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Article

Antimalarials and macrolides: a review of off-label pharmacotherapies during the first wave of the SARS-CoV-2 pandemic

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We critically analyzed clinical trials performed with chloroquine (CQ) and hydroxychloroquine (HCQ) with or without macrolides during the first wave of COVID-19 and discussed the design and limitations of peer-reviewed studies from January to July 2020. Seventeen studies were eligible for the discussion. CQ and HCQ did not demonstrate clinical advantages that justified their inclusion in therapeutic regimens of free prescription for treatment or prophylactic purposes, as suggested by health authorities, including in Brazil, during the first wave. Around August 2020, robust data had already indicated that pharmacological effects of CQ, HCQ and macrolides as anti-SARS-CoV-2 molecules were limited to *in vitro* conditions and largely based on retrospective trials with low quality and weak internal validity, which made evidence superficial for decision-making. Up to that point, most randomized and nonrandomized clinical trials did not reveal beneficial effects of CQ or HCQ with or without macrolides to reduce lethality, rate of intubation, days of hospitalization, respiratory support/mechanical ventilation requirements, duration, type and number of symptoms, and death and were unsuccessful in increasing virus elimination and/or days alive in hospitalized or ambulatory patients with COVID-19. In addition, many studies have demonstrated that side effects are more common in CQ- or HCQ-treated patients.

Keywords: Aminoquinolines. Coronavirus. Comorbidity. Adverse effect. Hospitalization.

ABBREVIATIONS

ACE2, angiotensin-converting enzyme 2; AST, aspartate aminotransferase; ARDS, acute respiratory distress syndrome; BMI, body mass index; CQ, chloroquine; COVID-19, coronavirus disease-2019; HCQ, hydroxychloroquine; ICU, intensive care unit; IQR, interquartile range; LRTI, lower respiratory tract infection; MERS, Middle East respiratory syndrome; MeSH, medical subject headings; NSAIDs, nonsteroidal anti-inflammatory drugs; NEWS, national early warning score; qSOFA, quick sequential organ failure assessment; SARS-CoV-1/-2, severe acute respiratory syndrome caused by coronavirus-1/-2; TMPRSS2, transmembrane protease serine 2; URTI, upper respiratory tract infection.

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INTRODUCTION

In December 2019, a new severe acute respiratory syndrome caused by coronavirus-2 (SARS-CoV-2), called coronavirus disease-2019 (COVID-19), caused the worst

pandemic in the last 100 years (Chen *et al*., 2020; Huang *et al*., 2020).

The new coronavirus belongs to the genus *Betacoronavirus*, family Coronaviridae and is genetically close to the viruses responsible for severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), which originated in 2002 in China. Middle East respiratory syndrome (MERS-CoV) emerged in 2012 in the Middle East and was similar to SARS in 2015-2016 in the Ukraine (Shereen *et al*., 2020). At the beginning of the disease or during mild infections, loss of taste, dry cough, fever, fatigue, headache, abdominal pain, anosmia, diarrhea, and myalgia are the most common symptoms (Mitjá *et al*., 2020; Yang, Gui, Xiong, 2020; Menni *et al*., 2020). Severe patients with COVID-19 have bilateral pulmonary pneumonia in approximately 2/3 of the cases, present multiple and irregular opacities (the two most common types of radiographic presentations) and have arisen associated with acute myocardial injury, chronic liver and kidney failures, septic shock, dyspnea, leukopenia, and lymphopenia (Chen *et al*., 2020). Hospitalization with mechanical ventilation, abnormal lung radiographic findings and disease progression are directly related to age and chronic diseases, and death rates increase in elderly patients over 60 years of age with chronic kidney and cardiovascular diseases (including coronary artery disease, congestive heart failure and arrhythmias), diabetes, immunosuppression, lung disorders, smoking, cancers, obesity, male sex, body mass index (BMI) > 30 kg/m^2, hyperlipidemia, and hemoglobin saturation < 94% (Chen *et al*., 2020; Huang *et al*., 2020; Qiu *et al*., 2020; Zheng *et al*., 2020).

With more than 6 million confirmed cases of SARS-CoV-2 worldwide (about 600,000 in Brazil) and 350,000 deaths (30,000 in Brazil) until July 2020 (when cases declined around the world after the first wave), simultaneous outbreaks led to the quick saturation of local health systems. To date, approximately 632 million COVID-19 cases have been registered, which resulted in 6,6 million deaths. In the worst health conditions, from February 26th, 2020 to July 5th, 2021, Brazil detected 18,742,025 cases and 523,587 deaths, an average of approximately 32,724 deaths/month, 1,091 deaths/day and a rate of 246.33 deaths per 100,000 inhabitants. Only in March 2021 did 75,371 Brazilians fall victim to COVID-19. Therefore, Brazil alone had 8,6% of cases and embittered 11,9% of deaths worldwide until November 2021 (WHO, 2021).

Without available vaccine or therapeutic treatments during 2020, several countries began to include affordable drugs used for other clinical purposes, including antimalarial (chloroquine phosphate, hydroxychloroquine), antibacterial (azithromycin, clarithromycin), antiviral (remdesivir, ribavirin, favipiravir, atazanavir, oseltamivir), anticoagulant (heparin) and antiparasitic drugs (ivermectin, nitazoxanide), to treat patients with COVID-19 (some based on preliminary *in vitro* studies) as a first-line, adjuvant or palliative option (D'Alessandro *et al*., 2020; Gao, Tian, Yang, 2020; Paumgartten *et al*., 2020; Tang *et al*., 2020; Wang *et al*., 2020). Chloroquine phosphate (CQ) and hydroxychloroquine sulfate (HCQ) have been used to treat malaria for more than 70 years (D'Alessandro *et al*., 2020). Therefore, these molecules have also been studied for extra approaches and were approved for the treatment of rheumatoid arthritis (Schrezenmeier, Dörner, 2020), systemic lupus erythematosus (Wallace *et al*., 1994), Sjogren's syndrome, and sarcoidosis (Ben-Zvi *et al*., 2012).

In vitro and *in vivo* antiviral mechanisms have also been suggested for chloroquine and hydroxychloroquine (Garulli *et al*., 2013; Devaux *et al*., 2020). In fact, some antiviral mechanisms (Figure 1) have been recently proposed against SARS-CoV-2, taking into consideration specific inhibition of autophagy steps, a well-known property of CQ, HCQ, and analogs, because they increase intralysosomal pH, which results in decreased phago-lysosome fusion and glycosylation impairment of the angiotensin-converting enzyme 2 receptor (Savarino *et al*., 2003; Vicent *et al*., 2005; Ben-Zvi *et al*., 2012; D'Alessandro *et al*., 2020; Schrezenmeier, Dörner, 2020; Wang *et al*., 2020), as seen for SARS-CoV-1 (Li, Moore, Vasilieva, 2003; Vicent *et al*., 2005), which may negatively influence virus-receptor binding and abrogate infection. Moreover, they have immune-modulating effects by inhibiting Toll-like receptor signaling and the production of cytokines, especially IL-1 and IL-6 (Savarino *et al*., 2003; Ferreira *et al*., 2021). However, it remains unclear how changes in the endosomal environment and pH affect the integrity of the SARS-CoV-2 viral genome (Pal *et al*., 2020).

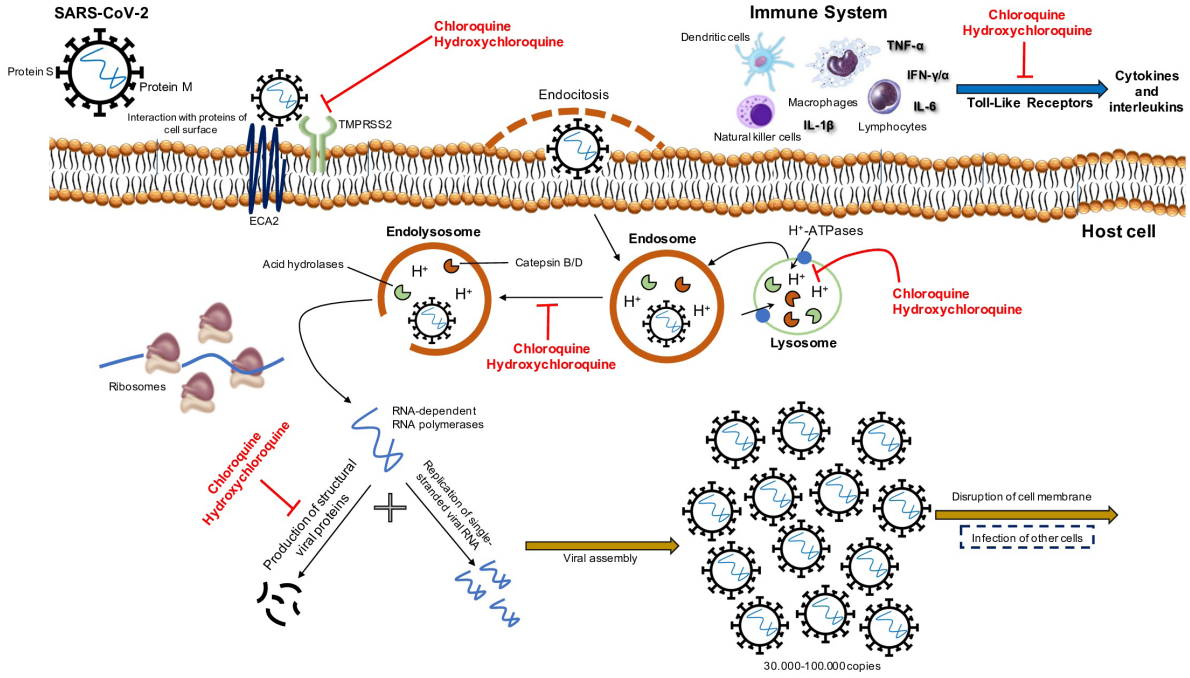

FIGURE 1 – Proposed mechanisms for the antiviral actions of chloroquine and hydroxychloroquine against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and other viruses. Some antiviral mechanisms that have been recently proposed present general parallelisms and depend on the specific inhibition of autophagy steps, a well-known property of chloroquine, hydroxychloroquine and analogs. SARS-CoV-2 enters cells by binding the S protein to the angiotensin-converting enzyme 2 (ACE2) receptor on the cell target surface, which is triggered by the transmembrane protease serine 2 (TMPRSS2). The virus exposes its RNA, and through transcription and replication, the complex forms RNA strands that will be translated for the structural proteins. Structural proteins and RNA in the cytoplasm assemble into new viral particles, which are released by exocytosis to infect other cells. Chloroquine/hydroxychloroquine has shown an *in vitro* ability to block/delay these steps of infection. The transportation of both drugs is completely via passive diffusion (i.e., no transporters are involved). However, it remains unclear how changes in the endosomal environment, particularly changes in pH, may affect the integrity of the SARS-CoV-2 viral genome. To date, no medication has been shown to prevent SARS-CoV-2 transmission or treat COVID-19 specifically.

The possible off-label use and pharmacological repositioning of CQ and HCQ have emphasized the multiple clinical arms of old drugs for new purposes (Ferreira *et al.*, 2021). In this review, we critically analyzed the clinical trials carried out with CQ and HCQ with or without macrolides during the first wave of the COVID-19 pandemic, considering their design and limitations.

MATERIAL AND METHODS

To carry out a comprehensive and consistent analysis of prospective or retrospective, single- or multicenter, observational or analytical clinical trials already published in peer reviewed journals, only original qualitative, quantitative or mixed articles written in English were used in this review. The descriptors were chosen as Medical Subject Headings (MeSH) terms as follows: "clinical trial, COVID-19, chloroquine" or "clinical trial, COVID-19, hydroxychloroquine" and used for bibliographic searches in PubMed, ScienceDirect, Scopus and Scielo databases by three researchers (P.M.P.F., R.W.R.S., and D.P.B.) independently. Afterwards, data were compared and contrasted. Only studies available between January and July 2020 and mentioning these descriptors in the abstract or in the title were initially included. Duplicated and nonrelated articles, as well as *in vitro* studies, opinions, comments, letters to the editor, and reviews, were not considered for review. Within the PICO strategy [(P, current population; I, therapeutic, diagnostic or prognostic intervention; C, comparison; O, outcomes (results)], our

hypothesis was that chloroquine and hydroxychloroquine are effective against SARS-CoV-2 in humans.

As an eligibility criterion and considering evidence-based practice to carry out a critical analysis, we observed the data, rigor, credibility, and study design. In addition, the method of patient selection, the relationships between researchers and patients, ethical criteria, statistical analysis, confounding factors, data presentation, and limitations were also examined (Singh, 2013). Since macrolides (mainly azithromycin or less often clarithromycin) were part of most reports as well as government documents, they were included in the discussion but not in the bibliographic investigations.

All studies were conducted according to the Brazilianrules (Law 466/2012, National Health Council) and international guidelines (World Medical Association, Declaration of Helsinki, and Universal Declaration on Bioethics and Human Rights/UNESCO).

RESULTS

Most articles published until July 2020 corresponded to *in vitro* studies, opinions, comments, letters to the editor, and reviews (or duplicated ones). The PRISMA diagram with details of the selection is shown in Figure 2. After exclusions, 17 clinical studies focusing on CQ or HCQ (with or without macrolides) were considered for the discussion. Each study was briefly described in Table I and sequentially commented throughout the text.

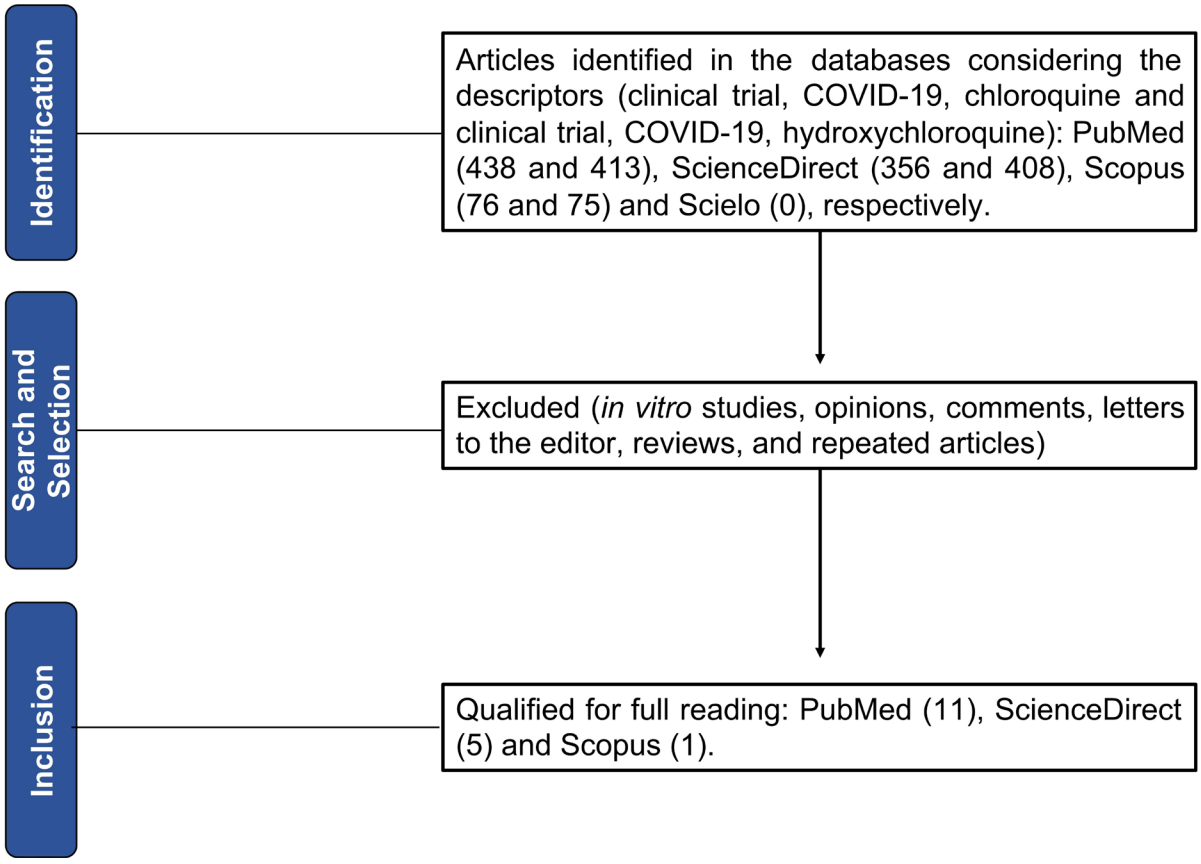

FIGURE 2 - General flowchart of the research process, screening and eligibility of articles to compose the results and the discussion of the thematic proposal.

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine (HCQ) sulfate plus Azithromycin France Gautret *et al*. (2020a)	36 patients a) HCQ: 20 patients received 200 mg of oral HCQ 3 times/ day for 10 days. Among the patients treated with HCQ, six received 500 mg of azithromycin on day 1, followed by 250 mg daily for the next 4 days. b) control group: 16 patients who refused the treatment or had an exclusion criteria. Patients with retinopathy, glucose-6-phosphate dehydrogenase (G6PD) deficiency prolongation of QT interval, breastfeeding, and pregnant were excluded.	Patients were grouped into three categories: asymptomatic (16.7 %), upper respiratory tract infection (URTI) when they had rhinitis, pharyngitis or fever (61.1 %) and low-grade myalgia, and lower respiratory tract infection (LRTI) when they had symptoms of pneumonia or bronchitis (22.2 %). The primary endpoint was the virologic clearance on day 6 after inclusion. Secondary outcomes were extra time for virologic clearance, clinical follow-up (body temperature, respiratory rate, hospitalization, and mortality) and side effects.	As determined by RT-PCR on day 6 after inclusion, 70 % of patients treated with HCQ were virologically cured compared to 12.5 % in the control group. 100 % of patients treated with HCQ plus azithromycin were virologically cured when compared to 57.1 % in patients treated only with HCQ and 12.5 % in the control group.	HCQ or HCQ plus azithromycin were effective for complete eliminating of viral nasopharyngeal load in three to six days. Drug's effect was better in patients with symptoms of URTI and LRTI in comparison with asymptomatic ones. COVID-19 patients can receive HCQ plus azithromycin to treat infection and limit virus transmission.
HCQ sulfate plus Azithromycin France Molina et al. (2020)	600 mg of HCQ daily for 10 days plus 500 mg azithromycin on day 1, followed by 250 mg/ day for the next 4 days. 7 men and 4 women: 8 with comorbidities (obesity: 2; solid cancer: 3; hematological cancer: 2; HIV infection: 1).	At the beginning of treatment, 10 of 11 patients had a fever and received nasal oxygen therapy.	In 5 days, 1 patient died, two were transferred to the intensive care unit (ICU); HCQ plus azithromycin were discontinued in one after 4 days due to the increasing QT interval from 405 ms to 460 and 470 ms. The mean plasma [HCQ] was 678 ng/mL between days 3 and 7.	Repeated tests with nasopharyngeal swabs in 10 patients by RT-PCR revealed positivity for SARS-CoV-2 in 8 of them on days 5 or 6 after the start of treatment. Despite *in vitro* antiviral activity of CQ against COVID-19, no evidence of clinical benefit was found after combination of HCQ and azithromycin.
Chloroquine diphosphate Ceftriaxone Azithromycin Oseltamivir (standard treatment) Brazil Borba *et al*. (2020)	81 patients a) 41 patients: high dose group, CQ (600 mg/4x150 mg, twice a day for 10 days), total of 12 g; b) 40 patients: low dose group, CQ (450 mg CQ/3x150 mg + 1 placebo) 2 times daily on day 0, 3x150 mg tablets + 1 placebo followed by 4 placebo tablets from days 1 to 4. Afterwards, 4 placebo tablets 2 times daily from day 5 to day 9, total of 2.7 g. All patients who met the same study criteria (acute respiratory distress syndrome) received ceftriaxone (1 g 2x for 7 days) + azithromycin (500 mg 1x for 5 days) from day 0. Oseltamivir (75 mg 2x for 5 days) was also prescribed when influenza infection was suspected.	Hospitalized patients with respiratory and heart rates > 24 rpm and/or> 125 bpm (without fever) and/or peripheral oxygen saturation < 90 % in room air and/or shock (mean arterial pressure < 65 mmHg, requiring vasopressors or oliguria or low level of consciousness). The primary outcome (reduction of lethality) assumed a lethality of 20 % in critical patients. Virologic measures included detection of viral RNA by qRT-PCR on days 0 and 4. The final lethality analysis was determined until day 28 and adverse effects and temporary or permanent discontinuation were also observed.	11 patients from the high dose [7 (63.6 %)] and 4 (36.4 %) in the low dose group died. The majority [62 of 81 (76.5 %)] showed COVID-19 confirmed in similar percentages in both groups. Creatine kinase and creatine phosphokinase isoenzyme MB increased by 39.4 % (13 of 33) and 38.4 % (10 of 26 patients), respectively. Increasing of creatine kinase was more frequent in patients under higher of CQ (50 %) than in the low dose group (31.6 %). The mortality rate was 27.2 %. 19 of out 22 dead patients had confirmed *antemortem* virology.	Only 6 patients (22 %) (samples paired in both arms of the study) presented negative respiratory secretion on day 4. The higher dosage of CQ for 10 days was associated with more toxic effects and lethality and cancelled. This dosage is not recommended to treat patients with severe COVID-19, mainly because old aging patients are the most common participants. No benefit with CQ was observed in relation to lethality.

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine plus Azithromycin Remdesivir Sarilumab (other concomitant studies/treatments) United States Geleris *et al*. (2020)	1376 patients a) 811 received HCQ 600 mg 2x on day 1, followed by 400 mg daily for 4 days. Azithromycin 500 mg on day 1 and then 250 mg/day for additional 4 days in combination with HCQ was an additional suggestion therapeutic option. b) 565 patients: control group Multivariate models were used with inverse probability weighting and adjusted for confounding factors using propensity score.	COVID-19 patients with moderate to severe degrees of respiratory disease, defined as an oxygen saturation at rest < 94 % while breathing ambient air. Patients were included in the study if admitted to the emergency sector up to 48 h before.	Among the 1376 patients, respiratory failure appeared in 346 individuals (25.1 %); a total of 180 patients were intubated and 166 died without intubation. As of April 25th, 232 patients had died (66 after intubation), 1025 had survived (hospital discharge), and 119 were hospitalized yet. Regression studies showed no association between HCQ and lower risk of intubation or death.	The risk of intubation or death was not significantly higher or lower among patients who received HCQ or azithromycin when compared to the those who did not. Therefore, the clinical guide of the hospital was updated and the indication HCQ was removed. The results do not indicate the use of HCQ except in randomized clinical trials to test its effectiveness.
Hydroxychloroquine plus Azithromycin United States Rosenberg *et al*. (2020)	Oral HCQ: 200-600 mg/day, 1 to 2 times/day; azithromycin: 200-500 mg/day, oral or i.v. 1 to 2 times a day. 1438 patients a) 735: received HCQ and azithromycin; b) 271: HCQ c) 211: azithromycin d) 221: no drug (control) Chloroquine was originally planned for the study, but the first selected 573 records indicated limited use and patients who received CQ were later excluded from the study.	Information was collected about the diagnosis of COVID-19, patient demographic data, pre-existing medical conditions, initial vital signs and results of laboratory tests within 24 h after admission and chest images. The primary outcome was hospital mortality. Additional secondary outcomes included cardiac arrest and abnormal electrocardiographic (ECG) findings (arrhythmia or prolonged fraction of the QT interval).	Patients who received HCQ plus azithromycin (30.7 %; 27.1 %) or HCQ (19.2 %; 18.8 %) presented higher levels of intensive care unit (ICU) admission and necessity for mechanical ventilation than those who received azithromycin alone (10.9 %; 6.2 %) and no medication (12.2 %; 8.1 %), respectively, even after statistical adjustment. Mortality adjusted at 21 days was 22.5 % for HCQ + azithromycin, 18.9 % for hydroxychloroquine, 10.9 % for azithromycin, and 17.8 % without drugs. Patients who received HCQ + azithromycin suffered from cardiac arrest (15.5 %) and abnormal ECG findings (27.1 %), as well as those in the HCQ group alone (13.7 and 27.3 %) compared to azithromycin alone (6.2 and 16.1 %) or no medication (6.8 and 14 %, respectively).	Obese, patients with pulmonary and cardiovascular disease and diabetics were more likely to receive HCQ + azithromycin and HCQ alone. Approximately 95 % of the HCQ + azithromycin group presented 3 imaging findings: lung opacity (63 %), pulmonary infiltrate (23.8 %) and bronchopneumonia/ pneumonia (20.7 %). Most patients (70 %) received HCQ alone and/or azithromycin. Among hospitalized patients, treatment with HCQ, azithromycin, or both, did not reduce mortality. Cardiac arrest events were more frequent in patients who received HCQ with azithromycin compared to patients who did not receive any medication, even after statistical adjustment.

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine Antiviral agents Arbidol Lopinavir-ritonavir Entecavir Virazole Ganciclovir (standard treatment varied for each hospital) China Tang *et al*. (2020)	HCQ 1200 mg/day for 3 days, followed by a maintenance dose of 800 mg/day. The total duration of treatment was 2 weeks for mild and moderate cases and 3 weeks for severe ones. HCQ dose was adjusted when related-drug adverse events arose. 150 patients a) 70 patients: HCQ and standard hospital treatment b) 80 patients: standard hospital treatment only (control)	Diagnosis of mild disease included patients with slight symptoms, and absence of pneumonia by imaging; moderate ones included fever, cough, sputum and other respiratory problems or nonspecific symptoms and pneumonia confirmed by image; severe cases had severe pneumonia defined as the $SaO_2/SPO_2 < 94$ % or PaO_2/FIO_2 ratio of 300 or less. The primary endpoint was the negative conversion of SARS-CoV-2 and clinical improvement within 28 days. The secondary outcome considered adverse events. Samples from URT, LRT or both were obtained during screening (day -3 to day 1) and during treatment and posttreatment follow-up by visits on days 4, 7, 10, 14, 21 and 28.	The probability of negative conversion of SARS-CoV-2 among patients with standard treatment plus HCQ was 85.4 % in 28 days and similar to the standard group (81.3 %). The most common adverse event in the HCQ group was diarrhea in 10 % of the patients. The average time for negative conversion, negative conversion in 21 days, symptom relief in 28 days, and average time for relief of clinical symptoms were similar in both groups.	There were no additional benefits to eliminate SARS-CoV-2 after adding HCQ to standard treatment in patients with mild to moderate forms of COVID-19. Overall, these data do not indicate HCQ to treat COVID-19.
Hydroxychloroquine plus Azithromycin Ceftriaxone Ertapenem Anticoagulants (standard treatment) France Million *et al*. (2020)	200 mg of oral HCQ 3 times daily for 3 days plus azithromycin 500 mg on day 1 followed by 250 mg per day for 4 days. Early treatment with HCQ plus azithromycin with or without symptoms in hospitalized patients or in infectious disease units (hospitalized patients) when necessary. High doses of anticoagulants were administered to critically ill patients. 1061 patients, excluding < 14 years old, pregnant women or patients with G6PD deficiency (only based on the patient's declaration). Patients initially treated in day-care hospital or discharged from conventional wards before day 10 were followed as ambulatory outpatients.	Patients were grouped by clinical presentation (symptoms of URTI or LRTI) and severity considering the national early warning score (NEWS) in 3 categories for clinical deterioration: low (NEWS 0-4), medium (NEWS 5-6) and high (NEWS ≥ 7) score. The primary outcomes were the necessity for oxygen therapy, transfer to the ICU or death after at least 3 days of treatment and prolonged hospitalization (10 days or more). The transmission capacity was assessed by RT-PCR and culture. A group with a poor clinical outcome (PClinO) was defined by death or transfer to the ICU or hospitalization for 10 days or more and a group with a poor virologic result (PVirO) was defined by viral persistence on the 10^{th} day. The others were assigned to a group with good overcomes (GO).	The majority did not report any adverse events that could be attributed to the treatment (97.6 %). No rhythmic cardiac events or sudden deaths were observed. The mean age (69 years) was higher in patients with PClinO than in the GO group (42 years). When compared to patients with GO, PClinO patients were more likely to have previous hypertension (50 %), diabetes (19.6 %), coronary heart disease (19.6 %), and cancer (15.2 %) and more likely receiving beta-blocking agents and angiotensin II receptor blockers, dihydropyridine, HMG-CoA reductase inhibitors, diuretics, and metformin.	Considering serum HCQ > 0.1 µg/mL within the therapeutic range, $[HCQ]_{plasma}$ on day 2 (0.20 µg/mL) was lower in the GO group. 1048 (98.7 %) patients who received the HCQ plus Azithromycin are cured to date. Tomographic scores revealed pneumonia in 35 patients from the PClinO group (90 %). It suggests generalized use of HCQ even in mild cases.

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine sulphate plus Azithromycin France Gautret *et al*. (2020b)	200 mg of HCQ 3 times daily for 3 days plus azithromycin (500 mg on day 1 followed by 250 mg for 4 days. 80 patients Nasopharyngeal swabs were collected daily until discharge. Patients were grouped into 2 categories: (i) URTI with isolated rhinitis and/or pharyngitis and/or fever and myalgia and (ii) LRTI with symptoms of pneumonia or bronchitis.	Three risk categories for clinical deterioration were stablished: low (NEWS 0-4), medium (NEWS 5-6) and high (NEWS ≥ 7) score. Radiological images classified as compatible (presence of peripheral multifocal opacities in ground glass, with or without cross-links) or presence of alveolar consolidation or mosaic paving pattern or not compatible with pneumonia. The primary outcomes: (i) aggressive clinical course with oxygen therapy or transfer to the ICU after at least 3 days of treatment; (ii) transmission assessed by RT-PCR and culture, and (iii) time of hospitalization.	Most patients presented low NEWS (92 %) and 53.8 % of them had compatible radiological pneumonia images. The majority (65/80 = 81.3 %) displayed a favorable result and was discharged with low NEWS (61/65 = 93.8 %, but 15 % needed oxygen therapy. A rapid reduction in nasopharyngeal viral load was described by 83 % on day 7. The presumable contagious declined to zero on day 12. Of the 65 patients discharged, the median time from onset to discharge was 4.1 days, with an average duration of 4.6 days under care for infectious disease.	A total of. 57.5 % of patients had at least one chronic condition (hypertension, diabetes, and chronic respiratory diseases were the most frequent) Coadministration of HCQ and azithromycin should be early used to treat and cure patients even before irreversible respiratory complications occur, and to slow or prevent the spread of the disease.
Hydroxychloroquine, Prednisone and Immunosuppressants (standard treatment) France Mathian *et al*. (2020)	Oral HCQ 200 - 400 mg/ day for most patients 17 patients with systemic lupus erythematosus (SLE) under long-term treatment with HCQ and confirmed nasopharyngeal SARS-CoV-2 by RT-PCR.	SLE patients had to meet the criteria for SLE ratings from the American College of Rheumatology (1997) or those of the 2019 European League Against Rheumatism/ American College of Rheumatology.	The main comorbidities were obesity and chronic kidney disease in 10 (59 %) and 8 (47 %) of the patients, respectively. Viral pneumonia was diagnosed in 13 (76 %), with complications in 16 patients [respiratory failure in 11 (65 %); ARDS in 5 (29 %)]. Fourteen (82 %) patients were hospitalized, including 7 (41 %) in ICU. Oxygen therapy was required for 11 of them (65 %, nasal cannula and invasive mechanical ventilation). Five (36 %) patients were discharged, seven (50 %) remained hospitalized and two (14 %) died.	The average duration of SLE was 8.2 years and the treatment with HCQ before COVID-19 was 7.5 years. Most SLE patients received long-term treatment with HCQ, whose blood concentrations were within the therapeutic range, which shows that HCQ does not seem to prevent COVID-19 infection.
Hydroxychloroquine Azithromycin Amoxicillin-clavulanate (standard treatment) France Mahévas *et al*. (2020)	HCQ 600 mg/day 181 patients a) 84 patients: HCQ within 48 h after hospitalization b) 97 patients: control Patients with COVID-19 pneumonia requiring oxygen by a face mask or nasal cannula as established by the WHO (progression score 5) were included in the study.	The start of follow-up (baseline or time zero) for each patient was the time of admission to hospital. All patients were followed-up from baseline until death, loss or end of follow-up, whichever occurred first. The primary outcome was survival without transfer to the ICU at day 21. Secondary outcomes were overall survival, survival without ARDS, weaning from oxygen, and discharge or rehabilitation at day 21.	At day 21, 17 of 173 (10 %) patients had died (9 from the treatment group and 8 from control group). In the nonweighted analyses (and in the inverse probability of treatment weighting analyses that took imbalance at baseline into account), among the 173 patients, the rate of survival without transfer to intensive care, overall survival rate, rate of survival without ARDS, and the oxygen weaning percentage were 80 and 75 % (76 and 75 %), 89 and 91 % (89 and 91 %), 70 and 74 %, (69 and 74 %), and 79 and 74 % (82 and 76 %) at 21 days, respectively, at day 21 for HCQ and control groups.	Of the 84 patients who received HCQ, eight (10 %) experienced electrocardiographic modifications. These side effects argue against the widespread use of HCQ in patients with COVID-19 pneumonia. Results also suggested that patients with fewer symptoms and better prognosis at admission did not respond to HCQ. HCQ did not reduce ICU transfer or deaths until the 21th day after admission, or ARDS in hospitalized patients with hypoxemic COVID-19 pneumonia.

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine Azithromycin Compassive therapy (without details) United States Magagnoli *et al*. (2020)	The median daily doses [interquartile range (IQR)) of HCQ were 400 (400-480) mg and 422.2 (400-480) mg in the HCQ and HCQ + AZ groups, respectively. The median (IQR) durations of treatment with HC were 5 (3–5) days and 5 (4–6) days in the HC and HC+AZ groups, respectively. 807 patients a) 198 patients: HCQ b) 214 patients: HCQ + azithromycin c) 395 patients: no HCQ	The outcomes were hospitalization (discharge or death), need for ventilation, type of ventilation, and the result of hospitalization among patients requiring ventilation. Mechanical ventilation included patients receiving both noninvasive and invasive forms of ventilation. For comorbid conditions, it was utilized ICD-10-CM codes and the Charlson comorbidity index.	Of the 807 patients, 124 (15.4 %) died, 517 (64.1 %) were discharged alive, and 166 (20.6 %) remained hospitalized at the end of the study period. After propensity score adjustment for clinical characteristics, the risk of death from any cause was higher in the HQC group but not in the HCQ + AZ group when compared to the no HCQ group (control). The propensity score-adjusted risk of mechanical ventilation was not different after the treatments. Moreover, the length of hospital stay was similar among groups.	After adjusting for several relevant confounders, benefits in HCQ groups (with or without azithromycin) were not observed for survival, need for mechanical ventilation, or length of stay among hospitalized COVID-19 patients.
Hydroxychloroquine or Chloroquine with or without macrolide (Azithromycin or Clarithromycin) Lopinavir/Ritonavir Ribavirin Oseltamivir (standard treatment) United States Mehra *et al*. (2020)	Within 48 h after the diagnosis of COVID-19, patients received: a) CQ: 1,868 patients (765 mg/6.6 days) b) CQ + macrolide: 3,783 patients (790 mg/6.8 days) c) HCQ: 3,016 patients (596 mg/4.2 days) d) HCQ + macrolide: 6,221 (597 mg/4.3 days) e) Control: 81,144 (none of drugs above)	The primary outcome was the association between use of a treatment regimen when initiated early after COVID-19 diagnosis with the endpoint of in-hospital mortality. The secondary outcome was the association between treatment regimens and the occurrence of ventricular arrhythmias during hospitalization [defined as the first occurrence of a nonsustained (at least 6 s) or sustained ventricular tachycardia or ventricular fibrillation]. They also analyzed rates of progression to mechanical ventilation use and length of stay in ICU.	Nonsurvivors to the treatments were nearly 2-fold for CQ (2.9 %), CQ + azithromycin (7.8 %), HCQ (5.1 %) and HCQ + macrolides (13.8 %) when compared to the control group (1.8, 3.4, 2.9 and 5.6 %), respectively) *De novo* ventricular arrhythmia (3.7 %), non-ICU length of stay (9.8 days), length of stay in the ICU (9.4 days), total length of stay (19.4 days), and need for mechanical ventilation (42.4 %) were statistically higher in nonsurvivors than in those who survived (1 %, 9 days, 11.1 days and 5.6 %, respectively).	In comparison with the control group (0.3 %), HCQ (6.1 %), HCQ + macrolide (8.3 %), CQ (4.3 %) and CQ + macrolide (6.5 %) groups were independently associated with increased risk of *de novo* ventricular arrhythmia during hospitalization. No evidence of benefit of HCQ or CQ when used either alone or with a macrolide and these regimens were associated with increased risk of ventricular arrhythmias and death, which suggest these therapies should not be used outside of clinical trials.

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine sulphate United States Boulware *et al*. (2020)	800 mg HCQ (4 tablets) at once followed by 600 mg 6 - 8 h later, then 600 mg/ day for 4 additional days 821 patients within 4 days of contact: a) 414 patients: HCQ b) 407 patients: placebo Participants have had household or occupational exposure to someone with confirmed COVID-19 at a distance of less than 6 ft for more than 10 min while wearing neither a face mask nor an eye shield (high-risk exposure) or while wearing a face mask but no eye shield (moderate-risk exposure).	The primary results were symptoms related to COVID-19 based on national guidelines whose cases had been confirmed by RT-PCR, for probable cases (the presence of cough, shortness of breath, or difficulty breathing, or the presence of two or more symptoms of fever, chills, rigors, myalgia, headache, sore throat, and olfactory and taste disorders), and possible cases (the presence of one or more compatible symptoms, which could include diarrhea). Secondary outcomes included the incidence of hospitalization for COVID-19 or death, the incidence of PCR-confirmed SARS-CoV-2 infection, the incidence of COVID-19 symptoms, the incidence of discontinuation of the trial intervention owing to any cause, and the severity of symptoms (if any) at days 5 and 14 according to a visual analog scale [scores ranged from 0 (no symptoms) to 10 (severe symptoms)].	A total of 87.6 % of volunteers (719/821) had high-risk exposures without using eye shields and surgical masks or respirators. Among them, 365 received HCQ and 354 received placebo. COVID-19 (confirmed by PCR or symptoms) developed in 13 % (107/821) during the 14 days. The incidence of new diseases compatible with COVID-19 did not differ significantly between who received HCQ (49/414 = 11.8 %) and placebo (58/407 = 14.3 %). The most common reason for discontinuation was attributed to side effects, which were more frequent in HCQ-treated group than with placebo, but no serious intervention-related adverse reactions or cardiac arrhythmias were noted.	There was no difference of symptoms in the group under preventive HCQ intervention if compared to the placebo. No significant changes regarding hospitalization, deaths or the time for onset of postexposure prophylaxis were detected between HCQ and placebo. High doses of HCQ did not prevent the development of COVID-19 when prophylaxis was started within 4 days after high- or moderate-risk exposure.
Hydroxychloroquine sulphate United States and Canada Skipper *et al*. (2020)	a) 244 patients: HCQ 800 mg/day (4 tablets) once; 600 mg/day 6 to 8 h later, and 600 mg (3 tablets) once daily for 4 additional days. b) 247 patients: placebo (folic acid, 400 ug). Persons with either laboratory-confirmed COVID-19 or compatible symptoms and contact with laboratory-confirmed COVID-19 person. Follow-up surveys were performed for 14 days to assess study medication adherence, adverse effects, presence and severity of COVID-19 symptoms (0-10 analogic scale), virologic results, and hospitalization.	Nonhospitalized adults who required to have 4 or fewer days of symptoms and either PCR-confirmed SARS-CoV-2 infection or compatible symptoms after a high-risk exposure (immediate household contact or a close occupational COVID-19 exposure) to a person with confirmed PCR within the past 14 days. The initial primary outcome was an ordinal outcome by day 14 of nonhospitalized, hospitalized, or ICU stay or death. Secondary end points were symptom severity on day 5 and 14 by 10-point visual scale, hospitalizations and deaths, and incidence of study medicine withdrawal. If hospitalized within 14 days, the follow-up continued to assess outcomes.	Of 341 persons, 145 were PCR-positive for SARS-CoV-2 and 280 had known high-risk exposure to a PCR-positive contact; 84 had both. The remaining 82 participants (19 %) were enrolled with suspected COVID-19: They had COVID-19-compatible symptoms and reported high-risk exposure. On day 5 and 14, 54 and 56 % and 24 and 30 % of participants receiving HCQ and placebo reported symptoms, respectively. HCQ (2.60 points) and placebo (2.33 points) groups had a similar and not significant mean reduction of points from baseline on the 10-point visual analog scale for symptom severity.	Additional *post hoc* analyses showed that self-reported use of zinc or vitamin C in addition to HCQ did not improve symptoms over use of HCQ alone. HCQ failed to decrease prevalence or severity of symptoms over the 14-day study period. The incidence of hospitalization or death did not differ between treated and placebo groups. Adverse effects were more common in HCQ than placebo through the 5-day regimen (43 % *vs*. 22 %, $p < 0.001$).

TABLE I - Details about clinical trials with chloroquine or hydroxychloroquine during the first wave of the COVID-19 pandemic.

Drug(s) Origin of the study Reference	Doses/days Study group	Clinical classification/outcomes	Results	Conclusions
Hydroxychloroquine Azithromycin Corticosteroids and Tocilizumab (standard treatment) United States Arshad *et al*. (2020)	HCQ 400 mg 2x on day 1, followed by 200 mg twice daily on days 2–5. Azithromycin 500 mg on day 1 followed by 250 mg once daily for additional 4 days. Their combination was reserved for selected patients with severe COVID-19 and with minimal cardiac risk factors. 2541 patients divided into: a) No drugs: 409 patients b) HCQ: 1202 c) Azithromycin: 187 d) HCQ + azithromycin: 783	Admission with a positive SARS-CoV-2 test and data collected from electronic medical records, including demographic and clinical characteristics. The maximal modified Sequential Organ Failure Assessment (mSOFA) scores on admission were collected. The primary endpoint was inpatient hospital mortality in each treatment group.	Multivariable Cox regression analysis showed HCQ alone decreased the mortality hazard ratio by 66 % and HCQ + azithromycin decreased the mortality hazard ratio by 71 %. Predictors of mortality were age ≥ 65 years, white race, kidney diseases, reduced O_2 saturation level on admission, and mechanical ventilation use during admission. Kaplan-Meier survival curves within the propensity matched setting displayed better survival in the HCQ treated group, and enhanced survival for 28 days from admission.	HCQ may have role to play in reducing COVID-19 mortality but such protocol should not be applied to patients outside of hospital settings.
Hydroxychloroquine sulphate Spain Mitjá *et al*. (2020)	HCQ 800 mg on day 1, followed by 400 mg once daily for six days 293 patients a) Control arm**:** 157 b) Intervention arm: 136 patients received HCQ 800 mg on day 1 and 400 mg once daily for 6 days. Patients aged 18 years or more with mild symptoms of COVID-19 (i.e., fever, acute cough, shortness of breath, sudden olfactory or gustatory loss, or influenza-like-illness) for less than 5 days before enrollment, nonhospitalized, and positive for SARS-CoV-2.	Participants were assessed on day 1 (baseline, HCQ was started), 3, 7, 14, and 28 for collection of epidemiological and monitoring of disease progression, safety, and self-reported treatment compliance. The primary outcome was the reduction of viral load in nasopharyngeal swabs at days 3 and 7. The secondary outcomes were clinical progression measured by a simplified version of the WHO progression scale and resolution time of symptoms within the 28-days follow-up period. Adverse events were assessed up to 28 days.	No differences between the control and HCQ groups at day 3 or 7 were observed in relation to the viral load from nasopharyngeal swabs. The risk of hospitalization was similar in the control (11/157 = 7.1 %) and intervention arm (8/136 = 5.9 %). Median time for resolution of COVID-19 symptoms was not significantly different between the groups (12 days *vs*. 10 days.). Twenty serious adverse events were reported (12 *vs*. 8 in the control and intervention arms, respectively). The most frequent treatment-related adverse events among participants given HCQ were gastrointestinal.	Any meaningful virologic or clinical benefit of HCQ in outpatients with mild COVID-19 was found if HCQ is given within five days from symptom onset. Moreover, this treatment regimen neither reduced the risk of hospitalization nor decreased the time to complete resolution of symptoms.

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Hydroxychloroquine plus Azithromycin Glucocorticoids, immunomodulators, antibiotic, and antiviral (standard treatment) Brazil Cavalcanti *et al*. (2020)	665 patients a) Control: 227 patients (standard treatment) b) HCQ 400 mg twice daily for 7 days: 221 patients c) HCQ 400 mg 2x daily plus azithromycin at a dose of 500 mg once a day for 7 days: 217 patients It included hospitalized persons with suspected or confirmed COVID-19 with 14 or fewer days of symptom.	The primary outcome was the clinical status at 15 days evaluated by a seven-level severity scale (from 1 to 7) taking into consideration the severity of symptoms, necessity for hospitalization, noninvasive and invasive mechanical ventilation, and death. Secondary outcomes included clinical status at 7 days evaluated by a six-level ordinal scale, and indication for intubation and supplemental oxygen, duration of hospital stays, in-hospital death, thromboembolic complications, acute kidney injury, and number of days alive and free from respiratory support up to 15 days.	There were no differences among the groups in the proportional odds of having a higher (worse) score on the seven-point ordinal scale at 15 days and need for mechanical ventilation requirements (HCQ + azithromycin: 11 %; HCQ: 7.5 %; control: 6.9 %). Adverse events were more common in patients who received HCQ + azithromycin (39.3 %) or HCQ alone (33.7 %), as well as prolongation of the QTc interval in HCQ with or without azithromycin. Elevation in liver enzymes was higher in patients receiving HCQ + azithromycin.	Thromboembolic or acute kidney injury events, days alive and free from respiratory support were similar among groups within 15 days. This 7-day course of HCQ either with azithromycin did not result in better clinical outcomes but these regimens were more associated with the occurrence of hepatic damages and more frequent events of QTc interval prolongation.

DISCUSSION

In the beginning of the SARS-CoV-2 pandemic, studies with African green monkey kidney Vero E6 cells infected with strains of the new coronavirus-2019 revealed antiviral activity and EC_{50} values of 23.9 and 5.47 μM and 6.14 and 0.72 μM in 24 and 48 h for CQ phosphate and HCQ sulfate, respectively (Wang *et al*., 2020). Similar studies indicated that the 50% maximal effective concentration for CQ (2.71, 3.81, 7.14, and 7.36 μM) was lower than that for HCQ (4.51, 4.06, 17.31, and 12.96 μM) (Liu *et al*., 2020). Some years before, the IC_{50} of CQ for *in vitro* inhibition of SARS-CoV-1 in infected Vero E6 cells indicated approximation of plasma concentrations of CQ reached during treatment of acute malaria (Keyaerts *et al*., 2004) since 6 - 6.5 mg/kg per day of HCQ sulfate could generate serum levels of 1.4 - 1.5 μM in humans **(**Laaksonen, Koskiahde, Juva, 1974).

Based on PBPK model results, a loading dose of 400 mg twice daily of oral HCQ sulfate followed by a maintenance dose of 200 mg given twice daily for 4 days was recommended for SARS-CoV-2 infection, since it reached 3-fold the potency of CQ phosphate when administered at the same dosages in patients with rheumatoid arthritis (500 mg/dose). Similarly, the computationally simulated chloroquine concentration in lung tissue was higher than that in plasma, where the lung to plasma ratio increased with time and reached a ratio of approximately 400 (Yao *et al*., 2020).

In this context, it is important to emphasize that in addition to being deficient in angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2), intrinsic nonspecific endocytic viral uptake mechanisms are responsible for viral entry in the Vero E6 line and a variety of other cell types (Murgolo *et al*., 2021). Indeed, chloroquine does block infection with SARS-CoV-2 in Calu-3 metastatic lung adenocarcinoma cells lacking ACE2 but expressing human TMPRSS2, and engineered expression of TMPRSS2 renders SARS-CoV-2 infection of Vero cells insensitive to CQ (Hoffmann *et al*., 2020a). These findings clearly indicate that CQ or HCQ will not exert antiviral activity in human lung tissue and will not be effective against COVID-19 (Hoffmann *et al*., 2002a), especially whether we take into consideration that the spike protein of SARS-CoV-2, which mediates viral entry, is activated by the endosomal-pH-dependent cysteine protease cathepsin L in some tissues but is pH-independent via TMPRSS2 in airway epithelial cells

(Hoffmann *et al*., 2020b). Calu-3 cells, as the airway epithelium, present low amounts of cysteine protease cathepsin L (Park *et al*., 2016), and SARS-CoV-2 entry mainly occurs by TMPRSS2 (Hoffmann *et al*., 2020b). Then, CQ and HCQ may block spike protein-driven entry, but this inhibition is a cell-specific pharmacodynamic response not observed in $TMPRSS2^{+}$ pulmonary cells. These effects indicate that some preclinical findings do not offer plausible reasons for clinical purposes before further mechanistic definitions.

All *in vitro* and computational analyses suggested doses and treatment schedules without tests on laboratory animals or humans, did not consider *in vivo* pharmacokinetic analyses, and claimed clinical recommendations and broad-spectrum conclusions about systemic anti-inflammatory actions of HCQ. Nonetheless, these early data encouraged researchers to carry out clinical trials with CQ and HCQ as supposable options to treat infected patients or prevent COVID-19.

The first clinical trial published after starting the COVID-19 pandemic was carried out in France. Gautret *et al.* (2020a) reported that HCQ (hydroxychloroquine sulfate) 600 mg/day for 10 days or HCQ plus azithromycin was effective in eliminating viral nasopharyngeal load as determined by reverse transcription polymerase chain reaction (RT-PCR), after 3-6 days in patients with COVID-19. It was an open, single and nonrandomized clinical trial, with a small sample (36 patients), higher average age in the treated group (51.2 years *vs*. 37.3 years), and omission of 6 (23%) patients excluded due to clinical worsening or loss of follow-up. No standardization of treatment according to the disease's severity was carried out, untreated patients enrolled involved another health center (it was not a control group), and all patients received the same type of treatment, regardless of the clinical condition, leading to worthless conclusions about the effectiveness of these drugs. In addition, this study did not provide details about comorbidities.

Shortly afterwards, another French open, prospective, and uncontrolled clinical trial considering identical dosages from Gautret *et al.* (2020a) showed conflicting results. Once again, a small group of 11 patients, without control or placebo. With a mean age (58.7 years) greater than the previous study, study, it was closer to the average age for most moderate and severe cases of COVID-19. They demonstrated that 2/3 (72.7%) of the patients presented a direct relationship between comorbidities and the severity of the disease (Molina *et al*., 2020). However, details about the use of supportive pharmacological therapies were not accessible. As in the previous study, there was no standardization of treatment according to COVID-19 severity, which excluded real correlations about progression of the pathology and the effectiveness of the treatment.

The first Brazilian clinical investigation called the CloroCovid-19 trial was carried out in Manaus, the largest city in the Amazon region. It enrolled 81 randomized patients who received high doses of CQ base (600 mg, twice per day for 10 days, total of 19 g CQ diphosphate or 12 g base) or lower doses (450 mg with or without placebo, divided into 10 days, total of 4.3 g CQ diphosphate or 2.7 g base). Among the 27 patients (paired sample analysis in both arms of the study), respiratory secretion on day 4 revealed negative conversion for only 6 patients (22%), and benefit for CQ-treated patients was not observed regarding lethality (Borba *et al*., 2020). This clinical trial was double-blind, analytical, without placebo/control group, parallel, and performed in a public hospital, representing the most common COVID-19 cases in Brazil. This indicates that a higher dosage of CQ is not medically aplicable for the treatment of severe COVID-19, especially among patients also receiving azithromycin and oseltamivir, because of safety concerns regarding QTc interval prolongation and increased lethality. Patients not excluded from the studies based on the QTc interval may represent the Brazilian reality, since most patients treated with CQ/HCQ with or without azithromycin are not submitted to cardiological follow-ups (e.g., ECG monitoring) before starting the treatment. The lack of lung radiological/tomographic examinations during the trial limited the monitoring of the clinical respiratory conditions (improvement or worsening). The fact that all patients received ceftriaxone, azithromycin or oseltamivir may have influenced the appearance of side effects and hampered the clinical interpretation of the results.

A large open, parallel, single, and observational cohort study carried out by Geleris *et al.* (2020)

with 1,376 patients, demonstrated that there was no association between HCQ and a lower rate of intubation or death in patients with COVID-19. As assumed by the authors, the results do not support the use of HCQ outside randomized clinical trials. Intriguingly, the decision to prescribe HCQ and/or azithromycin was given to the medical staff for each patient without clear rules. Patients who had already used sarilumab also received HCQ. In addition to the 27 patients who received remdesivir as a compassionate drug, thirty patients had already been included in a randomized, cohort, double-blind clinical trial with sarilumab. Therefore, some patients received 3 different treatments simultaneously. This study has 2 strong points: i) the statistical adjustment to reduce confounding factors for age, race, ethnicity, body mass index, diabetes, underlying kidney disease, chronic lung disease, hypertension, vital signs baseline, PaO_2:FIO_2 (oxygen arterial pressure:inspired fraction of oxygen) and inflammatory markers, although these inflammatory markers were not discussed in the article; ii) the use of propensity-score methods to reduce the effects of confounding because of the nonrandomized treatment.

In New York, a clinical trial conducted with 1,438 patients receiving HCQ, azithromycin, or both did not exhibit a reduction in lethality in hospitalized patients and revealed that cardiac arrest events were more frequent in HCQ plus azithromycin-treated patients than in those who did not receive such medications (Rosenberg *et al*., 2020). This report of HCQ-related side effects in patients with COVID-19 was obtained in a cohort, retrospective, multicenter, and observational analysis in 25 hospitals in New York state and supplemented by medical record reviews by trained chart abstractors. These data represented 88.2% of patients hospitalized in the region and provided good analysis of the heterogeneity of different communities and hospital protocols. Considering the innate complexity of this large clinical trial, some issues deserve to be highlighted. Data (deaths and side effects) were collected, as seen for most clinical studies conducted thus far, only from hospitalized patients. The necessity for intensive care unit (ICU) admission, mechanical ventilation or any treatment occurred shortly after admittance to the hospital, which impaired monitoring and time analysis and ICU admission-related factors and ventilation requirements. Although the supplementary material shows the use of NSAIDs (aspirin, celecoxib, diclofenac, ibuprofen, indomethacin, naproxen, oxaprazine, piroxicam) by patients who did not receive HCQ or azithromycin, patients receiving aspirin only (19.8%, 38/198) represented the most significant percentage of the volunteers. However, some people were also using the angiotensin-converting enzyme (ACE) inhibitor lisinopril (6.7%, 13/193) and the angiotensin receptor blocker losartan (2.6%, 5/192). As described in Borba *et al.* (2020), this study assumed a mortality rate of 20% in the group that did not receive HCQ. Interestingly, this study used statistical tools to control bias involving risk factors [≥ 65 years old, sex, diabetes, chronic lung disease, cardiovascular diseases (hypertension, coronary artery disease, congestive heart failure)] and the severity of signs and symptoms [respiratory rate > 22/min, O_2 saturation < 90%, abnormal chest imaging findings, aspartate aminotransferase (AST > 40 U/L and elevated creatinine levels)].

A multicenter, randomized, open controlled clinical trial in 16 hospitals from three Chinese provinces with 150 patients found no additional benefits of virus elimination after adding HCQ to the standard treatment of patients with moderate COVID-19 symptoms (Tang *et al*., 2020). A longer follow-up period up to 28 days (up to 7 days after the last dose of HCQ) was the main strength of the study, including visits that allowed the analysis of vital signs, functional biochemical markers, alterations and pulmonary symptoms, and side effects. This study focused on male patients (82%) with about 46 years-old and mild or moderate symptoms (99%; 1% with severe symptoms only), which explains, at least in part, the absence of COVID-19 progress/regression assessments. Another important difference from previous studies was that treatment with HCQ started 16 days after symptom onset, but this seems to not influence viral conversion results. A serious but inconclusive issue was that higher doses (1200 mg/day of HCQ for 3 days, followed by a maintenance dose of 800 mg/day) for longer periods compared to previous clinical trials (14 days) did not cause deaths during the clinical

trial. As expected, this finding is certainly related to the clinical profile of patients: most of them presented mild to moderate symptoms, which implied that highest doses of HCQ would not cause better antiviral effects, since self-healing is a well-established process in these cases. A critical methodology decision to require two consecutive molecular tests for SARS-CoV-2 with a 24-h interval to reduce the possibility of false negative outcomes and the presence of an independent security monitoring committee that supervised, reviewed and performed periodic statistical analysis of the project, gave greater credibility to the results.

Million *et al*. (2020) conducted a single, retrospective, uncontrolled, and descriptive study that recommends generalized usage of HCQ, even in mild cases. They reached this conclusion based on tomographic scores. Most patients (948 = 97.4%) showed a low national early warning score [NEWS < 4)] on admission to the hospital, suggesting that the majority of patients present a mild form of the disease at the beginning of treatment around the 6th day after the onset of symptoms, whose outcomes were confirmed by radiological findings. On the other hand, medical follow-up by computed tomography revealed that 36% had normal lungs, 43.2% showed minimal pulmonary impairment, and 19.2% had intermediate impairment. Bilaterally lung injuries were not mentioned. It is worth mentioning the inclusion of young people between 14 and 18 years old and the exclusion of patients with a risk of unpleasant side effects: cardiac complications (33 patients), likely pharmacological interaction with CQ/HCQ (15), hypokalemia (10), contraindications (6), allergy to HCQ + azithromycin (4), gastrointestinal tract intolerance to HCQ + azithromycin (4), and nonrecommended patients (66 were classified as nonspecific/no extra details were available). Broad-spectrum antibiotics (ceftriaxone or ertapenem) were routinely given to the patients with pneumonia and NEWS ≥ 5, and such interaction was not weighed.

At that time, Gautret *et al*. (2020b) suggested that the coadministration of HCQ sulfate and azithromycin can be used to treat and cure patients at early stages of COVID-19 before irreversible respiratory complications and to decrease or prevent the spread of the disease based mainly on viral conversion on days 7 (66/80 = 83%) and 9 (75/80 = 93%). However, there was no control group for comparison, and just one arm was included, whose patients received 200 mg of HCQ 3 times daily for 3 days plus azithromycin (500 mg on day 1 followed by 250 mg per day for 4 days). In this retrospective, descriptive, and observational analysis at a French hospital, they did not notice important adverse effects after drug exposure. Only 15% had fever, a rare finding since most previous pathological follow-ups display fever as one of the most characteristic symptoms in moderate to severe patients (Chen *et al*., 2020; Huang *et al*., 2020; Xie *et al*., 2020). Most (71.2%) were aged < 60 years, 5% of the patients were asymptomatic, 53.8% reported symptoms of lower respiratory tract infection, and 41.2% had symptoms of upper respiratory tract infection. Thus, nearly half of them had mild disease, although the National Early Warning Score (NEWS) interpretation was aberrantly high (92%, see Table I). The NEWS is a tool developed by the Royal College of Physicians at the National Health System of England to detect and respond to clinical deterioration in adult patients and is a key element of patient safety and improving patient outcomes.

On the other hand, patients receiving oral HCQ 200-400 mg/day to treat SLE for approximately 7.5 years have not demonstrated prophylactic protection against COVID-19 (Mathian *et al*., 2020). This small, descriptive, observational, nonrandomized single trial with SLE patients infected with COVID-19 also reports that, with the exception of a higher rate of dyspnea, headache, and diarrhea, the signs and symptoms of COVID-19 were similar to those described for people without SLE. This indicates that clinical follow-up and updated standard SLE therapy prevent acute attacks of SLE in patients with COVID-19, but it does not prevent COVID-19 progression from the viral to the inflammatory phase, even with HCQ plasma concentrations within the therapeutic range. In addition, among the 17 patients, 14 used prednisolone, 7 used immunosuppressants (5, mycophenolate mofetil; 2, methotrexate), 6 patients used ACE inhibitors, and 5 used anticoagulants. Fifth-three percent received antibiotics, although bacterial infection has been detected in just one (Mathian *et al*., 2020). The continuous use of corticosteroids by these patients also

raises the discussion about the premature use of steroids to prevent the progression and worsening of COVID-19.

An observational, multicenter, nonrandomized, and controlled study in four French higher education hospitals, with patients aged 60 years, of which 72% were men, showed that HCQ did not reduce ICU admission or deaths until the 21st day after admission or ARDS in hospitalized patients with hypoxemic COVID-19 pneumonia (Mahévas *et al*., 2020). Starting the treatment 48 h after hospitalization was a differential of the study because attacking the infection early has been critical for reducing viral load and could have a clinical benefit if began before 12 days after the onset of symptoms (Cao *et al*., 2020). The average interval between the onset of symptoms and hospitalization was 7 days, much earlier than in the study of Tang *et al*. (2020), but the decision to treat or not treat patients with HCQ was based on local medical consensus and personal opinions of physicians. This subjective aspect and lack of unanimity for therapy was certainly a confusing factor. To avoid loss of monitoring due to transferences to another hospital, hospital-hospital contacts were produced to obtain outcomes. However, this study does not declare if there was loss to follow-up or how many patients dropped out. The clinical features of the patients were consistent with other descriptions, such as the predominance of men and patients with cardiovascular comorbidities and obesity (Million *et al*., 2020; Tang *et al*., 2020), and they did not receive steroidal or nonsteroidal anti-inflammatory drugs before transference to the ICU, especially because nonsteroidal anti-inflammatory drugs (such as antipyretics) for adults are contraindicated for COVID-19 patients in France. Nevertheless, 18 and 76% of the patients belonging to the HCQ group and 52% and 28% in the control group received azithromycin and amoxicillin plus clavulanic acid, respectively, an appropriate clinical supportive treatment in cases of secondary bacterial pneumonia.

In the United States, a cohort multicenter study was designed to assess records from the integrated medical centers of the Veterans Health Administration of the United States (Magagnoli *et al*., 2020). Since 91% of all US veterans are male, the findings were influenced by the demographic composition (Garg *et al*., 2020): men, most black, with an average ranging between 68 and 71 years for our groups. After adjusting for several relevant confounders, no benefit from HCQ groups with or without azithromycin was observed in relation to survival outcomes, the need for mechanical ventilation, or length of stay among hospitalized COVID-19 patients. Despite limitations such as lack of randomization typical for retrospective studies, a total of 19 confounding relevant factors were statistically adjusted, including comorbidities, medications, and clinical and laboratory changes. In this context, it is important to highlight the high percentage of patients with diabetes (with or without complications) (534/807 = 66%), nephropathies, chronic cardiovascular (346/807 = 43%) and pulmonary (175/807 = 21.7%) diseases and cancers (127/807 = 15.7%) in all groups, in addition to the presence of smokers (128/807 = 15.9%) and hyperlipidemic individuals (124/807 = 15.4%). Clinically, the oxygen saturation was below 94% in 33.6%, ALT was > 40 U/L in 24.8%, and D-dimer was > 1000 ng/mL in 22.9% of the patients. These laboratory values at baseline were significantly different among the treatment groups, with the HQC and HQC + azithromycin groups having more patients with elevated hepatic enzymes and inflammatory markers. All received support therapy, but the study did not report which drugs were part of such therapy. Of out 807 patients, 121 (15%) and 67 (8.3%) were taking angiotensin converting enzyme inhibitors and angiotensin II receptor blockers, respectively. Since additional ventilation was not required in the group that received HCQ, it suggests that mortality in this group can be attributed to the side effects effects of treatment or to the disfunction in vital nonrespiratory organ systems. Considering the applicability for the general population, the main limitation of this study lies in the fact that it was done with older hospitalized men, which makes it a challenge to extrapolate some results to women and younger hospitalized persons or to pediatric patients. Another borderline factor for extrapolation is the disproportionality of blacks, but this directly reflects higher rates of COVID-19-related hospitalization among the black population in the United States during the first wave of the disease. This became clear throughout the year and now it is known that race and ethnicity are risk factors that affect health in USA, since American Indian or Alaska Native, Black or African American and

Hispanic, or Latino persons have as much as 3.5, 2.8, and 3-fold higher probability to need hospitalization and are 2.4, 1.9, and 2.3 more susceptible to die of COVID-19, especially because race and ethnicity are risk markers for other underlying conditions that affect health, including socioeconomic status, access to health care, and exposure to the virus related to occupation, e.g., frontline, frontline and essential infrastrutcture workers (COVID-NET, 2020; CDC, 2021).

A large and controversial observational, nonrandomized, multiracial, multicenter and intercontinental clinical trial analyzed, for a period of 4 months, data from patients admitted to 671 hospitals on 6 continents. Based on the results obtained at that time using artificial intelligence technological tools, the authors stated that there were no clinical benefits of CQ and HCQ alone or in combination with a macrolide. Additionally, such combination schedules were associated with an increased risk of ventricular arrhythmias and hospital death in patients with COVID-19 (Mehra *et al*., 2020). With an average age of approximately 55 years for all groups, the majority of patients were white (probable due to the greater number of patients from the USA). Data were collected in urban and rural, academic or community hospitals and in for-profit and nonprofit hospitals, which provided great heterogeneity of patients and generated significant population representativeness of the results. The standardization of clinical and laboratory signals of COVID-19, following the WHO guidelines, allowed us to reduce biases for the confirmation of COVID-19. Based on the underlying comorbidities described in the electronic medical record of each patient or hospital, in addition to exposure to treatment, the highest risk of death was associated with age > 60 years, obesity, male sex, body mass index > 30 kg/m^2, black or Hispanic ethnicity, diabetes, hyperlipidemia, coronary artery disease, congestive heart failure, immunosuppression, history of arrhythmias, chronic obstructive pulmonary disease, cigarettes, and SPO_2 < 94%. On the other hand, relevant data showed that women, people who use ACE inhibitors and statins and those with quick sequential organ failure assessment scores (qSOFA) < 1 had superior hospital survival. qSOFA is a bedside prompt that may identify patients with suspected infection who are at greater risk for a poor outcome outside the intensive care unit (ICU). It is used to assess the severity of organ dysfunction in a potentially septic patient: pressure (systolic blood pressure ≤ 100 mmHg), respiratory rate (≥ 22 breaths/min), or alterations of the central nervous system (Glasgow coma scale < 15) (Marik, Taeb, 2017). Each component allocated one point. Therefore, a qSOFA score of ≥ 2 points indicates organ dysfunction/failure.

In these retrospective investigations, Mehra *et al*. (2020) also suggested that drugs that stabilize cardiovascular and endothelial function may improve the prognosis and that ACE inhibitors and statins will be pharmacological classes with cardioprotective properties for patients with COVID-19. However, the study did not determine whether the association of an increased risk of hospital death with the use of medication regimens is directly related to cardiovascular risk, and a complete analysis of the dose response of risks was not performed. A few days after publication and enquiries, the authors published a note stating that an internal audit would not guarantee the veracity of primary data sources, since the company responsible for collecting and processing the raw data did not go through all the information required for a careful and independent analysis. Therefore, this article was retracted (Mehra *et al*., 2020). However, most of the findings detailed here were demonstrated in further controlled prospective clinical trials.

With prophylactic purposes, a prospective, multicenter, double-blind, placebo-randomized clinical trial was carried out with patients aged 40 years using 600 to 800 mg of HCQ sulfate daily for 5 days. Enrolled patients exhibited a low prevalence (< 30%) of chronic diseases (hypertension, diabetes and asthma) within four days after contact with sources of domestic or occupational contamination (Boulware *et al*., 2020). Considering that the risk for developing severe COVID-19 is related to age and coexisting pathological conditions, this study evaluated the risk of acquiring symptomatic infection, since this risk proved to be the same among adults, regardless of age. A question of great concern consisted that the majority (66.4%) of the participants were health professionals, which implies a high risk of contagion for 2/3 of the sample, and a high percentage of them (~ 60%) did not report using any

personal protective equipment (PPE) during exposure to COVID-19. For health professionals, exposure was predominantly associated with sick patients (76.7%) or infected coworkers (19.6%). Patients were followed-up until the 14th day, which allowed to detect symptomatic volunteers. In this case, they basically showed similar symptoms as previously described (Huang *et al*., 2020; Qiu *et al*., 2020; Yang *et al*., 2020; Zheng *et al*., 2020), including predominance of cough, fever, increased breathing, fatigue, sore throat, myalgia, and anosmia. In addition, the survey included up to 6 weeks of follow-up to detect any illness or hospitalization. Treatment adherence was lower in the HCQ group, probably because 40.1% (140 of 349) reported nausea, loose stools and abdominal discomfort as the most common side effects on the fifth day of treatment. As stated by the authors, cardiac arrhythmias or serious intervention-related adverse reactions were not detected, but it is important to highlight: the study does not report cardiac exams, such as electrocardiograms.

An international multicenter study was conducted with symptomatic, nonhospitalized adults with laboratory-confirmed COVID-19 or probable COVID-19 and high-risk exposure within 4 days of symptom onset (Skipper *et al*., 2020). Doses based on pharmacokinetic parameters to achieve and maintain HCQ sulfate concentrations above the estimated half-maximal effective concentration (EC_{50}) for SARS-CoV-2 (Al-Kofahi *et al*., 2020) revealed that HCQ was not able to reduce symptom severity. Only 12% in average improvement was detected, a modest clinical outcome when compared to other antiviral drugs against influenza, for example (Nicholson *et al*., 2000; Treanor *et al*., 2000). In addition, a difference in symptoms was not observed when the comparisons were limited to fever, cough, or breath changes at day 14 (16% for HCQ vs. 22% for placebo). An extra discovery involves the use of zinc or vitamin C plus hydroxychloroquine: they did not improve symptoms over the use of HCQ alone (Skipper *et al*., 2020). An essential methodology question must be highlighted: the double-blind placebo-controlled trial with a parallel design was categorically effective to reveal that adverse effects markedly differed between groups despite HCQ had not substantially substantially reduced symptom severity or prevalence over time in nonhospitalized persons with early COVID-19.

Skipper *et al*. (2020) designed a randomized double-blind placebo-controlled trial study with outpatient adults and concluded that self-reported use of zinc or vitamin C in addition to HCQ did not decrease symptom prevalence or severity over the 14-day study period. Between groups, there were no significant differences in age, sex, weight, comorbidities, duration, type or number of symptoms ($p > 0.05$), but persons identified as Black or African American were underrepresented (3%). Participants were younger when compared with other investigations, since 77% of them were under 50 years old and had few comorbid conditions, a disadvantage when generalization of results is wished. A remarkable limitation includes the absence of confirmed SARS-CoV-2 infection in all patients, although they met international and U.S. COVID-19 case definitions. As described in Table 1, gastrointestinal symptoms were the most commonly reported adverse effects in 31% (66 of 212) of participants, who reported upset stomach or nausea, and 24% (50 of 212) cited abdominal pain, diarrhea, or vomiting. The prevalence of such adverse effects decreased markedly after day 5 (last day for HCQ).

An American comparative, retrospective, nonblinded cohort study with patients presenting a median age of 64 years, 51% male, 56% African American, and accompanied for 28.5 days (IQR 3–53) indicated that HCQ may have a role in reducing COVID-19 mortality (Arshad *et al*. 2020). The majority of patients (52%, $n = 1.250$) had a body mass index ≥ 30, hypertension (65.4%), chronic kidney disease (43.3%), and diabetes mellitus (37.6%). A maximum SOFA score > 1 (2-5) was found for 73.6%, and O_2 saturation on admission of 90% was seen in all groups. An important reason for confounding considered a supporter therapy with corticosteroids (methylprednisolone and/or prednisone) and anti-IL-6 tocilizumab provided for 68% and 4.5% of patients, respectively. They monitored patients by telemetry and serial QTc checks; *torsades de pointes* were not documented, and even patients with severe COVID-19 and QTc > 500 ms (an elevated cardiac risk) were treated with HCQ and/or azithromycin. Nevertheless, cardiac

arrest (with a mean QTc interval from the last ECG reading 471 ms) was the primary cause of mortality for 18 out of 460 deaths. The strengths of this study include the inclusion of a multiracial composition of volunteers and control of confounding factors, including clinical characteristics, but the results are not confirmed by previous retrospective investigations with patients under similar clinical conditions (Geleris *et al*., 2020; Rosenberg *et al*., 2020). Such results should be interpreted with some caution and should not be applied to patients treated outside of hospital settings (Arshad *et al*. 2020).

The outcomes obtained by Arshad *et al.* (2020) were sharply criticized by Varisco *et al.* (2020) and Atkinson (2020) because i) the use of corticosteroids was common in patients who received HCQ with or without azithromycin (79% and 74%, respectively), but such adjunctive therapy was not disclosed, which strongly indicates that an initial clinical decline in HCQ arms was masked by corticosteroids; ii) dichotomizing age was alarming, given the established association between COVID-19 mortality and age; iii) Arshad *et al.* (2020) did not include azithromycin as a covariate in the propensity scored model (Varisco *et al*., 2020); iv) the volunteers were consciously allocated to the treatment protocols based on their basic pathological conditions, a bias not adjusted; v) they found COVID-19 increased risk of death due to cardiovascular comorbidities at 6 %, BMI of 30 or higher reduces the patient's risk of death by 22%, and being white increases it by 74%; vi) they make extensive adjustments to the death rate if the patient receives ventilator support, masking the real necessity of the HCQ group (Atkinson, 2020).

A well-designed multicenter randomized (1:1), open-label controlled trial in Catalonia (Spain) was mainly made up of healthcare workers [254 (86.7% of 293)] and did not find virologic or clinical benefit of HCQ in ambulatorial patients with mild COVID-19 if HCQ sulfate was initiated within five days from symptom onset. The quantification of the viral load in the upper URT provides strong evidence on the capacity of the treatment to affect the pathogen burden. Moreover, this treatment regimen neither reduced the risk of hospitalization nor decreased the time to complete resolution of symptoms (Mitjá *et al*., 2020). As expected, the volunteers were younger (mean age of 41.6 years), which explains the absence of deaths and the necessity of mechanical ventilation. Similarities in age, comorbidities, frequency of symptoms, and nasopharyngeal viral load were maintained from baseline to the 28th day of monitoring, and fever, cough, and sudden olfactory loss were the most common symptoms. The most frequent treatment-related adverse effects among participants receiving HCQ were gastrointestinal (e.g., diarrhea, nausea, metallic taste, and abdominal pain) and nervous system disorders (e.g., drowsiness and headache).

In Brazil, a large interventionist trial named "Coalition Covid-19 Brazil I", characterized by a multicenter, randomized (1:1:1), open-label, and controlled study, was performed in 55 hospitals from all Brazilian regions (Cavalcanti *et al*., 2020). Most patients (584/665 = 87.8%) underwent randomization within 10 days after symptom onset, and almost half of them (42%) were receiving supplemental oxygen at baseline. It revealed that hospitalized patients with mild-to-moderate COVID-19 did not present clinical benefits after a 7-day course of HCQ and/or azithromycin when compared with the standard care. After 15 days, 68%, 64% and 69% of control, HCQ, and HCQ + azithromycin patients, respectively, were comparably discharged, and no difference in the return of routine activities was observed. There was no medical consensus for standard care, which certainly had strong influence on the outcomes but confounding factors were not statistically assessed: > 50% of the patients received ceftriaxone, > 20% were exposed to oseltamivir or antibiotics not mentioned, and approximately 20% were also treated with corticosteroids.

On the other hand, analyses of patients with rheumatic disease receiving long-term HCQ considering serum and plasma, frozen serum samples from a pediatric systemic lupus erythematosus trial, and *in silico* simulated concentrations using a pharmacokinetic model during pregnancy found that most patients, including children and pregnant/nonpregnant adults, do not achieve adequate serumserum concentrations to inhibit SARS-CoV-2 *in vitro*, especially at standard dosages < 400 mg/day (400 mg orally every 24 h for 5 days is the standard-of-care dosing for most patients with rheumatic diseases) (Balevic *et al*., 2020). This showed only one-tenth or less exposure than that required for in vitro for *in vitro* viral inhibition,

and it would not achieve median target exposures reported by Gautret *et al.* (2020a) and Yao *et al.* (2020), but long-lasting exposure exerts a suppressive effect on interleukin 6 (IL-6) levels, possibly by affecting macrophage/monocyte release as opposed to lymphocyte-released cytokines (Wallace *et al*., 1994; Jang *et al*., 2006).

Drugs that decrease the viral index may be inappropriate when administered in the inflammatory phase during the called 'cytokine storm', generally on the second to third week after the manifestation of COVID-19 symptoms. Despite the immunomodulatory properties of HCQ, including the control of proinflammatory cytokines, such as IL-1, IL-2, IL-6, and TNFα, and inhibition of important reactions for the innate immune response, such as the endolysosomal physiological process of Toll-like receptors (Schrezenmeier, Dörner, 2020; Ferreira *et al*., 2021), treatment with HCQ has not shown clinical efficacy against the advanced inflammatory phase of the disease, mainly for hospitalized patients (Borba *et al*., 2020; Cao *et al*., 2020; Geleris *et al*., 2020; Mahévas *et al*., 2020; Magagnoli *et al*., 2020; Molina *et al*., 2020; Rosenberg *et al*., 2020) or mild-to-moderate COVID-19 (Skipper *et al*., 2020; Cavalcanti *et al*., 2020; Mitjà *et al*., 2020; Tang *et al*., 2020). Moreover, it seems unable to prevent COVID-19 infection or the progression of COVID-19 in patients who have been taken antimalarials chronically to treat autoimmune diseases (Balevic *et al*., 2020; Mathian *et al*., 2020). In 2018, investigations paradoxically showed that prophylactic treatment with CQ enhances Chikungunya virus replication in a nonhuman primate model, probably due to the downregulation of cellular and humoral immune system components (Roques *et al*., 2018), with similar results for patients.

As described in different studies, nearly 80% of patients with COVID-19 confirmed by RT-PCR tests are asymptomatic; 20% are symptomatic, of which 81% will have mild or moderate disease, 14% will have severe pneumonia and will need hospitalization, and approximately 5% will be severely affected and will require intensive care (Chen *et al*., 2020; Huang *et al*., 2020; Wu, McGoogan, 2020). While the global lethality of COVID-19 does not exceed 3%, it is appropriate to state that 97% of symptomatic patients will fully recover without antiviral treatment. Therefore, if a protocol was implemented for free prescription and universal use of CQ or HCQ (with or without azithromycin) for all symptomatic patients, the real effectiveness would be significant if the cure rate reached values greater than 97% (BSI, 2020). Therefore, even during the first wave, many results clearly indicated the use of CQ and HCQ with or without macrolides in clinical trials only, preferably randomized, double- or triple-blind trials, with control/placebo, and multicentric arms involving public and private institutions to represent, understand and cover regional, ethnic and gender differences, and with a representative number of volunteers clinically classified by disease severity.

Robust qualitative and quantitative studies have suggested caution when using CQ or HCQ plus azithromycin/clarithromycin or fluoroquinolones (ciprofloxacin, norfloxacin) since these drugs may increase the risk of cardiac complications due to synergistic effects for prolonging the QT interval and the onset arrhythmias and heart block (Borba *et al*., 2020; Mahévas *et al*., 2020; Rosenberg *et al*., 2020). CQ, HCQ, amodiaquine, and other aminoquinolines in clinical use clearly cause blurred vision, metallic taste, upset stomach or nausea, abdominal pain, diarrhea, and vomiting (Braga, Valle, 2007; Srinivasa, Tosounidou, Gordon, 2017; Mitjá *et al*., 2020; Skipper *et al*., 2020; Tang *et al*., 2020). Specifically, at loading doses > 800 mg, nervous system disorders, including drowsiness, agitation, insomnia, confusion, headache, hallucinations, paranoia, depression, catatonia, and suicide intention, were registered (Mitjá *et al*., 2020; Spanish Agency of Drug and Health Products, 2020); hypoglycemia due to the action of aminoquinolines (important in diabetics) (Schrezenmeier, Dörner, 2020), and clinical condition aggravation of patients with cardiovascular and kidney diseases (Borba *et al*., 2020; Rosenberg *et al*., 2020). Retinopathy is very common when aminoquinolines are chronically used (Schrezenmeier, Dörner, 2020), but behavior disorders may appear at any age, during acute or chronic use, and in patients without a history of psychiatric illness (Spanish Agency of Drug and Health Products, 2020).

Azithromycin and clarithromycin are the most commonly used antimicrobial macrolides and have

a broad spectrum of action against common bacteria in respiratory, enteric and genitourinary infections (Parnham *et al*., 2014). Azithromycin also has anti-inflammatory activity by inhibiting the production of GM-CSF and IL-β1 (Bosnar *et al*., 2009) and the enzyme phospholipase A_2 (PLA_2) (Banjanac *et al*., 2012). Nevertheless, there are insufficient data demonstrating the benefits of antibiotic therapy in patients with COVID-19 without bacterial infection. Indeed, there is no scientific evidence indicating such clinical protocols and large-scale usage of antibiotics may favor the development of microbial resistance, especially when they are administered to immunosuppressed patients due to the early use of steroids (Parnham *et al*., 2014; Tang *et al*., 2019; Brasil, 2020; Geleris *et al*., 2020; Molina *et al*., 2020; Million *et al*., 2020).

Viral conversion (negative results detected by RT-PCR), a basic finding to confirm cure, was not found in most clinical trials published until July 2020 after treatment with HCQ, and mortality is probably associated with CQ/HCQ with or without azithromycin, probably due to increases in the QTc interval (Bessière *et al*., 2020; Borba *et al*., 2020; Cavalcanti *et al*., 2020; Mercuro *et al*., 2020; Singh *et al*., 2020). This may be explained, at least in part, because extracellular lung concentrations do not reach effective concentrations. Indeed, computational simulations had already suggested in April 2020 that higher doses for treatment and prophylactic purposes would be mandatory (higher doses than those recommended for malaria) (Al-Kofahi *et al*., 2020). Hence, 200 mg three times daily is inappropriate to reach a supposed target blood level of 1-2 mg/L (Perinel *et al*., 2020), a likely interval estimated for EC_{50} blood levels to present virustatic/virucidal effects for CQ and HCQ (Wang *et al*., 2020; Yao *et al*., 2020). These findings were consistent with the > 20-fold lower *in vitro* EC_{50} for malaria compared with SARS-CoV-2 (Al-Kofahi *et al*., 2020), strongly indicating that treatment with CQ or HCQ has low potential for *in vivo* activity at standard dosing regimens (Fan *et al*., 2020). Furthermore, findings indicate that a concentration of 2 mg/L should not be exceeded to avoid ocular toxicity (Perinel *et al*., 2020), and physiological changes in infused, ventilated patients with multiple organ failure may modify HCQ pharmacokinetic parameters (Tukacs, 2018; Perinel *et al*., 2020).

On June 05, 2020, a large multicenter, multinational and randomized trial with several clinical arms (Randomized Evaluation of COVID-19 thERapY - RECOVERY) was partially cancelled for ethical reasons, since the HCQ arm displayed a lack of clinical effectiveness. In addition to HCQ and azithromycin, this study included lopinavir-ritonavir, dexamethasone, tocilizumab, and plasma from convalescent patients. This decision was based on partial results (80% of them), whose 1542 patients receiving HCQ, when compared to 3132 who received supportive treatment (control group), did not show a significant reduction in mortality, need for mechanical ventilation, or days of hospitalization after 28 days of follow-up (RECOVERY, 2020). Some months later, they concluded that HCQ was not superior to usual care and did not improve survival among patients hospitalized with COVID-19 infection. Additionally, patients receiving HCQ showed a longer duration of hospitalization than those receiving usual care (16 days vs. 13 days) and a worse probability of discharge alive within 28 days (59.6% vs. 62.9%) (The RECOVERY Collaborative Group, 2020).

By June 15, 2020, the FDA revoked the emergency authorization for CQ and HCQ to treat COVID-19 in hospitalized patients because large randomized clinical trials with hospitalized patients showed no benefit for decreasing the likelihood of death or speeding recovery (FDA, 2020). Corroborating these decisions, the WHO also discontinued the trial's HCQ and lopinavir/ritonavir arms in the Solidarity Trial because these drugs produce little or no reduction in mortality in hospitalized COVID-19 patients when compared to standard care (WHO, 2020a). Next, they published a complete report showing that remdesivir, HCQ, lopinavir, and interferon regimens did not reduce mortality overall and had no effects on the initiation of ventilation or duration of hospitalization (WHO Solidarity Trial Consortium *et al*., 2021). Therefore, it became clear that thousands of patients have received HCQ and CQ outside of clinical trials without evidence of beneficial effects.

Precisely about the Brazilian situation, surveys have demonstrated that Brazil performed approximately 11.3 RT-PCR tests per 100,000 inhabitants (Resende, 2021). This low coverage has been associated with the

off-label use of drugs (Borba *et al.*, 2020; Cavalcanti *et al.*, 2020; Cardoso, Fernandes, Santos, 2021) and vitamins (Skipper *et al.*, 2020) without scientific evidence, late adoption of sanitary emergencies (Aquino *et al.*, 2020), noncoordinated national/regional interventions, flow disruption of federal financial transfers and essential supplies, absence of national rules based on scientific decisions (Ferigato *et al.*, 2020), late governmental financial support for social isolation and purposeful dissemination of fake news. These issues and the political polarization certainty subsidized anti-vaccination movements, the propagation of SARS-CoV-2 (Guimarães *et al.*, 2020; Toueg, 2021), the encouragement to ignore social isolation or wear face masks, stimulated the emergence of new variants and strains (WHO, 2020b; Sabino *et al.*, 2021), and contributed to the depletion of medications for effective intubation (Bergamo, 2021) and for the collapse of the Brazilian Public Health System (*Sistema Único de Saúde*, SUS) (Ranzani *et al.*, 2021).

Since the general public has given much attention to the COVID-19 pandemic and the search for specific treatments, weak and early evidence about CQ or HCQ is continuously discussed in TV channels and social networks despite the unfavorable results continuously displayed by randomized clinical trials (Axfors *et al.*, 2021). Inside the storm of misinformation, key preclinical and clinical steps for the development and use of pharmaceutical products were relativized during the first wave of COVID-19 to save time, material, and human resources for drug repurposing aiming to reduce the dissemination of SARS-CoV-2. Therefore, not only pharmacodynamic discoveries but also essential data about pharmacokinetic profiles, therapeutic windows, and safety were put on the back burner. However, even for well-described drugs, extrapolation of preclinical experimental pharmacological results to humans is inexact (Clark, Steger-Hartmann, 2018) because of the systemic complexity of metazoan molecular pathways.

CONCLUSION AND FINAL CONSIDERATIONS

Chloroquine and hydroxychloroquine (with or without macrolides) did not demonstrate clinical advantages that justified their inclusion in therapeutic regimens of free prescription for the treatment of patients infected with COVID-19 or with prophylactic purposes, as suggested by some countries and authorities, including in Brazil, during the first wave. Most trials did not include children or pregnant/breastfeeding women and had a low capacity to understand the role of comorbid conditions and ethnicity as risk factors, which undoubtedly makes extrapolation difficult for the general population.

Although CQ and HCQ have received extraordinary attention as potential therapeutic agents after some inconclusive preclinical investigations and clinical trials, around August 2020, robust data had already indicated that pharmacological effects of CQ, HCQ, and macrolides as anti-SARS-CoV-2 molecules were limited to *in vitro* conditions and were largely based on retrospective clinical studies with low methodological quality and weak internal validity, which made evidence superficial for decision-making. Up to that point, most randomized and nonrandomized clinical trials did not reveal beneficial effects of CQ or HCQ with or without macrolides to reduce lethality, rate of intubation, days of hospitalization, respiratory support/mechanical ventilation requirements, duration, type and number of symptoms, and death and were unsuccessful in increasing virus elimination and/or days alive in hospitalized or ambulatory patients with COVID-19. Furthermore, many studies have demonstrated that side effects are more common in CQ- or HCQ-treated individuals, mainly in hospitalized patients, including cardiovascular and gastrointestinal alterations. Almost three years after the World Health Organization declared COVID-19 a public health emergency of international concern, the reduction of symptom severity and hospitalizations, adoption of sanitary emergencies, social isolation, and, more recently, preventive vaccination campaigns have been the key public mitigation strategies to overcome the SARS-CoV-2 pandemic since no specific and effective antiviral drugs have been discovered to date.

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CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could influence the work and outcomes reported in this paper.

AUTHORS' CONTRIBUTIONS

Paulo Michel Pinheiro Ferreira: Investigation, methodology, supervision, validation, visualization, writing-original draft and writing-review & editing; Rayran Walter Ramos de Sousa: Conceptualization, formal analysis, methodology, visualization and editing; Dalton Dittz, João Marcelo de Castro e Sousa and Francisco Leonardo Torres-Leal: Conceptualization, validation and visualization. Daniel Pereira Bezerra: Data curation, formal analysis, methodology, visualization and validation. All authors have read and agree with the published version of the manuscript.

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