Short Communication

Hydroxychloroquine against COVID-19: A critical appraisal of the existing evidence

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Abstract

Hydroxychloroquine (HCQ) has sparked much interest in the therapeutics of the Coronavirus Disease 2019 (COVID-19) pandemic. Its antiviral properties have been studied for years; regarding the Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2), it has been shown that HCQ may act at multiple levels. These extend from the initial attachment of the virus to the respiratory epithelium to the inhibition of its replication by the alkalinisation of the phagolysosome's microenvironment and the post-translational modification of certain viral proteins. Preliminary clinical evidence from China and France showed significant virological and clinical benefit in HCQ-treated patients, while other studies, mostly including critically ill patients, did not show favorable results. In this review, we critically appraise the existing evidence on HCQ against SARS-CoV-2 with particular emphasis on its protective and therapeutic role. Safety concerns that are relevant to the short-term HCQ use are also discussed. In the context of the rapid evolution of the COVID-19 pandemic that strains the health care systems worldwide and considering limited population-wide testing rates in most of the vulnerable countries, early empiric short-term administration of HCQ in symptomatic individuals, may be a promising, safe and low-cost strategy.

Keywords: Hydroxychloroquine, SARS-CoV-2, COVID-19, mechanisms of action, protection, efficacy

Introduction

In late December 2019, a cluster of patients with pneumonia-like disease of unknown origin were admitted in several hospitals of the city of Wuhan, China (1). This new clinical entity was quickly attributed to a novel virus of the Corona family (*Coronaviridae*), subsequently named Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2) by the World Health Organization (WHO) on February 11, 2020 (2). The resulting disease was named COVID-19 (Coronavirus Disease 2019). COVID-19 spread globally, mostly through commercial air travel, and the WHO declared a pandemic on March 11, 2020 (3). As of this writing, COVID-19 has affected 212 countries and territories worldwide with more than 4 million confirmed cases and more than 280,000 deaths (4). Given these unprecedented circumstances, a massive scientific effort was launched to elucidate the modes of transmission, pathogenesis, prevention and treatment of the new disease. A search in the PubMed database with the Medical Subject Headings (MeSH) terms ("SARS-CoV-2" OR "COVID-19") yielded 7686 articles from January 1 to May 1, 2020 (5).

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Regarding treatment, early observations from China reported that chloroquine, an antimalarial agent, was efficacious in improving lung imaging findings of pneumonia, promoting a virus-negative conversion and shortening the symptomatic phase of the disease (6). As a result, multiple randomized clinical trials have been launched in China to investigate the role of chloroquine and hydroxychloroquine (HCQ) in COVID-19 since early February 2020. The rational for these studies was based on an *in vitro* experiment that showed chloroquine to inhibit the replication of SARS-CoV-2 in Vero E6 cells culture by approximately 80% (7). HCQ, a less toxic derivative of chloroquine, was shown to exert similar results whereas it was 7-8 times more potent than chloroquine based on the half maximal effective concentration (EC_{50}) (8, 9). Shortly after, a small clinical trial from Marseilles, France on 36 patients with COVID-19 reported that HCQ was associated with a significant reduction of the viral load after six days of treatment; addition of azithromycin (AZI) in a subgroup of patients led to a 100% virological cure versus 57.1% in the HCQ only arm (10).

These outstanding results brought much hope to the scientific community and the combination of HCQ and AZI was characterized as "one of the biggest game changers in the history of medicine." The adoption of this claim by state leaders led to a worldwide run on these drugs, with pharmacies in North America

reporting shortages of HCQ within a few days (11). From a scientific perspective, 180 clinical trials investigating the role of chloroquine or HCQ in the management of COVID-19 have been registered as of May 1, 2020 (12). In this report, we review the antiviral properties of HCQ along with the currently available data on the treatment of COVID-19 patients with this agent. The safety of HCQ along with a rationale on its use is also analyzed.

Antiviral properties and mechanism of action of HCQ

The *in vitro* antiviral activity of chloroquine and HCQ has been identified since the 1960s (reviewed in 13). Many different viruses, including the 2003 SARS Coronavirus were shown to have reduced replication in cell cultures treated with chloroquine (14). In animal models, there has been evidence of clinical activity, particularly against certain strains of coronaviruses, enteroviruses, as well as the Zika and influenza A H5N1 viruses (13). However, there was no clinical benefit in the prevention of human influenza (15) or the treatment of human Dengue fever (16).

The mechanism of action of chloroquine (and HCQ) in viral infections is believed to be multifactorial. Against SARS-CoV-2, it has been postulated that HCQ may act at multiple levels that extend from the initial attachment of the virus to the respiratory epithelium to the inhibition of its replication by the alkalinisation of the phagolysosome's microenvironment andthe post-translational modification of certain viral proteins (17). Regarding the former, it has been shown that HCQ is a potent blocker of the SARS-CoV-2 Spike Glycoprotein (S protein)-ganglioside interaction that occurs in the initial steps of the viral replication cycle (i.e. attachment to the surface of the respiratory cells, which is mediated by the S protein of the virus) (18). Moreover, chloroquine interferes with the terminal glycosylation of angiotensin-converting enzyme-2 (ACE-2), which acts as a plasma membrane receptor for the SARS-CoV-2 (19). The next steps of the viral replication take place in the lysosome, where the acidic pH results in the fusion of the viral and endosomal membranes and the release of viral genome (and possibly enzymes that are essential for further

replication) in the cytosol (17). HCQ (and chloroquine) are able to inhibit this phase via the alkalinisation of the lysosomal pH (through the inhibition of lysosomal enzymes), a mechanism that has been confirmed in other enveloped viruses, such as the Dengue and Chikungunya viruses (17). Chloroquine-mediated post-translational modifications of certain viral proteins, mainly proteases and glycosyltransferases, have also been proven for the Human Immunodeficiency Virus (HIV), the Dengue and the Herpes Simplex 1 viruses (17). Chloroquine (and HCQ) also exerts immunomodulatory effects that produce an anti-inflammatory response (down-regulation of IL-1β, IL-6, TNF-α and several interferons) (20).

Moreover, several experimental studies suggested that during coronaviruses cell trafficking and replication, the endo-lysosomal (endocytic) pathway is interconnected and share complementary roles with autophagy, an intracellular degradation system aiming to maintain cellular homeostasis in response to metabolic stress and infection (21-23). Furthermore, induction of autophagy is tightly associated with neutrophil extracellular traps (NETs) formation, which might be implicated in the overwhelming inflammatory host response that characterizes severe COVID-19 (24-26). HCQ constitutes a well-defined inhibitor of the autophagic machinery impairing the autophagosome-lysosome fusion (22, 27, 28). However, the exact role of autophagy in COVID-19 remains to be elucidated (21, 22). Figure 1 summarizes the proposed actions of HCQ against COVID-19.

Is HCQ protective against SARS-CoV-2?

The interest in the antiviral properties of chloroquine and HCQ was fueled by the reproduction and wide dissemination of favorable results through the social media (29). Consequently, shortages of HCQ supplies were reported within a few days, while there was evidence that individuals might have started taking HCQ regularly to avoid contracting the new virus.
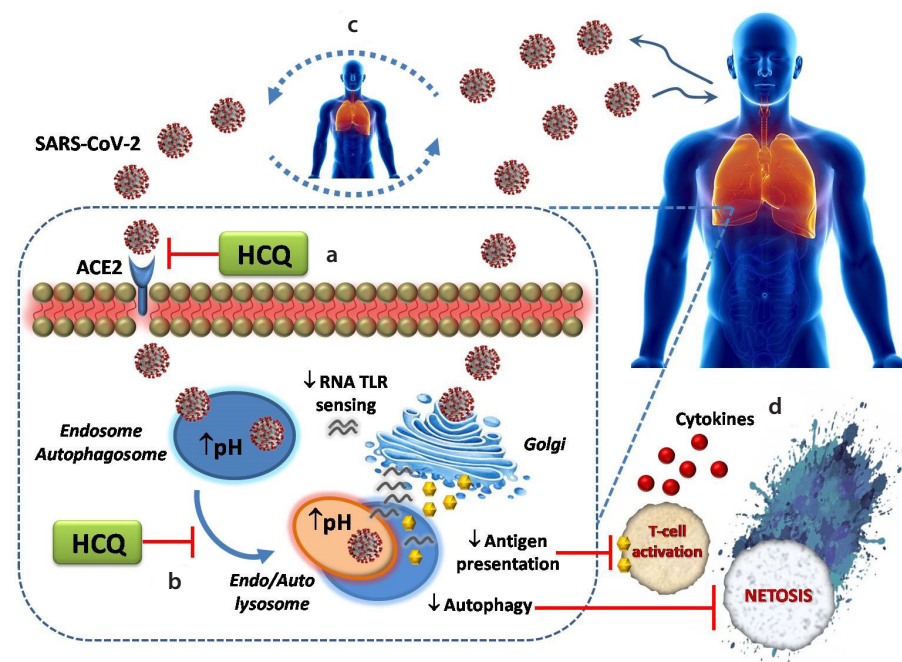

Figure 1. a-d. Suggested actions of HCQ against SARS-CoV-2 in COVID-19 disease. Extracellularly, HCQ interferes with the entry of virus in host cell by hindering the binding of virus on its membrane receptor ACE2 (a). Intracellularly, HCQ inhibits the replication rate of virus by increasing pH in acid compartments of the endo/auto-lysosomal trafficking system and blocking endosome/autophagosome-lysosome fusion. This prevents the release of viral RNA (grey jagged lines) and proteins (yellow polygons) and their assembly in the Golgi complex in order new mature virions be generated and released extracellularly via the secretory pathway (b). Ultimately, HCQ could reduce the rate of new infections in community by disrupting the vicious cycle of SARS-CoV-2 transmission (c). Furthermore, HCQ may exert anti-inflammatory actions. Diminished viral RNA sensing by TLRs, as well as reduced presentation of viral epitopes in T-cells attenuate proinflammatory cytokine production, whereas in neutrophils HCQ inhibits autophagy-mediated NET formation (NETosis) (d).
HCQ: hydroxychloroquine; ACE2: angiotensin-converting enzyme 2; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; NET: neutrophil extracellular traps.

Although based on very preliminary data, it appears that rheumatic patients on prolonged HCQ treatment have become infected with SARS-CoV-2 and may exert similar outcomes to non-HCQ treated patients. This information comes from the COVID-19 Global Rheumatology Alliance (https://rheum-covid.org), where rheumatologists from anywhere in the world can upload de-identified demographic, clinical and therapeutic data (along with the outcome of the infection) of patients with systemic autoimmune diseases who were diagnosed

with COVID-19 (30). Out of 777 patients (28.1% with rheumatoid arthritis, 14.9% with seronegative spondylarthritides, 13.5% with systemic lupus erythematosus and the rest with other systemic autoimmune diseases), 188 (24.2%) were on regular antimalarial (chloroquine or HCQ) treatment (31). Of note, many of these 777 patients were on treatment with glucocorticoids (30.8%) and other conventional (65.6%) and biologic disease-modifying anti-rheumatic drugs (DMARDs) (38.4%). Of the first 86 patients on HCQ, 53% and 19% were concomitantly taking conventional and biologic DMARDs respectively whereas 39% were on no other immunomodulating medication. The case fatality rate was 8.75% (68/777), significantly more than the global average of 6.9% (4). Another 275 patients (35.4%) were considered cured, while the outcome of the COVID-19 was unresolved or unknown for the remaining patients (31).

A recent small study from France on 17 systemic lupus erythematosus (SLE) patients with prolonged HCQ treatment (median of 7.5 years) and clinically quiescent disease who were diagnosed with COVID-19 reported that 13/17 (76.5%) developed pneumonia and 14/17 (82.4%) were hospitalized; half of them were admitted to an Intensive Care Unit (ICU) (32). Twelve (out of 17) and seven patients were concomitantly taking glucocorticoids and immunosuppressive medications respectively. At the time of publication, two of them (14%) had died, seven were still at the hospital and five were discharged (from the 14 hospitalized patients).

On the other hand, Izoulet (33) reported that the COVID-19 related deaths in the general population from the day of the third documented death for each country and for 10 days forward were inversely associated with the wide use of antimalarial drugs. He examined two groups of 16 countries each that were selected from the top 60 countries in confirmed cases as of April 13, 2020. The increase in the number of deaths was significantly less in the 16 countries that had implemented strategies for wide use of antimalarials; on the contrary, the growth was exponential in countries where antimalarials were not widely used. The difference remained statistically significant even after removal of the outliers. These findings were based on data from the early phase of the epidemic. By using an Auto Regressive Integrated Moving Average forecast model, he projected that the death curve will be stabilized for the "wide antimalarial users" whereas there will be a significant acceleration of death rates for "non-users" (33).

Based on the aforementioned preliminary data, it can be concluded that HCQ does not confer absolute protection against infection with SARS-CoV-2. However, there is a possibility that HCQ may reduce the rate of new infections, particularly in high-risk groups such as health care workers and the close contacts of infected individuals. Several clinical trials have been launched towards this direction; at least 61 of the trials that have been registered in "clinicaltrials.gov" up to May 1, 2020 are primarily focused on examining the benefit (or not) of HCQ in the pre- and post-exposure phase in high-risk individuals.

Is HCQ effective against SARS-CoV-2?

The first evidence that chloroquine is associated with better clinical outcomes in COVID-19 were published from China (6). However, that was only a commentary stating "…results from more than 100 patients have demonstrated that chloroquine phosphate is superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus-negative conversion, and shortening the disease course…" without further details (6). Subsequently, Gautret et al. (10) published the results of an open-label, non-randomized clinical trial of 36 patients, 20 receiving HCQ (600 mg/day for 10 days) and 16 controls. Most of these patients (approximately 80%) were mildly symptomatic or experienced symptoms limited in the upper respiratory tract. The authors concluded that 70% of the HCQ-treated patients were virologically cured (negative reverse transcription-polymerase chain reaction, RT-PCR, on nasopharyngeal swab) by day 6 compared to only 12.5% of the control group. Of note, six patients who were concomitantly treated with AZI (500 mg on day 1 and 250 mg on days 2-5) were all virologically cured by day 6. This article sparked severe criticism, particularly with regards to certain methodological flaws, lack of clinical outcomes and a subgroup of six patients who were excluded, and they eventually demonstrated unfavorable outcomes (three were admitted to the ICU and one died) (29).

A further report from the Marseilles centre on 80 COVID-19 patients who were treated with the combination of HCQ and AZI concluded that 93% of the patients had a negative RT-PCR by day 8 and viral cultures were negative by day 5 in 97.5% of the patients (34). Most patients had an initially mild-to-moderate disease (National Early Warning Score [NEWS] 0-4 in 92%) and about two thirds of them had radiological (based on computed tomography, CT) evidence of pneumonia. One 86y old patient had died and another 74y old patient was

in the ICU at the time of publication. All other patients recovered; the mean length of stay in the hospital was 4.6 days (34).

On April 20, the same investigators reported on 1061 new COVID-19 patients (not included in the previous reports) treated with the combination of HCQ and AZI in their centre (35). All patients were treated for at least three days and were followed for at least nine days. Their mean age was 43.6 years (46.4% males) and the vast majority had mild-to-moderate disease (NEWS 0-4 in 95% of the cases). Pneumonia (based on chest CT) was diagnosed in 469/714 patients (mild or moderate in 96.6% of them). A good clinical outcome, defined as no death, no transfer to the ICU, no prolonged hospitalization (less than 10 days) and no viral persistence beyond 10 days was observed in 973 patients (91.7%). Another 47 patients (4.4%) had a poor virological outcome but the vast majority of them recovered clinically; the initial virological load was significantly higher in these individuals. Patients who had a poor clinical outcome (n=46, 4.3%) were older (mean age 69 years), had a higher NEWS at presentation and more co-morbidities. Thirty-three of these patients were cured by the time of publication; cumulatively, 1048/1061 (98.7%) patients were deemed cured. Ten patients (0.9%) were transferred to the ICU and eight succumbed to COVID-19; the latter were older (median age 79 years), had certain co-morbidities and higher NEWS at presentation (35). At the time of publication, the overall case fatality rate for patients treated with HCQ+AZI for more than three days (including the patients from the previous reports) was 1.3% (16/1248), significantly lower than the 47/720 (6.3%) that was documented in a nearby centre where patients were treated with other treatment regimens. However, there was no control group neither information about the patients who were treated with other treatments or HCQ+AZI for less than three days. In their website, these investigators now report that 3248 COVID-19 patients have been treated in their centre with the HCQ plus AZI combination; 17 patients with more than three days of treatment have died up to May 8, 2020 for a case fatality rate of 0.52% (36).

A small randomized clinical trial (RCT) from China on 62 patients (31 on HCQ 400 mg/day and 31 controls) reported significantly reduced time to clinical recovery (by one day on average), progression to severe illness requiring ICU admission (0% vs. 12.9%) and deterioration of pneumonia as defined by repeated chest CT on day 6 after the initiation of treatment (6.5% vs. 29%), all in favor of HCQ (37).

The latest relevant report from China was a retrospective study of 568 critically ill patients with COVID-19; 48 of them were concomitantly treated with HCQ (400 mg/day for 7-10 days) (38). Demographic characteristics, co-morbidities, disease severity as well as additional medications including antiviral agents and antibiotics were all well balanced between the groups. In-hospital mortality rate was in favor of HCQ (18.8% vs. 45.8%, p<0.001); moreover, HCQ was associated with decreased serum levels of IL-6 (from a mean of 22.2 pg/mL before treatment to 5.2 pg/mL after the conclusion of HCQ therapy, p<0.05) (38).

However, other studies on HCQ efficacy in COVID-19, particularly in critically ill patients, did not yield similar results. Regarding virological cure, a small study from another French center showed that the combination of HCQ and AZI did not lead to any clinical benefit or rapid viral clearance; 10/11 patients still had positive RT-PCR after 6 days of combination treatment (39).

A new RCT from China including 150 patients (75 on HCQ and 75 controls) with a mean age of 46 years and mild disease in 99% of them reported no difference in virological cure after 28 days (85.4% vs. 81.3% in the control group) (40). Moreover, no significant differences were documented regarding the time to clinical recovery. However, there was some benefit for HCQ and time to recovery in a post hoc analysis that excluded patients who were taking other antiviral medications. Of note, the HCQ dose was surprisingly high (1200 mg/d for three days and 800 mg/d for 2-3 weeks depending on disease severity) and the authors reported a significant delay between the onset of symptoms and treatment initiation (16 days on average) (40).

A French study on 181 COVID-19 patients with hypoxic pneumonia (84 on HCQ 600 mg/d and 97 controls) reported no survival benefit (41). In the HCQ group, 2.8% of the patients died within seven days vs. 4.6% in the non-HCQ group (3 vs 4 events, RR 0.61, 95% CI 0.13-2.89). Acute respiratory distress syndrome was observed in 27.4% and 24.1% respectively at the same time period (24 vs 23 events, RR 1.14, 95% CI 0.65-2.00). Cumulatively, 20.2% patients in the HCQ group were transferred to the ICU or died within 7 days vs 22.1% in the non-HCQ group (16 vs 21 events, relative risk [RR] 0.91, 95% CI 0.47-1.80) (40). Of note, 75% of the study patients had moderate-to-severe bilateral pneumonia and more than 50% of them had significant co-morbidities. Almost all of them had elevated CRP, indicating that the second

stage of the COVID-19 (systemic inflammatory response syndrome or "cytokine storm") was already present.

Similar findings were recently reported from a retrospective analysis of 368 patients who were hospitalized in all the United States Veterans Health Administration medical centers until April 11 (42). The combination of HCQ and AZI was not associated with any reduced risk of mechanical ventilation or death compared to HCQ alone or no treatment. However, the study was received with severe criticism since a proportion of the patients on a ventilator started treatment with HCQ and AZI after intubation and eventually died. Had these patients been excluded from the analysis, a marginal benefit for the combination was demonstrated.

Another RCT from Brazil assessed the safety and efficacy of two different doses of chloroquine (600 mg twice daily for 10 days versus 900 mg on day 1 and 450 mg on days 2-5) in severe COVID-19 patients with acute respiratory distress syndrome (43). They recruited 81 individuals (out of 440 that was the initial target), most of them with multiple pre-existing conditions and all received AZI and ceftriaxone concomitantly. Patients on the high dose (n=41) developed more frequently severe QT_c prolongation (>500msec in 19%) and had a higher mortality rate compared to the low-dose group (39% vs. 15%). The study was prematurely terminated based on safety signals and all the surviving patients of the high-dose arm were re-allocated to the low-dose.

Finally, a retrospective study from the USA assessed the potential clinical benefit from HCQ in 1376 hospitalized regarding to the time to intubation or death (44). Patients on HCQ (n=811) had more severe disease as expressed by the median ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen compared to non-HCQ controls (n=565). The baseline differences were alleviated with propensity score matching, which showed that there was no significant association between HCQ use and intubation or death (HR 1.04, 95% CI 0.82 to 1.32). These results were further confirmed in multiple sensitivity analyses (44).

The results of the aforementioned studies (Table 1) should be interpreted with caution since methodological flaws were almost universally present. It seems, however, that most of the included patients in the negative studies had severe disease and/or were already admitted to an ICU, implicating severe pneumonia and respiratory insufficiency. In such settings, HCQ and AZI alone are unlikely to confer any clinical

benefit; only the retrospective study by Yu et al. (38) yielded promising results. Apart from the severity of the disease and the distinct dominant pathogenetic mechanisms (systemic inflammatory response syndrome), the pharmacokinetics might be significantly affected. In this context, Perinel et al. (45) showed that a dose of 600 mg/day is probably inappropriate for ICU patients with COVID-19 since only 61% achieved the recommended serum concentration of at least 1mg/l and this was achieved only after 48 hours.

Is HCQ safe to use in COVID-19?
The long-term side effects of HCQ are well known and include retinopathy, neuropathy, myopathy, cardiomyopathy and cutaneous hyperpigmentation. However, all of them usually develop after many years of consistent use and are probably irrelevant in the context of COVID-19. The most significant short-term adverse events, apart from idiosyncratic reactions, seem to be conduction system disorders, most notably the prolongation of the QT interval, Torsades de Pointes (TdP) and the subsequent generation of life-threatening ventricular arrhythmias (46). Sarayani et al. (47) reviewed data from the US Food and Drug Administration's Adverse Event Reporting System (FAERS) from 1969 until 2019 for death and/or QT/TdP associated with the use of HCQ or chloroquine and AZI. Of more than 13.3 million FAERS reports, they found that HCQ (or chloroquine) is not associated with any safety signals for death or QT prolongation/TdP (based on 78,848 reports). However, for AZI alone (53,378 reports), a significant safety signal was detected for QT prolongation/TdP but not for death. Of note, the combination of HCQ (or chloroquine) and AZI (600 reports) was deemed safe for all of these outcomes (47).

In the same line, a multinational, multicentre study utilizing data from administrative databases for 956,374 new HCQ users with rheumatoid arthritis reported no increased risk for 30-day cardiovascular mortality, angina or heart failure compared to new sulfasalazine users (48). Of these patients, 323,122 were concomitantly treated with AZI (351,956 individuals were concomitantly treated with amoxycillin, which served as a comparator and patients were matched according to propensity score). The investigators found an increased risk of 30-day cardiovascular mortality [HR=2.19 (95% CI 1.22-3.94)], angina [HR=1.15 (95% CI 1.05-1.26)] and heart failure [HR=1.22 (95% CI 1.02-1.45)] with the combination of HCQ and AZI (48). Unfortunately, neither QT prolongation/TdP nor the actual cause of cardiovascular death could be captured by the design of this study.

Table 1. Therapeutic studies with HCQ (or chloroquine) for COVID-19 (updated on May 8, 2020).

Author (Country), (Reference)	Patients	Main findings	Notes
Gao et al. (China) (6)*	>100 on CQ	CQ inhibits the exacerbation of pneumonia, improves lung imaging findings, promotes a virus-negative conversion and shortens disease course	No details published
Gautret et al. (France) (10)*	36 (20 HCQ, 16 controls)	Virological cure by day 6: HCQ 70%, controls 12.5% With concomitant AZI: 6/6 (100%)	Non-randomized, most patients with mild disease
Gautret et al. (France) (34)*	80 (all HCQ)	93% negative RT-PCR by day 8 97.5% negative viral cultures by day 5 78/80 recovered, 1 death, 1 in ICU	Non-randomized, most patients with mild disease
Million et al. (France) (35)*	1061 (all HCQ)	1048/1061 (98.7%) cured, 8 deaths in older patients (mean age 79 years) with more co-morbidities, 5 in the ICU	Non-randomized
Chen et al. (China) (37)*	62 (31 HCQ, 31 controls)	Time to clinical recovery: 1 day less for HCQ ICU admission: HCQ 0%, controls 12.9% Deterioration of pneumonia: HCQ 6.5%, controls 29%	Small sample size
Yu et al. (China) (38)*	568 (48 HCQ)	In-hospital mortality: HCQ 18.8%, non-HCQ 45.8% HCQ was associated with decreased IL-6 after therapy	Retrospective study, all critically-ill patients
Molina et al. (France) (39)**	11 (HCQ+AZI)	10/11 still had positive RT-PCR after 6 days	Small sample size
Tang et al. (China) (40)**	150 (75 HCQ, 75 controls)	Virological cure at 28 days: 85.4% HCQ, 81.3% controls Time to clinical recovery: in favor of HCQ for patients not taking antivirals	Delay between onset of symptoms and HCQ treatment (16 days on average)
Mahevas et al. (France) (41)**	181 (84 HCQ, 97 controls)	7-day mortality: HCQ 2.8%, controls 4.6% (RR 0.61, 95% CI 0.13-2.89) 7-day ARDS: HCQ 27.4%, controls 24.1% (RR 1.14, 95% CI 0.65-2.00) 7-day ICU or death: HCQ 20.2%, controls 22.1% (RR 0.91, 95% CI 0.47-1.80)	Mostly moderate-to-severe disease
Magagnoli et al. (USA) (42)**	368 (97 HCQ, 113 HCQ+AZI, 158 no HCQ)	Overall mortality: HCQ 27.8%, HCQ+AZI 22.1%, no HCQ 11.4% Mechanical ventilation: HCQ 13.3%, HCQ+AZI 6.9%, no HCQ 14.1%RR	Retrospective study, patient groups not well balanced
Borba et al. (Brazil) (43)**	81 (41 high dose CQ, 40 low dose CQ)	Overall mortality: high CQ 39%, low CQ 15% Severe QTc prolongation (>500msec): high CQ 19%, low CQ 11%	Terminated prematurely due to safety signals, both arms were treated with uncommonly high doses
Geleris et al. (USA) (44)**	1376 (811 HCQ, 565 no HCQ)	Intubation or death: HCQ 32.3%, non-HCQ 14.9% Multivariable analysis: HR 1.00 (95%CI 0.76-1.32) Propensity score analysis: HR 0.97 (95%CI 0.74-1.28)	Retrospective study, patients on HCQ had more severe disease

HCQ: hydroxychloroquine; CQ: chloroquine; AZI: azithromycin; RT-PCR: reverse transcriptase polymerase chain reaction; ICU: intensive care unit; ARDS: acute respiratory distress syndrome.
*Studies with favorable outcomes with HCQ.
**Studies where HCQ did not exert a significant benefit.

Finally, the evidence of hemolytic anemia in HCQ users with glucose-6-phosphate dehydrogenase (G6PD) deficiency is sparse. A small study from the USA in 11 G6PD-deficient patients with over 700 months of HCQ exposure did not report any event of hemolytic anemia (49). However, it is not known if these findings can be generalized in countries with higher prevalence and different genetic variants of G6PD deficiency.

Based on these data, caution is warranted when using the combination of HCQ and AZI for COVID-19 patients. This seems particularly significant for older patients with cardiovascular co-morbidities and potential use of other medications that may affect the risk for arrhythmias as well as patients with evidence of COVID-19-related myocardial damage.

Conclusion

Based on the existing data, regular use of HCQ does not seem to confer absolute protection against infection with SARS-CoV-2. If there is a relative protection through pre- and post-exposure prophylaxis in high-risk individuals remains to be seen; multiple clinical trials are awaited in the near future. From the efficacy perspective, HCQ (plus azithromycin) may decrease the viral shedding and contagiousness of COVID-19, reduce the rate of clinical deterioration in mild-to-moderate disease and minimize the need for ICU admission. In cases of severe disease (pneumonia with respiratory insufficiency, mechanically ventilated patients), the current data do not support its

widespread use. Regarding safety, it seems that HCQ alone is not associated with increased risk of arrhythmias; the combination with AZI, however, increases the risk for adverse cardiac events and should be monitored appropriately. Ongoing large RCTs (such as the WHO sponsored SOLIDARITY trial and the DISCOVERY trial, NCT04315948) are expected to provide more definitive answers and define the role of HCQ in the management of COVID-19. However, during the rapid evolution of the COVID-19 pandemic that strains the health care systems worldwide and considering limited population-wide testing rates in most of the vulnerable countries, early empiric short-term administration of HCQ in symptomatic individuals, could be a negotiable, safe and low-cost strategy.

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