to older age (OR 1.11), initial higher severity (OR 10.05) and low HCQ serum concentration. Poor clinical and virological outcomes were associated to the use of selective beta-blocking agents and angiotensin II receptor blockers ($P<0.05$). No cardiac toxicity was observed.

CONCLUSION: Early HCQ+AZ combination is a safe and efficient treatment for COVID-19.

TEXT

1. Introduction

COVID-19 is an emerging epidemic infection that has spread worldwide since January 2020 [1]. This epidemic questions therapeutic management. In the past 30 years, physicians specialized in infectious diseases have been used to resort to multicenter, randomized, controlled, double-blind studies to establish guidelines in the context of evidence based medicine and influence healthcare policy and practice [2,3]. However, in the course of history, most infectious diseases have been treated with a single option without simultaneous control arm [4,5]. The Ebola outbreak in West Africa (2013-2016) illustrated the controversy between randomized controlled trial (RCT)-supporters [6,7] and proponents of observational versus historical studies [8]. Some arguing that the results would not be interpretable if the trial was not randomized and controlled; others highlighting the unethical proposal of placebo in a disease well known for its severe prognosis [6–9]. Indeed, the concern was debated at the time of Ebola, but in developed countries, all the drugs likely to be efficient were used [10] while Africans were invited to take part in randomized studies, half of whom receiving placebos for a disease where mortality occurred in about 30% of cases. The issue of ethics was used on both sides, some arguing for their duty to treat and the others for the perceived necessity to test [11]!

The same question currently arises for COVID-19, and, more generally, the question of therapeutic implementation in acute severe emerging infectious diseases without known treatment, specifically as there are serious doubts on the superiority of randomized controlled trials over historical comparisons, in general [2,3]. As a consequence, several assays in China were single branch trials using chloroquine or hydroxychloroquine (HCQ) [12,13]. Given the spread of the current outbreak, we recently proposed, as soon as *in vitro* results were available [14], to use HCQ which was also considered effective in the preliminary results of Chinese

clinical studies [12]. We associated HCQ in severe cases with azithromycin (AZ) [15] that is recommended in children respiratory infections, including those of viral origin [16]. Thereafter, as AZ gave a significant effect in this first study despite the low number of cases [15], we treated 80 patients using a combination of HCQ+AZ with good clinical and virological outcomes [17]. Moreover, in a recent survey, most of the questioned physicians considered that HCQ and AZ are the two most effective treatments among available therapies for COVID-19 [18]. Finally, inhibition effect of both molecules and of their combination was demonstrated against SARS-CoV-2, *in vitro* [19,20]. Here, we report a cohort study including 1061 new patients with COVID-19, treated for at least 3 days with HCQ+AZ from the time of diagnosis and a further nine days at least of follow up. Endpoints were death, clinical worsening and viral shedding persistence.

2. Materials & methods

2.1. Patients and study design (Figure 1)

The study was conducted at Assistance Publique-Hôpitaux de Marseille (AP-HM), Southern France in the Institut Hospitalo-Universitaire (IHU) *Méditerranée Infection* (https://www.mediterranee-infection.com/). We have set up early unrestricted massive PCR screening for patients suspect of COVID-19 and for asymptomatic contacts of a confirmed case. The study was conducted on patients included from March 3rd to March 31st. Individuals with PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample as reported [21], were proposed HCQ+AZ early treatment, as standard care, on an ambulatory basis with treatment initiation at our day-care hospital or as in-patients when required. Patients were also referred to the IHU from other structures. Patients with at least three days of treatment and nine days of follow-up are described in this study. Demographics, chronic conditions and background treatments were documented. The patients described in previous studies [15,17] were not included in the present work. Inpatients discharged before day 10 were followed-up

on an outpatient basis. On April 18th, a new screening was made to update fatal cases and case fatality rates.

2.2. Clinical and radiological classification and follow-up

Details are available from our previous studies [15,17]. Briefly, patients were grouped according to clinical presentation at admission (upper respiratory tract infections or lower respiratory tract infections symptoms) and severity was assessed using the national early warning score (NEWS) for COVID-19 patients at admission and during follow-up [22]. We defined three risk categories for clinical deterioration: low score (NEWS 0-4), medium score (NEWS 5-6), and high score (NEWS≥7). The time between the onset of symptoms and treatment was documented. Patients underwent an unenhanced chest low-dose computed tomography (LDCT). The need for oxygen therapy, transfer to the intensive care unit (ICU), death, and overall length of stay in hospital (for in-patients) were documented. Virological follow-up included ≥1 test(s) performed on days 2, 6 and 10.

2.3. COVID-19 treatment and outcomes

Patients with no contraindications [15,17] were proposed a combination of 200 mg of oral HCQ sulfate, *tid* for ten days combined with AZ (500 mg day 1 followed by 250 mg daily for the next four days). No children, pregnant women or patients with G6PD deficiency were included. The systematic pre-therapy workup included an ionogram, and an electrocardiogram with corrected QT measurement (Bazett's formula). A specific inclusion protocol and follow-up for *torsade de pointes* risk was designed (Supplementary Material). Hydroxychloroquine dosage was performed as previously described [17,23] and a concentration of > 0.1 µg/mL was considered in the therapeutic range [24]. Broad spectrum antibiotics (ceftriaxone or ertapenem) were added for patients with pneumonia and NEWS score ≥ 5. Symptomatic treatments, including notably oxygen, were added as needed.

The primary outcomes were i) an aggressive clinical course requiring oxygen therapy, transfer to the ICU or death after at least three days of treatment, and prolonged hospitalization (10 days or more), and ii) contagiousness as assessed by PCR and culture.

2.4. Additional investigations on patients with treatment failure

Patients with clinical or virological failures were accurately characterized and a close clinical and viral follow-up was performed overtime. We defined a group with poor clinical outcome (PClinO) defined by either death or transfer to ICU or hospitalization for 10 days or more and a group with poor virological outcome (PVirO) defined by viral shedding persistence at day 10. Finally, individuals who belonged neither to the PClinO group nor the PVirO group were attributed to a group with a good outcome (GO). Factors associated with clinical failure were identified by comparing the PClinO to the GO group and factors associated with virological failure were identified by comparing the PVirO group to the GO group. We performed additional tests on patients with atypical evolution including late SARS-CoV-2 cultures on Vero E6 cells, as previously described [25], and broad-spectrum detection of other viruses by multiplex PCR [21] in respiratory samples. In addition, cDNA was reverse transcribed directly from total viral SARS-CoV-2 RNA rhinopharyngeal samples following the manufacturer's recommendations. cDNAs were purified by using Agencourt AMPure beads (Beckman Coulter, Villepinte, France). Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc, San Diego, CA,USA) with the Nextera Mate-Pair sample prep and Nextera XT Paired End kits (Illumina Inc., San Diego, CA, USA). The SARS-CoV-2 genomes were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/) or are available at EMBL-EBI under the BioProject : PRJEB37693. Phylogenetic reconstruction was performed using NEXSTRAIN (https://nextstrain.org/) and GISAID (Global Initiative; https://www.gisaid.org/) [26].

2.5. Comparison of COVID-19-related mortality with other centers

The information on overall COVID-19 patient mortality in the AP-HM was obtained from the Department of Statistics of our institution from March 7th (first death of a patient with COVID-19 in the AP-HM) to April 6th. We excluded 2 dead patients reported in our first series [17]. We compared patients who died with at least three days of HCQ+AZ treatment to others. The age and gender for these patients were collected. Data from the Bouches-du-Rhône department (Marseille being the largest city), Rhône department (Lyon being the largest city), and France overall were obtained from Santé Publique France [27]. Demographic data were obtained from the Institut National de la Statistique et des Etudes Economiques [28]. International data were obtained from the Center for Systems Science and Engineering at Johns Hopkins University, US [29].

2.6. Statistical methods

Continuous and categorical variables were presented as mean (std), median, min-max and n (%), respectively. We used the Student t-test, Mann-Whitney U test, Chi-square test, or Fisher's exact test to compare differences between the three groups (GO, PVirO, and PClinO) where appropriate. The GO group was chosen as the reference group for statistical testing (PVirO vs. GO and PClinO vs. GO respectively). To explore risk factors associated with the PVirO and PClinO groups, we also performed multivariable analyses using logistic regression models. All variables significant at $p< 10^{-2}$ in univariate analyses were introduced in the initial multivariate model. A stepwise approach was then used to assess the iteration of variables and to control potential confounders (both values of significance level for entry and stay were set at 0.05.) A two-sided alpha of less than 0.05 was considered statistically significant. All analyses were carried out using SAS 9.4 statistical software (SAS Institute, Cary, NC).

2.7. Ethics statement

This is a retrospective study on a cohort of patients receiving standard treatment following a research protocol previously registered (ANSM: 2020-000890-25, CPP Ile de

France: 10 20.02.28.99113, EU Clinical Trials Register: 2020 207 -000890-25; This study is referenced in [15]). The use hydroxychloroquine has now been authorized by the French government to treat COVID 19 hospitalized patients (such as it has been FDA approved in the USA and it many countries). The addition of an antibiotics (here azithromycin) regularly used to treat respiratory infection is also included in standard therapeutic management of patients. All the patients were anyway informed about the treatment they have received. There is no formal consent to sign in our institution by patients, to allow us perform anonymous observational retrospective studies in the context of standard therapeutic management of patients. The study was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (N°: 2020-13). The study was performed according to the good clinical practices recommended by the Declaration of Helsinki and its amendments.

3. Results

3.1. Participants

During the study period (March 3 to April 9, 2020), the laboratory of IHU *Mediterranée Infection* tested 59,655 samples for COVID-19 infection, including 38,617 individuals and 5,169 who tested positive including 3,165 being managed at IHU (Figure 1). Among 1,411 eligible patients with available data, 350 were excluded (Supplementary Table S1). For the present survey, a total of 1,061 patients were treated at least 3 days with the combination of HCQ+AZ at IHU, including 492 male (46.4%). The mean age was 43.6 years (standard deviation (sd), 15.6 years). Underlying conditions and symptoms declared by the patients (91.7%) are described in Table 1. The majority (95.0%) of patients had a low NEWS score. The time between the onset of the symptoms and the first day of treatment (day 0) was 6.4 days (sd, 3.8 days). A total of 469 patients (65.7%) had a LD CT scan consistent with pneumonia including 20.5 % and 2.2 % with a medium and severe score, respectively. The mean viral load obtained by PCR on nasopharyngeal swab at day 0 was 26.6 Ct with 5.0 as sd.

Successful isolation of virus in cell culture was obtained from 204 patients sampled at day 0. A total of 973 patients (91.7%) had a good clinical outcome (GO). Among 263 patients tested at day 2, HCQ was low (<0.1 µg/mL) in 30 patients including 3 in which it was undetectable.

3.2. Poor clinical outcome

Forty-six patients (4.3%) were classified into the PClinO group including 10 patients transferred into ICU, 8 patients who died (update April 18th), and 31 patients who were hospitalized for 10 days or more. Their median age (69.0 years; 31-95 years) was significantly higher than that of patients included into the GO group (42.0 years; 14-86, p<0.001) (Table 1). Sex ratio (M/F) was 1. When compared with patients in the GO group, PClinO group patients were significantly more likely to report previous hypertension (50%), diabetes (19.6%), coronary artery diseases (19.6%) and cancer (15.2%) (p<0.001). In addition, they were more likely to receive beta-blocking agents, dihydropyridine derivatives, angiotensin II receptor blockers, and HMG-CoA reductase inhibitors (p<0.001). The time between onset of symptoms and the beginning of the treatment was shorter and their NEWS score was less likely to be low than in the GO group patients (Table 1). However, upon multivariate analysis, only older age (OR= 1.11: 1.07-1.15), selective beta blocking agents (OR= 4.16: 1.19 – 14.55), angiotensin II receptor blockers (OR= 18.40: 6.28-53.90) and high and medium NEWS scores (OR= 10.05: 3.16-32.02) were significantly associated with the poor outcome (Table 2). Low dose CT scan score revealed pneumonia in 35 PClinO group patients (90%). Interestingly, the mean HCQ dosage at day 2 (0.20 µg/ml (0.17)) was significantly lower than in the GO group (Table 1) with 12/37 tested cases with a dosage lower than 0.100 µg/mL, and 3 without detectable HCQ.

Regarding specifically the 8 patients who died after having received HCQ+AZ ≥ 3 days, their median age was 79 years (74-95 years) (Supplementary Table S2). Six patients (75%) reported hypertension and one active cancer. Severity at admission was observed with a NEWS score ranging from 5 to 11 (mean 7.75) and low dose CT scan performed on 4

patients revealed intermediate to severe pneumonia involvement. Finally, mean HCQ dosage at day 2 was 0.162 including one patient with blood level lower than 0.10 µg/mL. As of 18th of April, 2020, 33 of 46 patients in the PClinO group are now cured. Accordingly, 1048 (98.7%) included patients are cured so far.

3.3. Comparative case fatality rates (CFRs)

During the survey, a total of 63/1,968 COVID-19 patients (3.2%) died at AP-HM. In this work, 8 of 1,061 patients who had received at least 3 days of HCQ+AZ died (CFR= 0.75%). One more patient of the previously published series of 80 patients also died since the publication (total deaths, 2/80), and 6 other patients (5.6%) died out of 107 patients treated with the same drug regimen at AP-HM apart from IHU. Altogether, 16/1248 (1.3%) patients died after having received at least 3 days of HCQ+AZ regimen. At IHU and in other units of AP-HM, 13/468 (2.8%) and 34/252 (13.5%) patients, respectively, died after having received another regimen (Table 3). In total, 47/720 patients (6.5%) died among those who did not receive at least 3 days of HCQ+AZ regimen. At IHU (p-value = 0.0017), at AP-HM apart from IHU (p-value = 0.030) and for whole AP-HM (p-value < 1.10^{-7}), CFR was significantly lower among patients who received at least 3 days of HCQ+AZ regimen when compared to those who received other regimen (Chi-2 test) .

We also compared the mortality per one million population by COVID-19 on 2020, between Marseille (59.1, by April 6th), the main city of the Bouches-du Rhône department (59.5) in Southern France, and the Rhone department (124.2), which has a similar size, population and number of hospitalized patients, in perspective with the rest of the world, including the highest level in Spain by April 6th, 2020 (278.1) (Supplementary Table S3).

3.4. Viral clearance

Forty-seven patients, including 5 who were also PClinO, exhibited a persistent nasal viral carriage at completion of treatment. Their sex ratio (M/F) and mean age were 0.68 and 47.9 +/- 17.5 years old, respectively. Of the 21 PVirO patients for whom specimens were

available after day 10, 20 had negative viral loads by day 15 post onset of treatment (95.2%). In addition, all eleven patients for whom daily culture was attempted were negative by day 10. When compared to GO group patients in this study, PVirO group patients exhibited a significantly higher viral load ($p < 10^{-2}$) at diagnosis, were less likely to have a low NEWS score, and they were treated earlier (Table 1). However, in multivariate analysis, only high viral load remained significantly associated with poor virological outcome. In two of eight tested PVirO individuals, but in none of 112 GO patients (p= 0.0007, Fisher exact test), a concurrent Bocavirus infection was detected by PCR. Whether this co-infection played a role in viral persistence is as-yet unknown. Comparative genomics between viral isolates from 3 non-treatment-responding patients (both PVirO and PClinO), one PClinO patient, one PVirO patient and 10 treatment-responding patients as well as 56 SARS-COV-2 strains from various geographical origins did not identify any specific viral variant linked to resistance to treatment (Supplementary Figure 1).

4. Discussion

In this work which is not a RCT but relates the real-life experience of physicians treating patients in the context of an emerging pandemic, we report the evolution of 1061 COVID-19 patients treated with an HCQ+AZ combination from the time of diagnosis. The spectrum of severity of COVID-19 ranges from mild symptoms to severe respiratory distress [1]. In order to assess treatment effectiveness, we assessed patients who received at least three days of treatment and eight days of follow-up. The majority of patients in our work had relatively mild disease at admission. Under these conditions, the treatment avoids worsening of the disease, as only 10 patients (0.9%) were transferred to the intensive care unit, but it also avoids death, as only eight (0.75%) patients died (case fatality rate updated April 18th, 2020). It also impaired persistent viral shedding. The mortality in patients treated with HCQ+AZ for at least three days in other AP-HM departments was 5.6%. By contrast, the mortality of AP-

HM patients apart from IHU who did not receive such a treatment was significantly higher (13.5%). Also, in the Bouches-du-Rhône department where mass SARS-CoV-2 testing was performed (about 2.5% of the Marseille population was tested at IHU, unpublished data) and HCQ+AZ treatment was frequently prescribed, the mortality in hospitalized COVID-19 patients was twice lower than in the Rhône department (Lyon area) where this strategy was not developed extensively (Supplementary Table S3). The two departments have roughly the same population and are at the same stage of the epidemic. This data strongly suggests that the combination of HCQ+AZ, when prescribed soon enough after the onset of symptoms, during at least three days leads to a more favorable outcome of COVID-19. Regarding viral shedding persistence, we observed that it was 4.4% at day 10 in treated patients, which is extremely low in comparison to Chinese studies, the largest of which showed that viruses are shed on average for 20 days with extremes of up to 38 days [1]. We believe that HCQ+AZ treatment is effective in shortening the duration of virus shedding which may play a role in the transmission of the disease. Surprisingly, the PVirO group was apparently diagnosed and treated earlier and had higher viral loads as compared to the GO group, but we did not find any specificity in the genomes of viruses in this group.

Indeed, we were surprised to find in the PClinO group that HCQ blood levels were lower than therapeutic target in 32.4% cases including two patients without any drug in the blood. This is not explained so far. We therefore recommend that close control of HCQ blood level be performed in treated patients so that drug dosage could be adapted accordingly.

As already described by others [1,30], we confirm that COVID-19 patients with PClinO are significantly more likely to be elderly patients. Moreover, when COVID-19 patients were treated belatedly and already showing clinical or radiological signs of pneumonia, the prognosis was poorer but genomes of viruses associated with PClinO were not apparently different from those in other patients (Supplementary Figure 1). Multivariate

analysis showed that selective beta-blocking agents and angiotensin II receptor blockers were independent factors associated with poor clinical and virological outcomes ($p<0.05$).

Nevertheless, the COVID-19-related mortality observed in AP-HM did not significantly differ from that related to influenza virus and respiratory syncytial virus (RSV) infections. Furthermore, the age of patients dying from SARS-Cov-2, influenza virus or RSV infection was in the same range (Supplementary Table S4).

To anticipate potential criticisms regarding the level of evidence regarding the effectiveness of HCQ+AZ treatment against COVID-19 we gathered the available data usually used when assessing the effectiveness of any treatments (Table 4). Based on this data, we believe that the current recommendation to prescribe HCQ+AZ treatment to COVID-19 patients should be of grade B. In the current context of frenetic search for potential COVID-19 treatments, we consider necessary to highlight the consistence between data from in vitro experimentation showing an activity of both HCQ and AZ against SARS-CoV-2, including a synergistic effect [19] and results of preliminary RCT conducted in China showing a significant clinical and radiological improvement of COVID-19 patients under HCQ treatment as compared to controls [31]. Based on our preliminary study showing a significant difference between no treatment, HCQ treatment and HCQ+AZ combined treatment in terms of SARS-CoV-2 viral load at day 6 post treatment, we believe that AZ reinforces the effect of HCQ [15]. HCQ as a COVID-19 treatment is prescribed in an increasing number of countries [18]. There is a coincidence in Italy between the decision to conduct mass SARS-CoV-2 testing and prescribing HCQ+AZ treatment to COVID-19 patients by Italian physicians and the recent rapid decrease in the number of COVID-19 cases and associated mortality (Supplementary Figure 2).

As a conclusion, based on our studies and on these observations in Europe it appears reasonable to follow the recommendations made in Asian countries for the control of COVID-19, notably in Korea and China that consist in early testing as many patients as possible and

treating them with available drugs where this strategy has produced much better results than in countries where no active policy has been implemented outside containment. In China, drugs that were recommended were primarily HCQ but also α-interferon, lopinavir, ritonavir and umifenovir [32], in Korea, recommended drugs were lopinavir/ritonavir and chloroquine [33]. We consider that a strategy consisting in not testing patients and not treating them is unethical. In the context of a pandemic with a lethal respiratory virus, we believe that early detection and treatment should be generalized in outpatient medicine, i.e. in mild individuals before signs of severity appear. Finally, there is a need to search what drugs can quickly cope with a large scale epidemic among already existing drugs. The commentaries that arose following our first publication [34,35], stating that rather than systematically treating patients based on our preliminary results, it would be more rational to wait for results of multicentrical double-blind, RCT of unapproved drugs seems immoral to us and in contradiction with the Hippocratic oath which states that a doctor must do as much as possible to treat patients according to the available knowledge in the field.

Funding

This work was supported by the French Government under the "Investments for the Future" program managed by the National Agency for Research (ANR), Méditerranée-Infection 10-IAHU-03, and was also supported by Région Provence-Alpes-Côte d'Azur and European funding FEDER PRIMMI (Fonds Européen de Développement Régional - Plateformes de Recherche et d'Innovation Mutualisées Méditerranée Infection).

Declaration of competing interest

The authors declare no competing interests. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Author contributions

Conceived and designed the study: DR. Designed and/or performed experiments: MM, JCL, PC, PEF, VEV, SH, FC, AGG, YR, EC, AL, AJ, JCD, FF, JMR, YO, MD, BLS, PB, PP. Took care of the patients and patients' recording data: MM, JCL, PP, SA, MM, MH, BD, CA, NC, CZ, PS, CD, IR, CT, CE, HTD, AS, PB. Analyzed and interpreted data: MM, JCL, PG, PC, PEF, SH, YO, JMR, PB, MD, BLS, PP, DR. Wrote the manuscript: MM, JCL, PG, PC, PEF, PP, DR. All authors read and approved the final manuscript.

Acknowledgments

We are thankful to Marion Bechet, Pascal Chanez, Véronique Filosa, Marc Gainier, Marion Gouitaa, Marie-Thérèse Jimeno, Cléa Melenotte, Matthieu Bardou, Marc Léone, Jean-Robert Harlé, Veronique Veit, all medical students from Aix Marseille University, all nurses, laboratory staff, administrative, technical and security staff of Assistance Publique-Hôpitaux de Marseille and IHU *Méditerranée Infection*, all medical doctors volunteers, and the *Bataillon des Marins Pompiers de Marseille* for their help.

References

[1] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395:1054-1062.

[2] Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. N Engl J Med 2000;342:1887-1892.

[3] Sacks H, Chalmers TC, Smith H, Jr. Randomized versus historical controls for clinical trials. Am J Med 1982;72:233-240.

[4] Florey HW, Abraham EP, Gardnier AD. Further observations on penicillin. Lancet 1941;6155:177-188.

[5] Woodward TE, Wisseman CL (1958) Chloromycetin (Chloramphenicol). In: Antibiotics, Monograph, no.8, Medical Encyclopedia, New York, USA, 1958.

[6] Ippolito G, Lanini S, Brouqui P, Di CA, Vairo F, Fusco FM, et al. Non-randomised Ebola trials--lessons for optimal outbreak research. Lancet Infect Dis 2016;16:407-408.

[7] Shaw D. Randomisation is essential in Ebola drug trials. Lancet 2014;384:1667-6736.

[8] Lagier JC, Raoult D. Deadly infectious diseases such as Ebola: the parachute paradigm. Clin Microbiol Infect 2015;21:389-390.

[9] Sissoko D, Laouenan C, Folkesson E, M'Lebing AB, Beavogui AH, Baize S, et al. Experimental Treatment with Favipiravir for Ebola Virus Disease (the JIKI Trial): A Historically Controlled, Single-Arm Proof-of-Concept Trial in Guinea. PLoS Med 2016;13:e1001967.

[10] Uyeki TM, Mehta AK, Davey RT, Jr., Liddell AM, Wolf T, Vetter P, et al. Clinical Management of Ebola Virus Disease in the United States and Europe. N Engl J Med 2016;374:636-646.

[11] Smith GC, Pell JP. Parachute use to prevent death and major trauma related to gravitational challenge: systematic review of randomised controlled trials. BMJ 2003;%20;327:1459-1461.

[12] Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 2020;14:72-73.

[13] Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care 2020;pii: S0883-9441(20)30390-7.

[14] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30:269-271.

[15] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020;105949. doi: 10.1016/j.ijantimicag.2020.105949. [Epub ahead of print]

[16] Bacharier LB, Guilbert TW, Mauger DT, Boehmer S, Beigelman A, Fitzpatrick AM, et al. Early Administration of Azithromycin and Prevention of Severe Lower Respiratory Tract Illnesses in Preschool Children With a History of Such Illnesses: A Randomized Clinical Trial. JAMA 2015;314:2034-2044.

[17] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: an observational study. Travel Med Infect Dis. 2020 Apr 11:101663. doi: 10.1016/j.tmaid.2020.101663. [Epub ahead of print]

[18] Sermo. Sermo COVID-19 Real Time Barometer. 2020. https://app.sermo.com/covid19-barometer?utm_campaign=wwwsermo_covid19. Last accessed: 17/04/2020.

[19] Andreani J, Le Bideau M, Duflot I, Jardot P, Rolland C, Boxberger M, et al. In vitro testing of Hydroxychloroquine and Azithromycin on SARS-CoV-2 shows 1 synergistic effect. IHU pre-print 2020. https://www.mediterranee-infection.com/wp-content/uploads/2020/03/La-Scola-et-al-V1.pdf

[20] Touret F, Gilles R, Barral K, Nougairede A, Decroly E, De Lamballerie X, et al. *In vitro* screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. bioRxiv preprint doi: https://doi.org/10.1101/2020.04.03.023846.

[21] Amrane S, Tissot-Dupont H, Doudier B, Eldin C, Hocquart M, Mailhe M, et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious diseases referral hospital in Marseille, France, - January 31st to March 1st, 2020: A respiratory virus snapshot. Travel Med Infect Dis 2020 Mar 20:101632. doi: 10.1016/j.tmaid.2020.101632. [Epub ahead of print]

[22] Liao X, Wang B, Kang Y. Novel coronavirus infection during the 2019-2020 epidemic: preparing intensive care units-the experience in Sichuan Province, China. Intensive Care Med 2020;46:357-360.

[23] Armstrong N, Richez M, Raoult D, Chabriere E. Simultaneous UHPLC-UV analysis of hydroxychloroquine, minocycline and doxycycline from serum samples for the therapeutic drug monitoring of Q fever and Whipple's disease. J Chromatogr B Analyt Technol Biomed Life Sci 2017;1060:166-172.

[24] Lagier JC, Fenollar F, Lepidi H, Giorgi R, Million M, Raoult D. Treatment of classic Whipple's disease: from in vitro results to clinical outcome. J Antimicrob Chemother 2014;69:219-227.

[25] La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-

CoV-2 patients from infectious disease wards. IHU pre-print 2020. https://www.mediterranee-infection.com/wp-content/uploads/2020/03/La-Scola-et-al.3-IHU-draft.pdf

[26] Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics 2018;34:4121-4123.

[27] Santé Publique France (2020) 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

[28] Institut national de la statistique et des études économiques (2020) Démographie. https://www.insee.fr/fr/accueil

[29] Johns Hopkins University (2020) Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). https://coronavirus.jhu.edu/map.html

[30] Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA 2020;2762130.

[31] Chen Z, Hu J, Zhang Z, Jiang S, Han S, Yan D, et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. medRxiv 2020.03.22.20040758; doi: https://doi.org/10.1101/2020.03.22.20040758

[32] Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther 2020;14:58-60.

[33] Sung-sun K. Physicians work out treatment guidelines for coronavirus. Korea Biomedical Review. http://m.koreabiomed.com/news/articleView.html?idxno=7428

[34] Decloedt EH, Reuter H, Allwood B, Parker A, Koegelenberg CFN, Blockman M, et al. Benefit v. risk when using chloroquine in patients with severe COVID-19 disease . S Afr Med J Published online 2 April 2020. https://doi.org/10.7196/SAMJ.2020.v110i5.14761

[35] Hulme OJ, Wagenmakers EJ, Damkier P, Madelung CF, Siebner HR, Helweg-Larsen J, et al. Reply to Gautret et al. 2020: A Bayesian reanalysis of the effects of hydroxychloroquine and azithromycin on viral carriage in patients with COVID-19. medRxiv preprint doi: https://doi.org/10.1101/2020.03.31.20048777

[36] Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). J Zhejiang Unniv (Med Sci) 2020;49:0.

[37] Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020;5801998:pii:ciaa237.

[38] Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020;6:16.

[39] Nicastri E, Petrosillo N, Bartoli TA, Lepore L, Mondi A, Palmieri F, et al. National Institute for the Infectious Diseases "L. Spallanzani", IRCCS. Recommendations for COVID-19 clinical management. Infect Dis Rep 2020;12:8543.

FIGURE LEGENDS

Figure 1. Design of inclusion, management and follow-up, and clinical and virological outcome

AP-HM, Marseille public hospitals; IHU, University Hospital Institute Méditerranée Infection ; HCQ, Hydroxychloroquine, AZ, azithromycin. * Five patients had both poor clinical and virological outcomes.

TABLES

Table 1. Baseline characteristics according to clinical and virological outcome of 1061 patients treated with HCQ+AZ ≥ 3 days at IHU Méditerranée infection Marseille, France with day 0 between March 3 and March 31, 2020.

	Poor virological outcome[a]	Good outcome	Poor clinical outcome[a,b]	Total
	n (%)	n (%)	n (%)	n (%)
Group size	47 (4.4%)	973 (91.7%)	46 (4.3%)	1061 (100%)
Age (years)				
Mean (SD)	47.9 (17.5)	42.4 (14.7)	69.2 (14.0)	43.6 (15.6)
Median [Min-Max]	**48.0 [18.0-89.0]***	42.0 [14.0-86.0]	**69.0 [31.0-95.0]*****	43.0 [14.0-95.0]
Male	19 (40.4%)	450 (46.3%)	23 (50%)	492 (46.4)
Chronic condition(s) and treatment(s)				
Chronic conditions				
Cancer	0 (0.0%)	21 (2.2%)	**7 (15.2%)*****	28 (2.6%)
Diabetes	3 (6.4%)	66 (6.8%)	**9 (19.6%)*****	78 (7.4%)
Coronary artery disease	2 (4.3%)	36 (3.7%)	**9 (19.6%)*****	46 (4.3%)
Hypertension	8 (17%)	120 (12.3%)	**23 (50.0%)*****	149 (14%)
Chronic respiratory diseases	8 (17%)	96 (9.9%)	8 (17.4%)	111 (10.5%)
Obesity	1 (2.1%)	57 (5.9%)	4 (8.7%)	62 (5.8%)
Comedication(s)				
Biguanides (metformin)	1 (2.1%)	15 (1.5%)	**4 (8.7%)****	20 (1.9%)
Selective beta blocking agents	**6 (12.8%)****	22 (2.3%)	**9 (19.6%)*****	34 (3.2%)
Dihydropyridine derivatives	3 (6.4%)	23 (2.4%)	**8 (17.4%)*****	34 (3.2%)
Angiotensin II receptor blockers	**6 (12.8%)****	22 (2.3%)	**14 (30.4%)*****	40 (3.8%)
HMG CoA reductase inhibitors	4 (8.5%)	28 (2.9%)	**7 (15.2%)*****	38 (3.6%)
Diuretics	2 (4.3%)	28(2.9%)	**5 (10.9%)***	35(3.3%)
Time between onset of symptoms and first day of treatment start (days)[c]				
Mean (SD)	4.3 (2.5)	6.5 (3.9)	5.9 (4.0)	6.4 (3.8)
Median [Min-Max]	**4.0 [0.0-9.0]*****	6.0 [0.0-27.0]	**5.0 [0.0-16.0]*****	6.0 [0.0-27.0]
Clinical classification (NEWS score)				
0 – 4 (low)	**43 (91.5%)***	948 (97.4%)	**19 (41.3%)*****	1008 (95.0%)
5 – 6 (medium)	2 (4.3%)	14 (1.4%)	10 (21.7%)	25 (2.4%)
≥ 7 (high)	2 (4.3%)	11 (1.1%)	17 (37.0%)	28 (2.6%)
Low-dose pulmonary CT-scanner within 72 hours of admission[d]				
Normal	11/37 (29.7%)	231/642 (36.0%)	**4/39 (10.3%)*****	245/714 (34.3%)
Limited	23/37 (62.2%)	277/642 (43.2%)	10/39 (25.6%)	307/714 (43.0%)
Medium	3/37 (8.1%)	123/642 (19.2%)	20/39 (51.3%)	146/714 (20.5%)
Severe	0/37 (0.0%)	11/642 (1.7%)	5/39 (12.8%)	16/714 (2.2%)
Viral load at inclusion (Ct - nasal)[e]				
Mean (SD)	23.4 (5.1)	26.8 (4.9)	25.6 (4.8)	26.6 (5.0)
Median [Min-Max]	**22.1 [14.8-34.0]*****	27.3 [12.8-34.0]	25.8 [15.0-33.2]	27.0 [12.8-34.0]
Hydroxychloroquine levels at day 2 (µg/mL)[f]				
Mean (SD)	0.25 (0.17)	0.26 (0.16)	0.20 (0.17)	0.25 (0.16)
Median [Min-Max]	0.19 [0.07-0.70]	0.22 [0.00-1.01]	**0.15 [0.00-0.75]****	0.21 [0.00-1.01]
Number ≤ 0.1µg/mL	4/24 (16.7%)	15/206 (7.3%)	**12/37 (32.4%)*****	30/263 (11.4%)

Poor virological outcome (PVirO): viral shedding persistence at day 10; Poor clinical outcome (PClinO): either death or transfer to intensive care unit (ICU) or hospitalization for 10 days or more; Good outcome: individuals who belonged neither to the PClinO group nor the PVirO group. SD: standard deviation. [a]Five patients belonged to both the PVirO and PClinO outcome so the sum of frequencies may be above 1061. [b]Including 8 deaths. [c]Data available for 928 patients (56 patients who did not declare any symptom before treatment start were excluded and 77 with missing data), [d]for 714 patients, [e]for 992 patients and [f]for 263 patients. On low-dose pulmonary CT-scanner, patients were classified as no involvement (lack of lung involvement (ground glass opacities, consolidation or crazy paving pattern); minimal involvement (subtle ground glass opacities); intermediate involvement (less than 50% of segment involvement in no more than 5 segments) and severe involvement (involvement of more than 5 segments). The denominator was mentioned when the result was not available for all patients. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ (Fisher's exact test, Student t-test, Wilcoxon-Mann-Whitney where appropriate; reference group is good outcome).

Table 2. Multivariable logistic regressions of variables found statistically different in the univariate analysis

	PViroO (versus GO)		PClinO (versus GO)	
	OR [95% CI]	p	OR [95% CI]	p
Age (years)	1.02 [1.00;1.04]	0.042	1.11 [1.07;1.15]	<0.0001
Comedication(s)				
Selective beta blocking agents	4.57 [1.54;13.60]	0.006	4.16 [1.19;14.55]	0.026
Angiotensin II receptor blockers (ARBs), plain	3.96 [1.34;11.68]	0.013	18.40 [6.28;53.90]	<0.0001
NEWS score				
0 – 4 (low)	1.0 (ref)		1.0 (ref)	
5 – 6 (medium)	NS		9.48 [3.25;27.66]	0.043
≥ 7 (high)			10.05 [3.16;32.02]	0.040
Viral load at inclusion (Ct, nasopharyngeal sample)[a]	0.86 [0.81;0.92]	<0.0001	NS	

NS: not statistically significant (p> 0.05) after stepwise selection.
[a] Missing values (n=69) were imputed based on the mean value (mean= 26.6, see Table 1).

Table 3. Case fatality rate among 1968 COVID+ patients diagnosed at AP-HM, Marseille France, with Day 0 treatment between March 3rd and March 31, 2020

	First cohort IHU [15]			IHU new cohort			AP-HM except IHU			TOTAL AP-HM		
	Dead	(N = 80)	CFR	Dead	(N = 1 529)	CFR	Dead	(N = 359)	CFR	Dead	(N = 1 968)	CFR
HCQ + AZ ≥ 3 days	2	80	2.5%	8	1 061	0.8%[a]	6	107	5.6%[c]	16	1 248	1.3%[e]
Versus												
Other treatment regimen*	-	-		13	468	2.6%[b]	34	252	13.1%[d]	47	720	6.3%[f]

*HCQ + AZ < 3 days or other treatment regimen. [a] vs [b], p-value = 0. 0.0017 (Chi-2 test) ; [c] vs [d], p-value = 0. 0.030; [e] vs [f], p-value < 1.10^{-7}; [a] vs [d], p-value < 1.10^{-7}; [a] vs [f], p-value < 1.10^{-7}

Table 4. Level of evidence for efficacy of a combination of hydroxychloroquine and azithromycin against COVID-19

Level of evidence	Type of evidence *	Available studies
Ia	Systematic review (with homogeneity) of RCTs	-
Ib	Individual RCT (with narrow confidence interval)	A preliminary French non-randomized clinical trial conducted in 36 COVID-19 patients showed a significant reduction in viral nasopharyngeal carriage at day 6 in patients treated with hydroxychloroquine at 600 mg per day during 10 days, (N=20, 70% testing negative), compared to untreated controls (N=16, 12.5% testing negative). In addition, of the twenty patients who were treated with hydroxychloroquine, six received azithromycin for five days (for the purposes of preventing bacterial super-infection) and all (100%) were virologically cured at day 6, compared to 57.1% of the remaining 14 patients [15] A Chinese RCT conducted in 62 COVID-19 patients showed significantly shortened body temperature recovery time, cough remission time and larger proportion of improved pneumonia as assessed by CT scan in patients treated with 400 mg hydroxychloroquine per day during five days (N=31) than in controls (N=31) [31] A Chinese RCT conducted in 30 COVID-19 patients showed no significant differences between patients treated with 400 mg hydroxychloroquine per day during five days (N=15) and controls (N=15) regarding pharyngeal carriage of viral RNA at day 7, however, patients received multiple additional treatments including antivirals [36].
Ic	All or none study	-
2a	Systematic review (with homogeneity) of cohort studies	-
2b	Individual cohort study (including low quality RCT; e.g., <80% follow-up)	Clinical results were reported in a news briefing by the Chinese government revealing that the treatment of over 100 patients with chloroquine phosphate in China had resulted in significant improvements of pneumonia and lung imaging, with reductions in the duration of illness [12] An uncontrolled French non-comparative observational study conducted in a cohort of 80 relatively mildly infected inpatients treated with a combination of hydroxychloroquine and azithromycin over a period of at least three days, all patients improved clinically except one 86 year-old patient who died, and one 74 year-old patient still in intensive care. A rapid fall of nasopharyngeal viral load was noted, with 83% negative at day 7, and 93% at day 8. Virus cultures from patient respiratory samples were negative in 97.5% of patients at day 5. Consequently patients were able to be rapidly discharged with a mean length of stay of five days [17]
2c	"Outcomes" research; ecological studies	Three studies have demonstrated that chloroquine phosphate inhibits SARS-CoV-2 [14,37,38] and two have demonstrated that hydroxychloroquine sulfate inhibits SARS-CoV-2 [37,38] *in vitro*. In addition, one study showed that the combination of hydroxychloroquine and azithromycin inhibits SARS-CoV-2 on SARS-CoV-2 *in vitro* [19].
3c	Systematic review (with homogeneity) of case-control studies	-
3b	Individual case-control study	-
4	Case-series (and poor quality cohort and case-control studies)	-
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles	The National Health Commission of the People's Republic of China published their recommendation mid-February, suggesting treating patients with 500 mg chloroquine phosphate twice per day, for a maximum of 10 days [32]. In Italy, the L. Spallanzani National Institute for the Infectious Disease published their recommendations for treatment on the 17th of March, which included the provision of 400mg of HCQ per day or 500mg CQ per day, in combination with another antiviral agent [39].

* https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/