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Original article

Chloroquine, hydroxychloroquine, and COVID-19: Systematic review and narrative synthesis of efficacy and safety

Michael Takla, Kamalan Jeevaratnam *

Faculty of Health and Medical Science, University of Surrey, Guildford GU2 7AL, United Kingdom

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ABSTRACT

The COVID-19 pandemic has required clinicians to urgently identify new treatment options or the repurposing of existing drugs. Of particular interest are chloroquine (CQ) and hydroxychloroquine (HCQ). The aims of this systematic review are to systematically identify and collate 24 studies describing the use of CQ and HCQ in human clinical trials and to provide a detailed synthesis of evidence of its efficacy and safety. Of clinical trials, 100% showed no significant difference in the probability of viral transmission or clearance in prophylaxis or therapy, respectively, compared to the control group. Among observational studies employing an endpoint specific to efficacy, 58% concurred with the finding of no significant difference in the attainment of outcomes. Three-fifths of clinical trials and half of observational studies examining an indicator unique to drug safety discovered a higher probability of adverse events in those treated patients suspected of, and diagnosed with, COVID-19. Of the total papers focusing on cardiac side-effects, 44% found a greater incidence of QTc prolongation and/or arrhythmias, 44% found no evidence of a significant difference, and 11% mixed results. The strongest available evidence points towards the inefficacy of CQ and HCQ in prophylaxis or in the treatment of hospitalised COVID-19 patients.

1. Introduction

In the final week of December 2019, the Hubei Integrated Chinese and Western Medicine hospital in Wuhan reported a clustered point-source outbreak of pneumonia (Wu and McGoogan, 2020), with an unknown viral origin. Within 30 days, the rapid geographic expansion of the disease, which the International Committee on Taxonomy of Viruses later coined Coronavirus Disease 2019 (COVID-19) (Gorbalenya et al., 2020), implied human-to-human transmission. On March 11, 2020, the World Health Organisation (WHO) designated COVID-19 a pandemic (Cucinotta and Vanelli, 2020). As of August 9, 2020, COVID-19 has been confirmed as the cause of over 19,000,000 cases and 725,000 deaths (Dong et al., 2020) globally.

In the absence of specific antiviral pharmacotherapy, the repositioning of existing drugs represents an attractive clinical option. Selecting which drugs to repurpose, however, hinges on the compatibility of their mechanisms of action with the disease progression of severe acute respiratory syndrome (SARS), and with the biology of the recently emerged pathogenic agent that causes it.

With a likely evolutionary origin in bats (Zhou et al., 2020b), the novel (beta)-coronavirus, SARS-CoV-2, probably acquired the ability to zoonotically infect humans via natural selection of the receptor-binding domains of its spike (S) proteins in an intermediate mammalian host (Tang et al., 2020b). Indeed, compared to SARS-CoV-1, the highly homologous (Jaimes et al., 2020) coronavirus responsible for the SARS pandemic (Marra et al., 2003), the S protein has a 10-20-fold greater affinity for the ACE2 receptor (Wrapp et al., 2020) predominantly expressed by pulmonary and intestinal epithelia and vascular endothelia (Hamming et al., 2004). In fact, *in silico* analysis has demonstrated that the expres-

Abbreviations: CoV, coronavirus; COVID-19, Coronavirus Disease 2019; CQ, chloroquine; FDA, Food and Drug Administration; HCQ, hydroxychloroquine; ICU, intensive care unit; MERS, Middle East Respiratory Syndrome; PICOT, Population, intervention, comparison, outcome, time; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; QTcF, The corrected QT interval by Fredericia; SARS, Severe Acute Respiratory Syndrome; WHO, World Health Organisation; VT, ventricular tachyarrythmia.

* Corresponding author at: Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7AL, United Kingdom.
E-mail address: drkamalanjeeva@gmail.com (K. Jeevaratnam).

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sion of sialic acid further facilitates viral entry, whereby binding of human gangliosides impairs inhibitory interactions between the S protein and the plasma membrane (Fantini et al., 2020). The resulting receptor-mediated endocytosis precedes endosomal cathepsin and TMPRSS2-mediated (Hoffmann et al., 2020) cleavage of the S protein, permitting fusion of the viral lipid envelope and human vesicular membrane, whereupon RNA entry into the cytosol enables viral replication, maturation (Coutard et al., 2020), and budding. The initial innate immune response to the subsequent dissemination of SARS-CoV-2 throughout the patient's extracellular fluid elicits a wave of pro-inflammatory cytokines, including IL-1(beta), IL-6, and TNF-(alpha), that recedes upon lymphopenia, only to return at higher concentrations in a cytokine storm (McGonagle et al., 2020) that predisposes to a potentially lethal acute respiratory distress syndrome (ARDS). Additionally contributing to the mortality of critically ill COVID-19 patients is the significantly elevated incidence of often pulmonary thromboembolic events (Poissy et al., 2020; Klok et al., 2020; Zhang et al., 2020).

Chloroquine (CQ), and its less oculotoxic (Raines et al., 1989; Yam and Kwok, 2006) derivative, hydroxychloroquine, (HCQ) were among the first drugs selected for repurposing to treat COVID-19 patients. There is significant substantiation for the *in vitro* efficacy of these aminoquinolines against the Chikungunya virus (Ozden et al., 2008), and, importantly, SARS-CoV-2 (Wang et al., 2020), raising the possibility that they could minimise the rise in viral load at the beginning of the infection course. However, the relative lack of *in vivo* data, particularly in humans, prevents definitive conclusions on the applicability of such results to COVID-19 patients. Yet, even if inefficacious at limiting viral replication, the anti-inflammatory (Yang et al., 2018), and anti-thrombotic (Carter and Eban, 1974; Pilcher, 1975; Rand et al., 2008; Bertrand et al., 1990) effects of CQ and HCQ imply a possible role in curbing symptoms related to the inflammatory response in the latter stages of infection. But as of August 8, 2020, there is no *in vivo* evidence to support this. Even so, the ability of 4-aminoquinolones to prolong the QT interval (Chen et al., 2006; Negoescu et al., 2013) increases the risk of *de novo* ventricular tachyarrhythmias (VTs). As such, despite the initial federal push to administer CQ and/or HCQ to COVID-19 patients, both their potential efficacy (Aljofan and Gaipov, 2020) and cardiac safety (Jeevaratnam, 2020) have been called into question.

Here, we systematically review existing clinical trial and observational study data to provide a detailed synthesis of the evidence, or lack thereof, for the efficacy and safety of CQ and HCQ in hospitalised COVID-19 patients. We also aim to examine whether the use of such drugs in in the absence of rigorous evidence may pose a cardiac safety risk to treated patients, and thereby contribute to excess mortality in such a clinical setting.

2. Materials and methods

2.1. Objectives

This systematic review seeks to clarify the strength of evidence for the relative efficacy and safety of CQ and HCQ treatment in patients suspected of, and diagnosed with, COVID-19.

2.2. Methods

In line with the PICOT format (Riva et al., 2012) of framing subjects for clinical research, this study centres on answering the question: 'In patients suspected of, and diagnosed with, COVID-19, How efficacious, relative to standard symptomatic care, and safe are CQ and HCQ in patients at risk or suspected of, and diagnosed with, COVID-19?'

The subsequent elaboration of the systematic review and narrative synthesis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Larissa et al., 2015) for evidence-based assessment of published research.

2.3. Search strategy

In light of the current global health emergency, and the requisite rapid turnover of publications to meet the consequently urgent need to obtain and analyse the results that they present, several authors have resorted to the use of preprint servers to disseminate their findings. Despite the evident shortfalls inherent in referring to data, whose quality has not been peer-reviewed, the present paucity of published original research on the efficacy and safety of CQ and HCQ in COVID-19 patients demands exceptional measures. As such, this systematic review will take into account non-peer-reviewed work, provided that they have been submitted to a preprint server, where they are available in open-access form. Nevertheless, given its focus on data quality, this review will make unambiguous every instance in which data from such sources are used.

Therefore, on August 8, 2020, MEDRXIV and BIORXIV, along with PubMed and Web of Science, acted as the databases for the initial search of items relevant to the PICOT-formatted question. The preliminary use of the search terms "COVID", "chloroquine", and "hydroxychloroquine" yielded a large number of results that bore little relevance to the research topic. Combining such terms into phrases – *"COVID" AND "chloroquine"*, and *"COVID" AND "hydroxychloroquine"* in the title or abstract – and requiring the term *"outcome"* or *"outcomes"* anywhere in a free full text appearing within the last year considerably focused the responses. The subsequent application of identical phase stenography to each database ensured internal consistency.

2.4. Search attrition criteria

As this review aims to establish the weight of evidence for the use of a therapy in patients, data able to answer such a question must derive from primary research. Moreover, owing to the international extent of the present health crisis, any imposition of an original language requirement would exclude useful and otherwise rare resources. As such, following the collation of items in Mendeley and the removal of duplicates, application of these criteria excluded unique items for which there was either no English version or no original data.

Screening of the resulting papers against the criteria established by the PICOT-formatted question – namely, the requirement that data be collected from COVID-19 patients treated with CQ and/or HCQ – included only controlled trials and observational studies. Of all case series considered, only those directly assessing CQ or HCQ safety addressed each facet of the question and were thus included.

2.5. Article processing and selection

Having applied the exclusion and inclusion criteria to all search results and removing duplicates at all stages where necessary, two investigators independently reviewed the final repertoire of studies.

2.6. Quality appraisal

Rather than merely verifying the relevance and scope of the material in the final library, holistic analysis of each item of

Table 1
Quality appraisal of the 26 papers passing through search attrition.

Publication (first author)	Problem Statement, Conceptual Framework, and Research Question	Reference to the Literature and Documentation	Relevance	Research Design	Instrumentation, Data Collection, and Quality Control	Population and Sample	Data Analysis and Statistics	Reporting of Statistical Analyses	Presentation of Results	Discussion and Conclusion: Interpretation	Title, Authors, and Abstract	Presentation and Documentation	Ethical Approval	Total Criteria Met
Horby	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Mitja	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Boulware	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Kamran	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Tang	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Molina	✓	✓	✓	✗	✓	✓	✓	✓	✗	✓	✗	✓	✓†	10
Mehra	✓	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✗	10
Sbidian	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Singh	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Arshad	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Ip	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Bernaola	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	12
Geleris	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Yu	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Huang	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Magagnoli	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Ho An	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Saleh	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Mahevas	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Bhattacharya	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Hsia	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Mercuro	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Oteo	✓	✓	✓	✓	✓	✓	✓	N.A.*	✓	✓	✓	✓	✗	11**
Kim	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13
Mfeukeu-Kuate	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	13
Mallat	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓†	13

✓† : Mention of ethical approval, but explicit waiver of informed consent.
*: Case series without necessity for statistical comparison to a control group.
**Of the 12 criteria relevant to the paper, Oteo et al., 2020 satisfied 11.

Table 2
Data extraction from the 24 papers passing quality appraisal.

Study	Sample size	Age (mean/ median)	Male (%)	Symptom severity	Relevant treatment	Efficacy in meeting outcome	Safety	Limitations
Horby (RCT)	4,716	65	62	Variable or unstated	HCQ 800 mg 2 in 6 hrs, then 400 mg at 12 hrs on day 1, then 400 mg 2 day^{-1} on days 2–10, or until discharge.	No significant difference in mortality risk between treatment (27%) and control (25%) groups up to 28 days. Significantly lower risk of hospital discharge of alive patients given treatment up to 28 days. Significantly higher risk of symptoms progressing to invasive mechanical ventilation in patients given treatment.	No significant excess of *de novo* cardiac arrhythmias in the treatment group.	Absence of a placebo.
Mitja (RCT)	2,314	49	27	No symptoms (88%)	HCQ 800 mg day^{-1} on day 1, then 400 mg day^{-1} on days 2–7	No significant difference in probability of PCR-confirmed symptomatic COVID-19 by 14 days. No significant difference in SARS-CoV-2 transmission prevention.	Significantly higher incidence of adverse events in the treatment group. No serious adverse events observed.	Absence of a placebo, reducing the rate at which adverse events in the control group were declared. Lack of masking.
Boulware (RCT)	821	40	48	No symptoms	Within 4 days of expected exposure: HCQ 800 mg once, 600 mg 6–8 hrs later on day 1, then 600 mg day^{-1} for 4 days	No significant difference in incidence of symptomatic COVID-19 between HCQ (12%) and placebo control (14%) patients at high-risk exposure to an individual confirmed to have been infected.	Significantly higher probability of side-effects by day 5 in HCQ (40%) compared to placebo control (17%) patients. No observation of serious adverse drug effects, including arrhythmias.	Possible misclassification of symptomatic cases as COVID-19 due to lack of diagnostic testing availability, culminating in reliance on the U.S. case definition of probable infection.
Kamran (RCT)	500	36	93	Mild	HCQ 400 mg 2 day^{-1} on day 1, then 200 mg 2 day^{-1} on days 2–5	No significant difference in progression of symptoms. No significant difference in viral clearance by 14 days, though higher in the treatment group at 7 days.	No serious side-effects observed.	Absence of a placebo. Standard of care unusually consisted of Zinc, and vitamins C and D. Overwhelmingly male sample prevents generalisation to both sexes. A subset of day 7 PCR negatives retested positive on day 14, suggesting either false negatives on day 7 due to variable kit sensitivity, or false positives on day 14 as non-replicable viral nucleic acid is detected by PCR after 10 days. No evidence of monitoring for cardiac arrhythmias or retinopathy.
Tang (RCT)	150	46	55	Mild to moderate	Standard of care + HCQ 1200 mg day^{-1} loading dose for 3 days, then 800 mg day^{-1} maintenance dose for remainder of 2 weeks if mild/moderate, or 3 weeks if severe	No significant difference in probability of negative conversion of SARS-CoV-2 at end time-point, and at all specific time-points. No significant difference in median time to negative conversion. No significant difference in median time to symptom recession	30% of HCQ patients reported adverse events, compared to only 9% given the standard of care only. The most frequent adverse event in HCQ patients was diarrhoea (10%), with HCQ discontinued in 1 patient with blurred vision. 2 patients in the HCQ group experienced serious events related to COVID-19 progression and upper respiratory tract infection. No cardiac arrhythmic events, including QT prolongation, in either group.	Absence of placebo. Lack of masking. Underpowered sample size. Premature termination of trial censored data on primary outcome. No assessment of antiviral efficacy within 48 h of onset due to enrolment of hospitalised patients. Short-term period of follow-up underestimates frequency of QT prolongation. Possible underestimation of retinal damage suggested by detection of early harm caused by 800–1200 mg day^{-1} HCQ in a sensitive screening test.

(*continued on next page*)

Table 2 (*continued*)

Study	Sample size	Age (mean/ median)	Male (%)	Symptom severity	Relevant treatment	Efficacy in meeting outcome	Safety	Limitations
Sbidian (OS)	4,642	66	59	Variable or unstated	HCQ 600 mg day^{-1} on day 1, then 400 mg day^{-1} on days 2–10± Azithromycin 500 mg day^{-1} on day 1, then 250 mg day^{-1} on days 2–5	No significant difference in mortality by 28 days between HCQ only and control. Significantly higher mortality risk for patients given HCQ + azithromycin compared to control. Significantly higher discharge rate in the group given HCQ only.	N.A.	Lack of randomisation. Limited sample size for the HCQ + azithromycin subgroup necessitates caution in interpreting higher mortality risk compared to control. Dosage may have varied from guidelines due to physician discretion. Significantly higher proportion of younger, male and smoking patients in the treatment cohorts than the control, though no significant differences in biological parameters. No monitoring of cardiac arrhythmias or retinopathy.
Singh (OS)	3,372	62	52	Variable or unstated	Unspecified dose of HCQ In 71% of patients, combination with azithromycin	No significant difference in mortality or need for mechanical ventilation.	No significant difference in incidence of *de novo* VT, fibrillation or SCD in HCQ compared to control patients.	Lack of randomisation.
Arshad (OS)	2,541	64	51	Variable or unstated	HCQ 400 mg 2 day^{-1} on day 1, then 200 mg 2 day^{-1} on days 2–5± Azithromycin 500 mg day^{-1} on day 1, then 250 mg day^{-1} on days 2–5	Significantly lower mortality risk in both HCQ only (14%) and HCQ + azithromycin (20%) treatment groups.	Of the 4% of patients who died from cardiac arrest, mean QTc of the final ECG was 471 ms. No observation of major cardiac arrhythmias.	Lack of randomisation. Combination of HCQ and Azithromycin was exclusive to severe COVID-19 patients.
Ip (OS)	2,512	64	62	Variable or unstated	Median 5 days after symptoms, variable doses.	No significant difference in mortality.	Significantly higher proportion of mortality attributable to cardiac causes in patients treated with HCQ (21%) compared to the control group (16%). Similar incidence of arrhythmias and cardiomyopathy in treated (5% and 1%) and control (4% and 1%) patients.	Lack of randomisation. Significantly lower age, but later presentation in clinical course and greater symptomatic disease in the treatment group. Possible misclassification due to manual abstraction of HER data. Possible sampling bias due to use of a convenience sample for data collection.
Bernaola (OS)	1,498	Not given in main text	62	Variable or unstated	Unspecified dose of HCQ	Significant, but small, reduction in risks of intubation and mortality.	N.A.	Lack of randomisation.
Geleris (OS)	1,376	Not given in main text	57	Variable or unstated	HCQ loading dose 600 mg 2 day^{-1} on day 1, 400 mg day^{-1} on days 2–5± Azithromycin 500 mg on day 1, 250 mg day^{-1} on days 2–5	No significant difference in probability of mortality or intubation between HCQ and control patients, under primary multivariable analysis. However, the confidence interval was relatively wide (0.82 to 1.32).	N.A.	Lack of randomisation. Some HCQ patients were also administered sarilumab. Single-centre design reduces representativeness of the sample. Lower baseline $P_aO2:F_iO2$ in HCQ compared to control patients. Missing data for some variables. Possible inaccuracies in electronic health records.

Table 2 (*continued*)

Study	Sample size	Age (mean/ median)	Male (%)	Symptom severity	Relevant treatment	Efficacy in meeting outcome	Safety	Limitations
Yu (OS)	550	68	63	Severe	HCQ 200 mg 2 day^{-1} for 7–10 days	Significantly lower mortality rate in HCQ (19%) compared to control (47%) patients. No significant difference in average hospital stay. Among patients who died, significantly longer hospital stay for the HCQ (15 days) compared to the control (8 days) group. Significant reduction in plasma IL-6 in HCQ, but not control patients. Among patients with IL-6 > 60 pg ml^{-1}, HCQ treatment, but not control treatment reversed the trend after 10 days, and significantly reduced fatality. Early start of HCQ within 5 days of admission reduced fatality compared to a late start. However, this difference was not statistically significant.	N.A.	Lack of randomisation. Considerable imbalance in the sample size of the treatment and control groups. Interferon application reached 11% in the control, but 0% in the treatment group.
Huang (OS)	373	44–46	45–49	Moderate	CQ 500 mg day^{-1} OR CQ 500 mg 2 day^{-1}	Significantly higher probability of viral clearance by days 10 and 14 in the treatment group. Significantly shorter duration of fever symptoms in the treatment group.	No serious adverse events observed in the treatment group. However, higher incidence of GI disturbances. Significantly lower incidence of adverse events in patients in the treatment group on half-dose, compared to those given the full dose.	Lack of randomisation. Only 1 patient in the treatment, compared to 9 patients in the control, group experienced aggravated (i.e. moderate to severe) symptoms. Impossibility of dissociating the effects of CQ from those of other antiviral drugs used prior to its administration. However, patients given CQ within 3 days of symptom onset still exhibit faster viral clearance.
Magagnoli (OS)	368	68–70	100	Variable or unstated	Unspecified dose of HCQ± Unspecified dose of azithromycin	No significant difference in ventilation risk, or mortality following ventilation, in either treatment group compared to the control. Higher mortality risk in HCQ only, but not HCQ + azithromycin, groups compared to the control.	N.A.	Lack of randomisation. Higher probability of prescribing HCQ ± azithromycin to patients with more severe metabolic, haematological, and ventilatory symptoms of COVID-19. Significant differences in demography, vital signs, prescription drug use, comorbidities, and disease severity. However, all adjusted by propensity score. The vast majority of the sample were African American and > 65 yrs, with both groups exhibiting disproportionately high rates of hospitalisation.
Ho An (OS)	226	35–43	16–40	Mild to moderate	HCQ 200 mg 2 day-^{1}±Azithromycin 500 mg day^{-1} for up to 5 days± Cefixime 100 mg 2 day^{-1}	No significant difference from standard conservative treatment in length of time to viral clearance, duration of hospital stay, and/or symptoms.	No serious adverse events or death.	Lack of randomisation. Small sample size, with large imbalance between treatment (31) and control (195) groups limiting the number of factors included in the propensity score model. A few patients in the treatment group (7.5% of total) also received treatment with lopinavir/ ritonavir, though duration of use included in multivariate Cox analysis.

(*continued on next page*)

Table 2 (*continued*)

Study	Sample size	Age (mean/ median)	Male (%)	Symptom severity	Relevant treatment	Efficacy in meeting outcome	Safety	Limitations
								Baseline systolic ABP and haematocrit varied significantly between treatment and control group, even after matching. No monitoring of cardiac rhythm, or of retinopathy.
Saleh (OS)	201	59	58	Moderate to severe	CQ 500 mg 2 day^{-1} on day 1, and 500 mg day^{-1} on days 2–5 OR HCQ 400 mg 2 day^{-1} on day 1, and 200 mg 2 day^{-1} on days 2–5± Azithromycin 500 mg on days 1–5	N.A.	Significant increase in QTc to peak and post-treatment. Significantly shorter maximum QTc in CQ/HCQ only compared to CQ/HCQ ± azithromycin patients. 8% of patients experienced de novo atrial fibrillation, and 3% had monomorphic ventricular tachycardia, which was non-sustained for all but one patient. No observation of *torsades de pointe*. However, 4% of patients had to prematurely discontinue HCQ owing to QT prolongation.	Lack of randomisation. Absence of a control. Small sample size relative to total cohort population treated. Lack of reporting of instances of torsades de pointe may be influenced by reporting bias. QT intervals in MCOT patches while on therapy were not correlated to baseline ECGs.
Mahevas (OS)	173	60	72	Moderate	Within 48 h of admission: HCQ 600 mg day^{-1}	No significant differences in overall survival rate, survival rate without transfer to ICU, survival rate without ARDS, time to weaning from O2 therapy, or time to discharge. Likewise for patients with better prognoses upon admission and less severe COVID-19 symptoms.	10% of HCQ patients experienced averse ECG modifications requiring cessation of treatment after a median of 4 days. Among them, 88% had a QTc prolongation > 60 ms (including > 500 ms in one patient). One of these patients presented with a 1st degree AV block after 2 days despite a lack of concomitant proarrhythmic medication.	Lack of randomisation. Small sample size. Lower probability of co-administration of azithromycin in the HCQ (18%) compared to the control (29%) group. Higher probability of co-administration of amoxicillin and clavulanic acid in the HCQ (52%) compared to the control (28%) group. HCQ patients had lower prevalence of comorbidities, except hepatic cirrhosis. The 4 covariates exceeding the standardised difference threshold were excluded from the final propensity score model. Imbalance in the number of HCQ patients between centres not taken into account by the propensity score model.
Bhattacharya (OS)	106	26–28	46–52	No symptoms	(At least) HCQ 400 mg 2 day^{-1} on day 1, then 400 mg $week^{-1}$ on weeks 1–7	Significantly (81%) lower probability of SARS-CoV-2 infection in the treatment group.	Significantly higher proportion of mostly mild adverse events in the treatment group, such as GI disturbances (19%), skin rash (6%), and headache (4%). No serious adverse effects observed.	Lack of randomisation. Small sample size. Atypically low prevalence of comorbidities in atypically young demographic. No taking into account of differences in behaviour-associated risk. No monitoring of cardiac arrhythmias or retinopathy.
Hsia (OS)	105	67	55	Variable or unstated	Variable dose of CQ±HCQ 400 mg 2 day^{-1} on day 1, then 400 mg day^{-1} on days 2–5±Azithromycin 500 mg day^{-1} on day 1, then 250 mg day^{-1} on days 2–5	N.A.	Significantly higher probability of QTc prolongation in any treatment group as compared to baseline. Significantly higher mortality risk with QTc prolongation > 60 ms.	Lack of randomisation. Small sample size. Inclusion only of patients requiring hospitalisation increases risk of selection bias. Higher probability of ECG-monitored patients being in a specialised care unit with more testing availability.

Table 2 (*continued*)

Study	Sample size	Age (mean/ median)	Male (%)	Symptom severity	Relevant treatment	Efficacy in meeting outcome	Safety	Limitations
							No significant differences in the prevalence of specific arrhythmic events, such as atrial fibrillation, tachycardia or torsade de pointes.	3% and 10% of patients were coadministered methadone and ondansetron, respectively, both of which prolong QTc, creating a confounder effect.
Mercuro (OS)	90	60	51	Moderate to severe	HCQ 400 mg 2 day^{-1} on day 1, 400 mg day^{-1} on days 2–5±Azithromycin	N.A.	11% had QTc prolongation > 60 ms, including 3% of HCQ only and 13% of concomitant HCQ + azithromycin patients. Final QTc exceeded 500 ms in 20% of patients, including 19% of HCQ only and 21% of concomitant HCQ + azithromycin patients. 11% of patients ceased HCQ before day 5 due to arrhythmic and GI adverse events, as well as a case of hypoglycaemia. One such patient developed *torsades de pointe* 3 days after cessation.	Lack of randomisation. Small sample size. Possible underestimation of QTc due to short follow-up period. Possible role of COVID-19-associated myocarditis and/or stress cardiomyopathy in observed adverse events. Baseline QTc was shorter in HCQ + azithromycin compared to HCQ only patients. Most patients had at least 1 cardiac comorbidity, and were taking 2 or more drugs prolonging QTc.
Oteo (OS)	80	52	47	Moderate	HCQ 400 mg 2 day^{-1} on day 1, then 200 mg 2 day^{-1} on days 2–6 +Azithromycin 500 mg day^{-1} on day 1, then 250 mg day^{-1} on days 2–6	N.A.	15% of patients, all but one of whom had pneumonia, had predominantly GI disturbances. One patient was admitted to hospital, with significant QTc prolongation. No patient was required to terminate the original therapeutic strategy.	Lack of randomisation. Small sample size.
Kim (OS)	65	54	39	Mild to moderate	HCQ 400 mg day^{-1}	Significantly longer time to viral clearance than patients given lopinavir-ritonavir 400 and 100 mg 2 day^{-1}, respectively. No significant difference in delay until improvement of disease symptoms.	No significant difference in the incidence of adverse events. Significantly lower incidence of lymphopenia and hyperbilirubinaemia than in patients given lopinavir-ritonavir.	Lack of randomisation. Small sample size. Significantly greater probability of confirmed pneumonia on CT scans in patients given lopinavir-ritonavir than those given HCQ.
Mfeukeu-Kuate (OS)	51	39	57	Mild	HCQ 200 mg 2 day^{-1} for 7 days +Azithromycin 500 mg day^{-1} for 1 day, then 250 mg day^{-1} for days 2–5	N.A.	No significant QTc prolongation. Significant fall in heart rate, prolonging the duration of the QRS complex. No symptomatic arrhythmic events; only clinically insignificant ECG abnormalities in patients with low Tisdale	Lack of randomisation. Small sample size.

(*continued on next page*)

Table 2 (*continued*)

Study	Sample size	Age (mean/ median)	Male (%)	Symptom severity	Relevant treatment	Efficacy in meeting outcome	Safety	Limitations
							risk.	
Mallat (OS)	34	37	74	Mild to moderate	HCQ 400 mg 2 day^{-1} on day 1, HCQ 400 mg day^{-1} for 10 days	Significantly longer time to viral clearance in HCQ (17 days) compared to control (10 days) patients, even after adjustment for confounders, such as symptoms and pneumonia or oxygen therapy. By day 14, significantly lower proportion of HCQ (48%) compared to control (91%) patients tested negative for SARS-CoV-2.	No observed side-effects of HCQ. No significant changes in plasma counts of leukocytes, lymphocytes, or concentrations of CRP and ferritin in either HCQ or control groups.	Lack of randomisation. Small sample size. No direct measurement of QTc prolongation. HCQ group had both significantly higher comorbidities and D-dimer levels.

Abbreviations: RCT = RCT; OS = observational study; SCD = sudden cardiac death; MCOT = mobile cardiac outpatient telemetry.

research in line with the framework set out in the Checklist of Review Criteria provided by the Task Force of Academic Medicine and GEA-RIME committee (Bordage et al., 2001) ensured stringent appraisal of study quality.

Indeed, the identification of – among other facets of robust research – appropriate study design, statistical analysis, and quality control (Table 1) permitted only papers with sufficient scientific merit to pass onto the data extraction stage.

2.7. Data extraction

Among the research items constituting the final library for analysis, there exists a wide variation in study design, results, and, crucially, the extent to which each distinct aspect of the PICOT-formatted question is answered. As such, a specialised data extraction table collates and summarises the most important information in every paper (Table 2). In particular, emphasis on the different sample sizes and structures, symptomatic stages, doses of drug used, primary and/or secondary outcomes and overall design limitations, facilitates both clarity and caution when making comparisons between the sets of results presented.

3. Results

3.1. Search breakdown

The results of each search, and the number failing and passing exclusion and inclusion criteria, respectively, have been summarised in a flowchart (Fig. 1).

Searches for phrases one (a: (“COVID”[Title/Abstract] AND “Chloroquine”[Title/Abstract]) AND “outcome”[Text Word]; b: (“COVID”[Title/Abstract] AND “Chloroquine”[title/abstract]) AND “outcomes”[Text Word]) and two (a: (“COVID”[Title/Abstract] AND “Hydroxychloroquine”[Title/Abstract]) AND “outcome”[Text Word]; b: (“COVID”[Title/Abstract] AND “Chloroquine”[Title/Abstract]) AND “outcomes”[Text Word]) with the aforementioned time filters yielded 264 results on PubMed, and 42 on Web of Science. The subsequent removal of 162 duplicates left 144 unique items from these two databases. In parallel, use of the same stenography, but with ‘outcome(s)’ added to the same engine in searching the titles, abstracts, or full texts in MEDRXIV and BIORXIV yielded, following imposition of a time filter and manual withdrawal of duplicates, 69 unique results.

Of the unique papers discovered in the first two databases, 72 failed the exclusion criteria, distributed categorically as follows: 22 commentaries; 19 literature reviews; 18 study proposals; 11 systematic reviews and/or meta-analyses; and three models of COVID-19 spread, pathological dynamics, and/or therapy. Likewise, of those unique items found on the preprint servers, 28 were excluded by the same criteria, distributed categorically as follows: 17 systematic reviews or meta-analyses; five study proposals; three commentaries; two models; and one literature review.

As part of the search attrition methodology, parallel application of the inclusion criteria to each set of remaining unique results left 13 and sixteen items from the first two, and preprint, databases, respectively. Of these 29 studies, three were duplicates, culminating in a library of 26 papers from all four databases.

3.2. Quality appraisal

The use of 13 criteria created in accordance with the aforementioned Checklist of Review Criteria elaborated by the GERA-RIME committee, whereby meeting all but one of those relevant to the study type in question was judged to be indicative of scientific

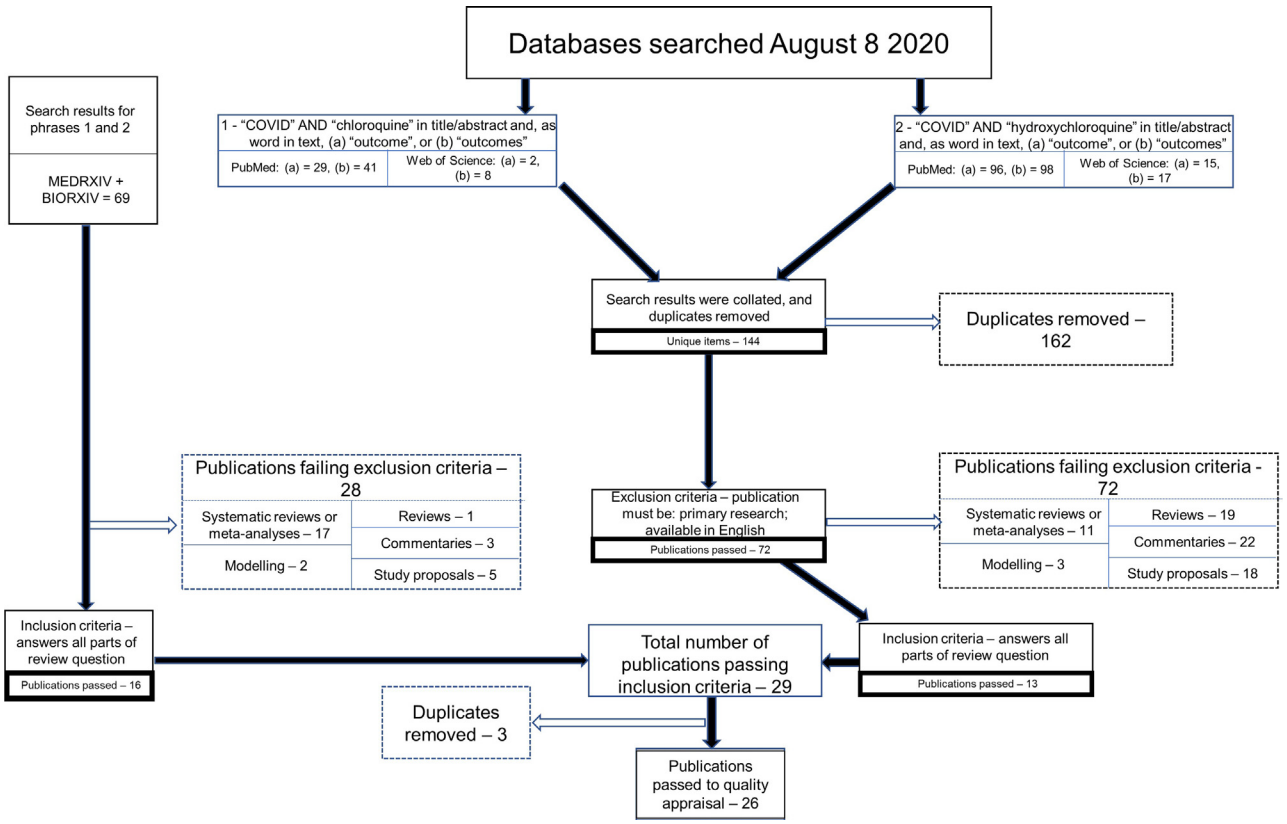

Fig 1. The attrition processes for publication. This comprised: (1) searching of three databases for peer-reviewed papers; (2) acquisition of submitted, but not yet peer-reviewed, items from a preprint server; and (3) application of exclusion and inclusion criteria. The removal of duplicates at each step, where necessary, left 26 unique items to pass onto the quality appraisal stage.

rigour, excluded two (Mehra et al., 2020; Molina et al., 2020) of the remaining 26 items (Table 1).

However, it is noteworthy that, of the 23 studies approved by an ethics committee, 11 explicitly waivered informed consent – another basic tenet of data collection from patient trials – citing the retrospective nature of their research. Confusingly, three studies claiming ethical board approval failed to explicitly mention consent (Sbidian et al., 2020; Arshad et al., 2020; An and Kim, 2020), while one investigation (Boulware et al., 2020) only sought consent after randomised allocation had already taken place.

3.3. Study design

As a result of the relatively broad scope of the research question, the authors of the 19 papers passing quality appraisal employed a variety of study types, therapeutic doses, and primary and/or secondary outcomes (Table 2). It is thus essential to distinguish results by study design in order to prevent invalid inferences drawn from comparison of data sets.

3.4. Study type

Although the research question requires the use of CQ and/or HCQ in patients suspected of, and diagnosed with, COVID-19, there exists a range of possible approaches to the collection of data obtained from such patients.

The gold standard of primary clinical research into the efficacy and safety of drugs administered to humans is the randomised controlled trial (RCT), evidence from which may be further buttressed by masking of subjects, experimenters, or both, as well as the use of a placebo in the control group. However, only 21% of items passing quality appraisal were clinical trials, of which, though all were randomised, only one (Boulware et al., 2020) employed either masking or an exclusively placebo control. While unacceptable under normal circumstances, the absence of both masking and a placebo is admissible in light of the ethical violation that would otherwise result from the use of either in the context of patient consent being unlikely. Indeed, the paper reporting use of masking and a placebo was likely able to do so given the asymptomatic presentation of the uninfected patients constituting the sample from which the control group derived.

The remaining 79% of papers were observational studies, of which the vast majority retrospectively searched hospital databases to collect clinical data obtained by following up on cohorts of patients from the time they received CQ and/or HCQ, or the standard of care only, until a defined end-point. Despite 74% using a case-control structure, a minority (26%) constituted case series focusing on the cardiac safety of drugs administered to hospitalised COVID-19 patients for a given duration of time.

3.5. Therapeutic doses

The deliberate absence of a specified dose in the research question accounts for the diversity of administration regimens among the 24 papers. Indeed, the doses used largely reflect the studies being conducted on different dates, which, in turn, influences the relative sway of either federal healthcare guidelines or the results of prior clinical research on regimen selection.

Every clinical trial tested a distinct dosing scheme: a loading dose of 1200 mg HCQ per day for three days, followed by a maintenance dose of 800 mg per day for two or three weeks if symptoms are mild/moderate or severe, respectively (Tang et al.,

2020a); 800 mg HCQ once and 600 mg six to eight hours later for one day, then 600 mg per day for four days (Boulware et al., 2020); 800 mg HCQ twice in six hours, then 400 mg at 12 hrs on day one, followed by 400 mg twice a day for nine days, or until discharge (Horby et al., 2020); 800 mg HCQ once on day one, then 400 mg per day for six days (Mitja et al., 2020); and 400 mg twice a day on day one, followed by 200 mg twice a day for four days (kamran et al., 2020). Likewise, each clinical trial treated its control group differently, from: a placebo folate tablet administered in a regimen identical to that of the treatment group (Boulware et al., 2020), to only a standard of care, itself varying with clinical centre and national guidelines.

By contrast, the retrospective and often multi-centre nature of many observational studies has resulted in 21% using variable or undeclared doses, some collecting data from some patients taking azithromycin in combination with the HCQ. The remaining 79% of items relied on highly divergent dosing regimens (Table 2). One popular iteration administered 200 mg HCQ twice a day, with: no antibiotics, for one week to 10 days (Yu et al., 2020a); necessarily (Mfeukeu Kuate et al., 2020), or optionally (An and Kim, 2020), with azithromycin (Mfeukeu Kuate et al., 2020) and/or cefixime (An and Kim, 2020) for up to five days. The most popular higher dose of choice involved giving 400 mg HCQ, rarely once (Kim et al., 2020), and more commonly twice, a day for one day, followed either by: 200 mg twice a day for four days, with or without 500 mg azithromycin (Saleh et al., 2020); 400 mg per day for four days, with or without azithromycin (Mercuro et al., 2020; Oteo et al., 2020; Hsia et al., 2020); 400 mg per day for ten days (Mallat et al., 2020); or 400 mg per week for seven weeks (Bhattacharya et al., 2020). In contrast, two studies relied on a much higher loading dose of 600 mg HCQ, either: once per day (Mahévas et al., 2020) throughout; only on day one, then 400 mg per day for nine days (Arshad et al., 2020); or twice per day on day one, followed by 400 mg per day for four days (Geleris et al., 2020), with or without azithromycin. Only three sets of authors also analysed data for patients taking CQ variably (Hsia et al., 2020), or, more commonly, at a full dose of 500 mg twice a day, either: for one day, followed by half-dose for four days, with or without 500 mg azithromycin per day for five days (Saleh et al., 2020); or at a half-dose for the duration of treatment (Huang et al., 2020a). Similarly, the 74% of observational studies with a control treated the group differently, giving standard of care without, or with declaration of additional antivirals, antibiotics, or both.

3.6. Primary and/or secondary outcomes

Given the vast array of possible measures of CQ and HCQ efficacy and safety in COVID-19 patients, the different authors outlined distinct primary and/or secondary outcomes.

The primary outcome in 80% of clinical trials was $C_t > 40$ for PCR amplification of SARS-CoV-2 RNA, indicating either prophylaxis or viral clearance. Likewise, 63% of observational studies directly measured a specific indicator of efficacy other than mortality rate, using similar outcomes to the clinical trials, as well as the duration of hospital stay, need for mechanical ventilation, and probability of transfer to an intensive care unit (ICU).

As regards direct measurement of safety, all clinical trials and 74% of observational studies explicitly recorded adverse events as an indicator of CQ and/or HCQ safety in COVID-19 patients, with 50% of the total actively focusing on cardiac pathology.

Notably, 42% of studies used mortality rate as a key end-point. In isolation, however, risk of death could be indicative of either safety or efficacy. As such, this review reports the findings on mortality rate separately from those pertaining to outcomes that are specific measures of one of efficacy or safety.

3.7. Results

Of the clinical trials providing data on a specific indicator of CQ and/or HCQ efficacy in patients suspected of, and diagnosed with, COVID-19, all showed no significant difference in the probability of viral transmission or clearance in the prophylactic (Boulware et al., 2020; Mitja et al., 2020) or therapeutic (Horby et al., 2020; kamran et al., 2020; Tang et al., 2020a) treatment, respectively, compared to the control, groups. Notably, therapeutic administration of CQ and/or HCQ in the context of single-centre clinical trials largely focused on patients with mild to moderate symptoms. However, these results hold in spite of wide variations in both symptom severity, as in the largest multi-centre study to date (Horby et al., 2020), and dosage across the range of papers.

In contrast, only 58% of observational studies employing an endpoint specific to efficacy recorded no significant difference in the attainment of outcomes, such as duration of hospital stay, need for mechanical ventilation, and probability of transfer to an intensive care unit (ICU), between COVID-19 patients given a range of CQ and/or HCQ doses, and the control groups. Indeed, of the remaining papers, 60% found evidence of a higher probability of discharge rate (Sbidian et al., 2020), viral clearance and shorter symptom duration (Huang et al., 2020a) in a therapeutic context, and a lower incidence of SARS-CoV-2 infection in a prophylactic context (Bhattacharya et al., 2020). Nonetheless, two investigations suggested that CQ and/or CQ delayed viral clearance, finding a lower proportion of treated patients among those with a negative PCR test, compared to standard of care (Mallat et al., 2020) or lopinavir-ritonavir (Kim et al., 2020) control groups.

Although 60% of clinical trials found evidence of higher mild adverse drug-related events in the treatment group, none of those specifically focusing on cardiac-side effects discovered any significant difference relative to the control. Likewise, 50% of observational studies examining an indicator unique to drug safety discovered a higher probability of adverse events in those treated patients suspected of, and diagnosed with, COVID-19. Of those retrospective studies measuring cardiac side-effects, 44% failed to find a significantly different incidence between the treatment and control groups, while another 44% indicated a significantly greater probability, with QTc prolongation the most common finding, in addition to its potentially lethal consequences of VT and cardiac arrest. In contrast, one such paper presented mixed findings, with an elevated risk of cardiac events despite no apparent rise in the risk of QTc prolongation.

Of the total studies using mortality rate as a key end-point, 60% reported no significant change in the risk of death, while 30% showed a depression, in treated relative to control patients. Interestingly, one investigation (10%) yielded a mixed result of raised mortality risk in patients given HCQ only, but no significant difference in those coadministered azithromycin (Magagnoli et al., 2020).

4. Discussion

The absence of a pharmacological treatment tailored to COVID-19 has rendered urgent the search to find alternative therapies by repositioning drugs with the theoretical potential to alleviate symptoms. However, hypothetical plausibility is insufficient grounds for translation into clinical practice. Indeed, any therapeutic repurposing must only proceed in light of strong evidence for the pre-clinical basis, and clinical efficacy and safety, of the drug in question. This review finds that, while such evidence certainly exists for the former, it does not for the latter, calling into question any clinical use of CQ and/or HCQ in COVID-19 patients prior to the collection and analysis of high-quality RCT data.

4.1. Pre-clinical indications of the potential of CQ and HCQ to treat COVID-19

Pre-clinical studies performed *in vitro* provide strong evidence for the theoretical utility of CQ and HCQ in inhibiting all stages of viral entry, maturation, and spread.

In vitro, CQ blocks infection both at, and after, entry of SARS-CoV-2 into Vero E6 cells, with an EC_{50} of 1.13 μM (Wang et al., 2020). Indeed, although therapeutic doses of CQ do not seem to alter S protein glycosylation (Vincent et al., 2005), whose pattern is distinct from that of SARS-CoV-1 (Kumar et al., 2020), they may inhibit biosynthesis of sialic acid (Kwiek et al., 2004), *N*-glycosylation of ACE2, as well as downregulating the expression of PICALM (Wolfram et al., 2017) in the clathrin-dependent endocytosis machinery.

Furthermore, immunofluorescence analysis of the amount of nucleoprotein in distinct vesicular compartments of the host cell has demonstrated that treatment of infected cells with CQ and HCQ stalls transfer of viruses from early to late endosomes (Liu et al., 2020). In fact, by increasing the pH of the early endosome, CQ has the potential to reduce acid-dependent proteolytic cleavage of the S protein by cathepsin and TMPRSS2, thereby inhibiting viral uncoating, genomic replication and particle maturation (Wang et al., 2008). Despite its similar effect on viral distribution, as well as its comparable cytotoxicity (Liu and Li, 2020), to CQ, HCQ appeared to amplify and enlarge the late endosomes, implying a slightly distinct mechanism of action. Furthermore, there exists conflicting evidence for the relative *in vitro* efficacy of the two drugs (Yao et al., 2020).

It is nonetheless clear that the initial basis for investigating the translatability of CQ and/or HCQ to the treatment of hospitalised COVID-19 patients was predicated on high-quality evidence for its pre-clinical antiviral efficacy.

4.2. Clinical evidence of the efficacy of CQ and HCQ in the treatment of COVID-19

That CQ and HCQ can reduce viral entry, trafficking, and budding *in vitro* constitutes evidence of translational potential relies on the underlying assumption that symptom severity is a function of viral replication. Yet, while viral load may influence severity in the very early stages of COVID-19 (He et al., 2020) – as in SARS (Cheng et al., 2004), the largest prophylactic randomised clinical trials to date have found no evidence of the efficacy of high-dose HCQ in preventing the development of symptoms in individuals exposed to others confirmed to have been infected with SARS-CoV-2 (Mitja et al., 2020; Boulware et al., 2020). The failure of drug administration in those at high risk of exposure to prevent infection points towards a potential lack of translatability of the antiviral facets of aminoquinoline mechanism of action. Nonetheless, confirmation of the repeatability and external validity of the study data by, for instance, the COPCOV trial, is a prerequisite to any definitive judgment on the efficacy of CQ and/or HCQ in inhibiting SARS-CoV-2 entry into, and replication within, host cells.

Importantly, however, in COVID-19 patients, symptoms subsequent to the causal infection result firstly from the initial cytokine wave of the innate immune response (Yang et al., 2020), then a state of immunodeficiency and lymphopenia (Hadjadj et al., 2020), and, finally, a potentially lethal cytokine storm (Zhou et al., 2020a). The causal distinction between these symptomatic phases highlights not only the difficulty in repurposing a single drug for use at all time-points, but also the need to approach with caution the comparison of trial data collected from patients given drugs at different times post-infection. The sample of one of the first clinical trials performed on patients testing positive upon PCR amplification of SARS-CoV-2 RNA comprised asymptomatic patients (17%), as well as those with upper (61%) and lower (22%) respiratory tract infections, thereby capturing the range of symptom severity. After 6 days of treatment, patients given HCQ alone had a higher probability of viral clearance compared to those given the standard of care only (57% vs. 13%), rising to 100% in patients also given azithromycin (Gautret et al., 2020). That the authors additionally discovered a greater drug effect on patients with upper and lower respiratory tract infections than on asymptomatic individuals raises the possibility that the potential therapeutic benefit of HCQ in COVID-19 patients lies in its capacity for immunomodulation. On a theoretical level, the anti-inflammatory effects of HCQ render such an effect possible. Indeed, through alkalinisation of early endosomes, CQ and HCQ could impair: PAMP-induced activation of TLR7 and TLR9 (Saitoh and Miyake, 2009), and, by extension, MMP-9 expression (Lim et al., 2006); antigen presentation by major histocompatibility complexes (MHCs) (Roche and Furuta, 2015; Guerriero, 2019); prostaglandin and thromboxane production (Nosál' and Jančinová, 2002); and T and B cell activation (Goldman et al., 2000), differentiation, and proliferation (Yang et al., 2018). Importantly, both SARS-CoV-2 (Chen et al., 2020; Conti et al., 2020) and related coronaviruses, such as SARS-CoV-1 (Wang et al., 2014) and MERS-CoV (Mahallawi et al., 2018), may incur pulmonary damage through TNF-(alpha) (Malaviya et al., 2017), which CQ and HCQ can down-regulate through p38 MAPK inhibition (Kono et al., 2008).

However, this study had several considerable limitations. In addition to the lack of randomisation and the use of a C_t of 35 rather than 40 as the threshold for viral clearance, the sample size of 36 was very small. Moreover, 23% of patients in the treatment, but none of those in the control, group were lost in the follow-up due to transfer to the ICU, disenrollment, or premature cessation, leaving the sample even further underpowered. Indeed, Bayesian reanalysis of the data demonstrates that the statistical evidence for efficacy weakens to anecdotally positive upon the exclusion of untested patients, and even to anecdotally negative with the assumption that untested patients were infected with SARS-CoV-2 (Hulme et al., 2020).

Nonetheless, one of the largest retrospective observational studies to date has shown that, despite no significant difference in 28-day mortality rate, among 4,642 COVID-19 patients of varying symptom severity, those treated with HCQ only exhibited a significantly higher rate of hospital discharge within the investigative timeframe (Sbidian et al., 2020). Yet a significantly higher proportion of treatment subjects were younger, perhaps confounding the causal link between grouping and clinical outcome. In agreement, however, many highly-powered case-control studies, with sample sizes of 2,541 (Arshad et al., 2020) and 1,498 (Bernaola et al., 2020) provide evidence of a fall in mortality and intubation risk associated with the administration of HCQ, irrespective of symptomatic phase or degree. Although another paper found a significantly lower mortality rate in patients given HCQ (19%), compared to those given the standard of care only (47%), there was no significant difference in the duration of hospital stay. Furthermore, among patients who died during the study, those given HCQ had remained in hospital significantly longer (15 days) than the control group (eight days) (Yu et al., 2020b). Moreover, this research suffered from a considerable imbalance in the sample sizes of treatment and control groups, while coadministration of interferons reached 11% in the latter, but was absent from the former. Other research coming to the same conclusion regarding the efficacy of CQ and/or HCQ in COVID-19 patients exhibited numerous shortcomings. Indeed, 67% of the observational studies showing higher probability of viral clearance or prophylaxis with low dose CQ and/or HCQ treatment were severely underpowered, sample sizes of only 373 (Huang et al., 2020a) and 106 (Bhattacharya et al., 2020), respectively. Likewise, outside the purview of this study's

database search, one RCT found that, compared to the control group administered lopinavir/ritonavir, patients with moderate and severe symptoms given CQ exhibited more than double the rate of improvement in CT scan indicators of pulmonary health, the sample consisted of only 22 individuals (Huang et al., 2020b).

Irrespective of statistical power, all observational studies suffer from a lack of randomisation, thereby generating a confounding effect that hinders valid inference of a causal association. By contrast, all RCTs in the library have failed to find any significant difference in the attainment of primary and secondary clinical outcomes between COVID-19 patients treated with CQ and/or HCQ and those given the standard of care (with or without additional antivirals and/or antibiotics). Indeed, the multi-centre RECOVERY trial discovered no significant difference in the 28-day risk of mortality between 4,716 patients of varying symptom severity in the treatment (27%) and control (25%) groups (Horby et al., 2020). In fact, those administered HCQ exhibited a significantly higher risk of symptomatic progression necessitating the use of invasive mechanical ventilation as a form of therapeutic intervention. Smaller RCTs with mild-to-moderate phenotype also reveal no significant improvement in viral clearance or time to recession of clinical symptom presentation (kamran et al., 2020; Tang et al., 2020a). Notably, however, no RCT employed the use of either a placebo or masking to prevent unwanted confounding.

Nevertheless, many observational studies support the absence of significant evidence CQ and/or HCQ efficacy compared to that of standard of care alone. Furthermore, the homogeneity of, and correction for, baseline characteristics in the case and control cohorts further buttresses the reliability of the evidence presented by studies with sample sizes ranging from 1,376 (Geleris et al., 2020) to 3,372 (Singh et al., 2020b). One such study, however – with a sample size of 2,512 – used a treatment cohort with a significantly lower age but greater symptomatic disease compared to the control (Ip et al., 2020). Similarly, the vast majority of papers contending that CQ and/or HCQ delayed SARS-CoV-2 clearance in COVID-19 patients were severely underpowered and exhibited significant differences in sample structure and/or comorbidities, nullifying their influence on the conclusion of this review (Mahévas et al., 2020).

As such, it is reasonable to conclude that, at present, the highest quality evidence does not support the efficacy of either CQ or HCQ in the prophylaxis or treatment of patients at high risk of, or diagnosed with, COVID-19, relative to the standard in-hospital management of symptoms. Nevertheless, the completion of more RCTs will be necessary to further substantiate this claim.

4.3. Clinical evidence of the safety of CQ and HCQ in the treatment of COVID-19

Robust evidence for the safety of an otherwise efficacious drug is a prerequisite for its widespread application in any clinical setting. In light of the unsubstantiated benefit of CQ and/or HCQ administration in patients with COVID-19, there exists an even more compelling imperative to ensure that any compassionate use did, and does, not contribute to excess mortality.

Despite their adequate safety records in an anti-inflammatory context, as aminoquinolines, CQ, and its derivative, HCQ, are proarrhythmic (Khobragade et al., 2013). Arrythmias arise from an imbalance of the normal physiological variables influencing the activation and inactivation kinetics of the cardiac ion channels that permit the transmembrane currents forming the foundation of the cardiac action potential. In a healthy *milieu intérieur*, the waveform of this cardiac action potential is quadriphasic (Noble, 1962). Electrical diastole (IV) precedes a rapid depolarisation (0), after which a refractory period is followed by a slow hyperpolarisation (I), a 300 ms plateau (II), and then a period of repolarisation (III) to resting membrane potential (E_m). By blocking – in order of increasing potency – the delayed (I_{Kr}) and inwardly-rectifying (I_{K1}) K^+ currents (Sánchez-Chapula et al., 2001), and the latter preferentially at depolarised E_m, CQ and HCQ significantly prolong the QT interval and slow ventricular conduction, thereby predisposing to early-after-depolarisation and, by extension, *torsades de pointess*. Combined with their tonic block of voltage-gated Na^+ and L-type Ca^{2+} currents at low channel opening frequencies, QT interval prolongation thus significantly increases the risk of potentially fatal VTs.

Indeed, despite the only randomised clinical trials examining prophylaxis having found no increases in adverse cardiac events upon HCQ administration to patients at risk of COVID-19 (Mitja et al., 2020, Boulware et al., 2020), there is some evidence that aminoquinoline treatment predisposes those diagnosed with confirmed infections to tachyarrhythmia. In fact, a significant association of high doses of CQ with lethality in patients with severe symptoms forced the premature termination of a RCT (Borba et al., 2020). Despite this relationship with mortality risk disappearing upon correction for age, there remained a significantly higher proportion of patients in the high (19%) compared to the low (11%) dose group with QTcF > 500 ms, including three percent of patients who experienced VT before death. However, given the abortion of the study, as well as the co-administration of QT-prolonging oseltamivir (Hama and Bennett, 2017) confounding the causal link to cardiac side-effects, these data, alone, are insufficient to conclude that CQ is unsafe in COVID-19 patients.

Notably, the only paper failing this review's quality appraisal suggested a significant excess mortality in patients treated with CQ and/or HCQ relative to controls. A highly-powered observational study of 96,032 patients with no significant differences in comorbidities between groups, it claimed that patients given CQ (four percent) or HCQ (six percent) alone had a significantly augmented risk of *de novo* in-hospital ventricular arrhythmias, compared to controls (three-tenths of one percent) given standard therapy, including remdesivir (Mehra et al., 2020). However, within one day of investigative journalists' discovery of inconsistencies in the reporting of the data from international hospitals involved in the study, over 140 signatories penned an open letter to the authors of the paper and the journal in which it was published, also criticising the statistical analysis and lack of ethics review. Despite the study's consequent correction of the continental assignment of one hospital, which had no impact on its overall findings, questions remain over the lack of transparency of the company that managed the original databases. On June 4, 2020, three of the four co-authors retracted the paper (Mehra et al., n. d.), thereby necessitating caution when interpreting its original findings. Nonetheless, a more recent observational study of 2,512 patients suspected of, or diagnosed with, COVID-19, has elucidated a significantly higher proportion of cardiac-attributable mortality in the HCQ treatment (21%) than the control (16%) group. Moreover, despite being individually underpowered, several smaller retrospective database searches focusing specifically on the QTc duration have consistently and independently supported a significant prolongation in hospitalised COVID-19 patients treated with a range of CQ and/or HCQ doses (Saleh et al., 2020; Mercuro et al., 2020). Regardless, drawing causal inferences from such observational studies is inadvisable given the lack of randomisation and absence of a placebo in the control groups, leaving the data susceptible to unmeasured confounders.

By contrast, the most highly powered RCT found no evidence of a significant excess of *de novo* cardiac arrhythmias in COVID-19 patients of varying severity given HCQ for 10 days, or until discharge (Horby et al., 2020). Likewise, many of the largest retrospective case-control studies passing quality appraisal demonstrated an absence of a statistically significant difference in the incidences of VTs, fibrillation, or sudden cardiac deaths (SCDs) between hospitalised patients administered HCQ and those

provided with the standard of care alone (Singh et al., 2020a; Arshad et al., 2020).

In any case, from a theoretical standpoint, the cardiac safety risk of CQ and HCQ use is unlikely uniform among COVID-19 patients. Indeed, there is a significant positive correlation between baseline QTc and age (Reardon and Malik, 1996), QTc prolongation and anti-dysrhythmic, antipsychotic or macrolide antibiotic co-administration (Al-Khatib et al., 2003), and QT interval dispersion and mortality risk in type II diabetes mellitus (Giunti et al., 2012). Given the relative risk conferred by both older age ("The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China," 2020) and type II diabetes mellitus (Richardson et al., 2020) on symptom severity and consequent probability of hospitalisation of COVID-19 patients, the data for in-hospital CQ/HCQ safety may not be extrapolable to many infected individuals in the population due to selection bias (Henderson and Page, 2007). As such, the wide variation in average sample patient age, symptom severity and drug dosing regimen (Table 2) further complicates inferences of reliable agreement between the papers.

Therefore, as of Aug 8, 2020, there is a lack of strong evidence that, relative to standard in-hospital management of symptoms, the use of CQ and HCQ to treat hospitalised COVID-19 patients has been either distinctly safe or unsafe. The substantiation, of presently unclear strength, for the tendency of the aminoquinolines to prolong QTc in this therapeutic context may still provide cause for concern in patients whose comorbidities predispose them to VTs. The ongoing inquiries into the validity of data sets indicating a lack of drug safety, however, should preclude definitive judgment on the matter until the completion of more high-quality RCTs.

5. Limitations

Crucial to the understanding of the conclusions drawn in this systematic review is an appreciation of its many limitations, which relate to both the search methodology and data analysis.

Insofar as peer-reviewed publications are concerned, this review searched two databases to yield only 144 unique results, leaving the authors to also seek the findings of 69 papers on two preprint servers. Despite facilitating the collection of a more representative sample of current research on the subject in question, the absence of documented expert scrutiny ought to prevent their data from influencing clinical decisions. Nevertheless, to compensate for the lack of peer review, rigorous application of the quality appraisal criteria established by the GEA-RIME committee and the Task Force of Academic Medicine ensured that only data from adequately designed studies were taken into account. Importantly, however, that peer-reviewed journal material was no longer a prerequisite for inclusion may have slightly reduced the effects of positive publication bias (Mlinarić et al., 2017) on the results of this systematic review.

In light of the recent investigation into the integrity of the data collection and statistical analysis of the largest observational study to date (Mehra et al., 2020), it is also necessary to advocate caution in the interpretation of results, even if the paper in which they are presented has undergone peer review. Indeed, worries concerning the decline in review duration and even scrutiny, as a result of the rush to publish, should elicit wariness in considering even the most fundamental of underlying assumptions; namely, that a data set is valid.

However, the predominant shortcoming of the review is its inability to completely disentangle the large differences in study design when making comparisons between different data sets from the included papers. Indeed, despite stressing the obvious invalidity of cutting across distinct sample sizes, baseline characteristics, drug doses, and individual limitations, a systematic review, by nature, does exactly that. The categorisation of the results and data analysis by randomisation, COVID-19 symptom severity, and HCQ/CQ dosage constitutes an attempt to reduce this problem of comparative inferences as greatly as possible.

6. Conclusions

On March 18, 2020, the WHO announced the launch of an international phase III-IV RCT, with four arms, measuring the efficacy and safety of: (1) remdesivir; (2) lopinavir and ritonavir; (3) lopinavir, ritonavir, and IFN-(beta); and (4) CQ or HCQ (Kupferschmidt, 2020). In the meantime, amidst a dearth of high-quality evidence from completed randomised clinical trials, the U.S. Food and Drug Administration (FDA) issued an emergency use authorisation of CQ and HCQ in COVID-19 patients (Piller, 2020).

Since then, data from the most robust of the completed case-control studies have failed to find any significant evidence of the efficacy of CQ and/or HCQ in the treatment of hospitalised COVID-19 patients. Meanwhile, on May 22, 2020, in the midst of a large number of research groups finding evidence of significant QTc prolongation upon administration of aminoquinoline drugs, a recent large retrospective observational study indicated a possible lack of drug (particularly cardiac) safety in this clinical context. On May 25, 2020, the WHO suspended the fourth arm of the Solidarity Trial, citing these safety concerns. In the UK, the Medicines and Healthcare products Regulatory Agency (MHRA) followed suit, calling the COPCOV trial to a halt. Less than one week later, however, an international coalition of scientists raised concerns regarding the integrity of the collection and analysis of the inaccessible data on which the suspension was based, and the responsible paper has since been retracted. The MHRA has authorised the COPCOV trial to recommence. Following the WHO's recontinuation of the Solidarity Trial arm, interim results provided little evidence of a reduction in mortality risk for hospitalised COVID-19 patients administered CQ and/or HCQ, ultimately leading to its indefinite discontinuation on July 4, 2020.

The urge to begin all international and national clinical trials arose from the necessary desire to rapidly compensate for the prior and present scarcity of randomised data on the efficacy and safety of CQ and/or HCQ in patients infected with SARS-CoV-2. In the absence of evidence for the safety of CQ and HCQ in COVID-19 patients, the FDA's initial decision to authorise their use in hospitalised patients was of questionable scientific prudence.

Indeed, this review finds that, despite data from different *in vivo* studies conflicting and even contradicting each other, the strongest evidence does not support the efficacy of either CQ or HCQ in the prophylaxis or treatment of patients at high risk of, or diagnosed with, COVID-19, relative to the standard in-hospital management of symptoms. Likewise, there is a lack of strong evidence that the use of CQ and HCQ to treat hospitalised COVID-19 patients has had a distinct effect on safety outcomes.

Yet it is precisely because of this dearth of evidence that there still exists a pressing demand for RCTs. In fact, the saga ensuing from the hastiness in accepting the findings of the now-retracted observational study is a case in point of the need to collect randomised clinical data in order to substantiate or contend any claims of drug efficacy and/or safety.

Declaration of Competing Interest

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

References

Al-Khatib, S.M., Allen LaPointe, N.M., Kramer, J.M., Califf, R.M., 2003. What clinicians should know about the QT interval. J. Am. Med. Assoc.. https://doi.org/10.1001/jama.289.16.2120.

Aljofan, M., Gaipov, A., 2020. Chloroquine and COVID-19: a light at the end of the tunnel, or is it another train?. Electron. J. Gen. Med. 17.

An, M.H., Kim, M.S., Park, Y., Kim, B.-O., Kang, S.H., Kimn, W.J., Park, S.K., Park, H.-W., Yang, W., Jang, J., Jang, S., Hwang, T.-H., 2020. Treatment Response to Hydroxychloroquine and Antibiotics for mild to moderate COVID-19: a retrospective cohort study from South Korea. medRxiv 2020.07.04.20146548. https://doi.org/10.1101/2020.07.04.20146548..

Arshad, S., Kilgore, P., Chaudhry, Z.S., Jacobsen, G., Wang, D.D., Huitsing, K., Brar, I., Alangaden, G.J., Ramesh, M.S., McKinnon, J.E., O'Neill, W., Zervos, M., 2020. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. Int. J. Infect. Dis. IJID Off Publ. Int. Soc. Infect. Dis. 97, 396–403. https://doi.org/10.1016/j.ijid.2020.06.099.

Bernaola, N., Mena, R., Bernaola, A., Lara, A., Carballo, C., Larranaga, P., Bielza, C., 2020. Observational study of the efficiency of treatments in patients hospitalized with Covid-19 in madrid. medRxiv 2020.07.17.20155960. https://doi.org/10.1101/2020.07.17.20155960..

Bertrand, E., Cloitre, B., Ticolat, R., Kouad Bile, R., Gautier, C., Obou Abiyou, G., Yao Bohui, B., 1990. Antiaggregation of chloroquine. Med. Trop..

Bhattacharya, R., Chowdhury, S., Mukherjee, R., Nandi, A., Kulshrestha, M., Ghosh, R., Saha, S., 2020. Pre exposure Hydroxychloroquine use is associated with reduced COVID19 risk in healthcare workers - a Retrospective cohort. medRxiv 2020.06.09.20116806. https://doi.org/10.1101/2020.06.09.20116806.

Borba, M.G.S., Val, F.F.A., Sampaio, V.S., Alexandre, M.A.A., Melo, G.C., Brito, M., Mourão, M.P.G., Brito-Sousa, J.D., Baía-da-Silva, D., Guerra, M.V.F., Hajjar, L.A., Pinto, R.C., Balieiro, A.A.S., Pacheco, A.G.F., Santos, J.D.O., Naveca, F.G., Xavier, M. S., Siqueira, A.M., Schwarzbold, A., Croda, J., Nogueira, M.L., Romero, G.A.S., Bassat, Q., Fontes, C.J., Albuquerque, B.C., Daniel-Ribeiro, C.-T., Monteiro, W.M., Lacerda, M.V.G., 2020. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. JAMA Netw. Open. https://doi.org/10.1001/jamanetworkopen.2020.8857..

Bordage, G., Caelleigh, A.S., Steinecke, A., Bland, C.J., Crandall, S.J., McGaghie, W.C., Pangaro, L.N., Regehr, G., Shea, J.A., 2001. Joint task force of academic medicine and the GEA-RIME committee. Acad. Med..

Boulware, D.R., Pullen, M.F., Bangdiwala, A.S., Pastick, K.A., Lofgren, S.M., Okafor, E. C., Skipper, C.P., Nascene, A.A., Nicol, M.R., Abassi, M., Engen, N.W., Cheng, M.P., LaBar, D., Lother, S.A., MacKenzie, L.J., Drobot, G., Marten, N., Zarychanski, R., Kelly, L.E., Schwartz, I.S., McDonald, E.G., Rajasingham, R., Lee, T.C., Hullsiek, K. H., 2020. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. N. Engl. J. Med.. https://doi.org/10.1056/NEJMoa2016638.

Carter, A.E., Eban, R., 1974. Prevention of postoperative deep venous thrombosis in legs by orally administered hydroxychloroquine sulphate. Br. Med. J.. https://doi.org/10.1136/bmj.3.5923.94.

Chen, C.Y., Wang, F.L., Lin, C.C., 2006. Chronic hydroxychloroquine use associated with QT prolongation and refractory ventricular arrhythmia. Clin. Toxicol. https://doi.org/10.1080/15563650500514558..

Chen, G., Wu, D., Guo, W., Cao, Y., Huang, D., Wang, H., Wang, T., Zhang, Xiaoyun, Chen, H., Yu, H., Zhang, Xiaoping, Zhang, M., Wu, S., Song, J., Chen, T., Han, M., Li, S., Luo, X., Zhao, J., Ning, Q., 2020. Clinical and immunologic features in severe and moderate Coronavirus Disease 2019. J. Clin. Invest.. https://doi.org/10.1172/JCI137244.

Cheng, V.C.C., Hung, I.F.N., Tang, B.S.F., Chu, C.M., Wong, M.M.L., Chan, K.H., Wu, A.K. L., Tse, D.M.W., Chan, K.S., Zheng, B.J., Peiris, J.S.M., Sung, J.J.Y., Yuen, K.Y., 2004. Viral replication in the nasopharynx is associated with diarrhea in patients with severe acute respiratory syndrome. Clin. Infect. Dis. https://doi.org/10.1086/382681..

Conti, P., Gallenga, C.E., Tetè, G., Caraffa, A., Ronconi, G., Younes, A., Toniato, E., Ross, R., Kritas, S.K., 2020. How to reduce the likelihood of coronavirus-19 (CoV-19 or SARS-CoV-2) infection and lung inflammation mediated by IL-1. J. Biol. Regul. Homeost. Agents. https://doi.org/10.23812/Editorial-Conti-2..

Coutard, B., Valle, C., de Lamballerie, X., Canard, B., Seidah, N.G., Decroly, E., 2020. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res.. https://doi.org/10.1016/j.antiviral.2020.104742.

Cucinotta, D., Vanelli, M., 2020. WHO declares COVID-19 a pandemic. Acta Biomed.. https://doi.org/10.23750/abm.v91i1.9397.

Dong, E., Du, H., Gardner, L., 2020. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect. Dis. https://doi.org/10.1016/S1473-3099(20)30120-1..

Fantini, J., Di Scala, C., Chahinian, H., Yahi, N., 2020. Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. Int. J. Antimicrob. Agents. https://doi.org/10.1016/j.ijantimicag.2020.105960..

Gautret, P., Lagier, J.-C., Parola, P., Hoang, V.T., Meddeb, L., Mailhe, M., Doudier, B., Courjon, J., Giordanengo, V., Vieira, V.E., Dupont, H.T., Honoré, S., Colson, P., Chabrière, E., La Scola, B., Rolain, J.-M., Brouqui, P., Raoult, D., 2020. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int. J. Antimicrob. Agents. https://doi.org/10.1016/j.ijantimicag.2020.105949..

Geleris, J., Sun, Y., Platt, J., Zucker, J., Baldwin, M., Hripcsak, G., Labella, A., Manson, D., Kubin, C., Barr, R.G., 2020. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. N. Engl. J. Med..

Giunti, S., Amione, C., Gruden, G., Ghezzo, G., Fornengo, P., Cavallo-Perin, P., Barutta, F., Bruno, G., 2012. Increased QT nterval dispersion predicts 15-year cardiovascular mortality in type 2 diabetic subjects: The population-based Casale Monferrato Study. Diabetes Care. https://doi.org/10.2337/dc11-1397.

Goldman, F.D., Gilman, A.L., Hollenback, C., Kato, R.M., Premack, B.A., Rawlings, D.J., 2000. Hydroxychloroquine inhibits calcium signals in T cells: A new mechanism to explain its immunomodulatory properties. Blood. https://doi.org/10.1182/blood.v95.11.3460.011k26_3460_3466.

Gorbalenya, A.E., Baker, S.C., Baric, R.S., de Groot, R.J., Drosten, C., Gulyaeva, A.A., Haagmans, B.L., Lauber, C., Leontovich, A.M., Neuman, B.W., Penzar, D., Perlman, S., Poon, L.L.M., Samborskiy, D. V., Sidorov, I.A., Sola, I., Ziebuhr, J., 2020. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat. Microbiol. https://doi.org/10.1038/s41564-020-0695-z..

Guerriero, J.L., 2019. Macrophages: Their Untold Story in T Cell Activation and Function. Int. Rev. Cell Mol. Biol. https://doi.org/10.1016/bs.ircmb.2018.07.001..

Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Pere, H., Charbit, B., Bondet, V., Chenevier-Gobeaux, C., Breillat, P., Carlier, N., Gauzit, R., Morbieu, C., Pene, F., Marin, N., Roche, N., Szwebel, T.-A., Smith, N., Merkling, S., Treluyer, J.-M., Veyer, D., Mouthon, L., Blanc, C., Tharaux, P.-L., Rozenberg, F., Fischer, A., Duffy, D., Rieux-Laucat, F., Kerneis, S., Terrier, B., 2020. Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients. medRxiv. https://doi.org/10.1101/2020.04.19.20068015..

Hama, R., Bennett, C.L., 2017. The mechanisms of sudden-onset type adverse reactions to oseltamivir. Acta Neurol. Scand.. https://doi.org/10.1111/ane.12629.

Hamming, I., Timens, W., Bulthuis, M.L.C., Lely, A.T., Navis, G.J., van Goor, H., 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J. Pathol.. https://doi.org/10.1002/path.1570.

He, X., Lau, E.H.Y., Wu, P., Deng, X., Wang, J., Hao, X., Lau, Y.C., Wong, J.Y., Guan, Y., Tan, X., Mo, X., Chen, Y., Liao, B., Chen, W., Hu, F., Zhang, Q., Zhong, M., Wu, Y., Zhao, L., Zhang, F., Cowling, B.J., Li, F., Leung, G.M., 2020. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat. Med. https://doi.org/10.1038/s41591-020-0869-5..

Henderson, M., Page, L., 2007. Appraising the evidence: What is selection bias? Evid. Based. Ment. Health. https://doi.org/10.1136/ebmh.10.3.67..

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., Müller, M.A., Drosten, C., Pöhlmann, S., 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. https://doi.org/10.1016/j.cell.2020.02.052.

Horby, P., Mafham, M., Linsell, L., Bell, J.L., Staplin, N., Emberson, J.R., Wiselka, M., Ustianowski, A., Elmahi, E., Prudon, B., Whitehouse, A., Felton, T., Williams, J., Faccenda, J., Underwood, J., Baillie, J.K., Chappell, L., Faust, S.N., Jaki, T., Jeffery, K., Lim, W.S., Montgomery, A., Rowan, K., Tarning, J., Watson, J.A., White, N.J., Juszczak, E., Haynes, R., Landray, M.J., 2020. Effect of Hydroxychloroquine in Hospitalized Patients with COVID-19: Preliminary results from a multi-centre, randomized, controlled trial. medRxiv 2020.07.15.20151852. https://doi.org/10.1101/2020.07.15.20151852..

Hsia, B.C., Greige, N., Quiroz, J.A., Khokhar, A.S., Daily, J., Di Biase, L., Ferrick, K.J., Fisher, J.D., Krumerman, A., 2020. QT prolongation in a diverse, urban population of COVID-19 patients treated with hydroxychloroquine, chloroquine, or azithromycin. J. Interv. Card. Electrophysiol. Int. J. Arrhythm. pacing 1–9. https://doi.org/10.1007/s10840-020-00822-x.

Huang, M., Li, M., Xiao, F., Liang, J., Pang, P., Tang, T., Liu, S., Chen, B., Shu, J., You, Y., Li, Yang, Tang, M., Zhou, J., Jiang, G., Xiang, J., Hong, W., He, S., Wang, Z., Feng, J., Lin, C., Ye, Y., Wu, Z., Li, Yaocai, Zhong, B., Sun, R., Hong, Z., Liu, J., Chen, H., Wang, X., Li, Z., Pei, D., Tian, L., Xia, J., Jiang, S., Zhong, N., Shan, H., 2020a. Preliminary evidence from a multicenter prospective observational study of the safety and efficacy of chloroquine for the treatment of COVID-19. medRxiv 2020.04.26.20081059. https://doi.org/10.1101/2020.04.26.20081059..

Huang, M., Tang, T., Pang, P., Li, M., Ma, R., Lu, J., Shu, J., You, Y., Chen, B., Liang, J., Hong, Z., Chen, H., Kong, L., Qin, D., Pei, D., Xia, J., Jiang, S., Shan, H., 2020. Treating COVID-19 with Chloroquine. J. Mol. Cell Biol.. https://doi.org/10.1093/jmcb/mjaa014.

Hulme, O.J., Wagenmakers, E.-J., Damkier, P., Madelung, C.F., Siebner, H.R., Helweg-Larsen, J., Gronau, Q., Benfield, T.L., Madsen, K.H., 2020. Reply to Gautret et al. 2020: A Bayesian reanalysis of the effects of hydroxychloroquine and azithromycin on viral carriage in patients with COVID-19. medRxiv. https://doi.org/10.1101/2020.03.31.20048777..

Ip, A., Berry, D.A., Hansen, E., Goy, A.H., Pecora, A.L., Sinclaire, B.A., Bednarz, U., Marafelias, M., Berry, S.M., Berry, N.S., Mathura, S., Sawczuk, I.S., Biran, N., Go, R. C., Sperber, S., Piwoz, J.A., Balani, B., Cicogna, C., Sebti, R., Zuckerman, J., Rose, K. M., Tank, L., Jacobs, L., Korcak, J., Timmapuri, S.L., Underwood, J.P., Sugalski, G., Barsky, C., Varga, D.W., Asif, A., Landolfi, J.C., Goldberg, S.L., 2020. Hydroxychloroquine and Tocilizumab Therapy in COVID-19 Patients - An Observational Study. medRxiv 2020.05.21.20109207. https://doi.org/10.1101/2020.05.21.20109207..

Jaimes, J.A., André, N.M., Chappie, J.S., Millet, J.K., Whittaker, G.R., 2020. Phylogenetic analysis and structural modeling of SARS-CoV-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop. J. Mol. Biol.. https://doi.org/10.1016/j.jmb.2020.04.009.

Jeevaratnam, K., 2020. Chloroquine and hydroxychloroquine for COVID-19: implications for cardiac safety. Eur. Hear.J. Cardiovasc. Pharmacother. https://doi.org/10.1093/ehjcvp/pvaa041..

Kamran, Sultan mehmood, Mirza, Z.E.H., Naseem, A., Saeed, F., Azam, R., Ullah, N., Ahmad, W., Saleem, S., 2020. Clearing the fog: Is HCQ effective in reducing COVID-19 progression: A randomized controlled trial. medRxiv 2020.07.30.20165365. https://doi.org/10.1101/2020.07.30.20165365..

Khobragade, S.B., Gupta, P., Gurav, P., Chaudhari, G., Gatne, M.M., Shingatgeri, V.M., 2013. Assessment of proarrhythmic activity of chloroquine in in vivo and ex vivo rabbit models. J. Pharmacol. Pharmacother.. https://doi.org/10.4103/0976-500X.110892.

Kim, J.-W., Kim, E.J., Kwon, H.H., Jung, C.Y., Kim, K.C., Choe, J.-Y., Hong, H.-L., 2020. Lopinavir-ritonavir versus hydroxychloroquine for viral clearance and clinical improvement in patients with mild to moderate coronavirus disease 2019. Korean J. Intern. Med. https://doi.org/10.3904/kjim.2020.224..

Klok, F.A., Kruip, M.J.H.A., van der Meer, N.J.M., Arbous, M.S., Gommers, D.A.M.P.J., Kant, K.M., Kaptein, F.H.J., van Paassen, J., Stals, M.A.M., Huisman, M. V., Endeman, H., 2020. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb. Res. https://doi.org/10.1016/j.thromres.2020.04.013..

Kono, M., Tatsumi, K., Imai, A.M., Saito, K., Kuriyama, T., Shirasawa, H., 2008. Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: Involvement of p38 MAPK and ERK. Antiviral Res.. https://doi.org/10.1016/j.antiviral.2007.10.011.

Kumar, S., Maurya, V.K., Prasad, A.K., Bhatt, M.L.B., Saxena, S.K., 2020. Structural, glycosylation and antigenic variation between 2019 novel coronavirus (2019-nCoV) and SARS coronavirus (SARS-CoV). VirusDisease. https://doi.org/10.1007/s13337-020-00571-5.

Kupferschmidt, K., 2020. WHO launches global megatrial of the four most promising coronavirus treatments. Science (80-). https://doi.org/10.1126/science.abb8497.

Kwiek, J.J., Haystead, T.A.J., Rudolph, J., 2004. Kinetic Mechanism of Quinone Oxidoreductase 2 and Its Inhibition by the Antimalarial Quinolines. Biochemistry. https://doi.org/10.1021/bi035923w.

Larissa, S., David, M., Mike, C., Davina, G., Alessandro, L., Mark P., et al. 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation BMJ 349 :g7647..

Lim, E.J., Lee, S.H., Lee, J.G., Chin, B.R., Bae, Y.S., Kim, J.R., Lee, C.H., Baek, S.H., 2006. Activation of toll-like receptor-9 induces matrix metalloproteinase-9 expression through Akt and tumor necrosis factor-α signaling. FEBS Lett. https://doi.org/10.1016/j.febslet.2006.06.100.

Liu, J., Cao, R., Xu, M., Wang, X., Zhang, H., Hu, H., Li, Y., Hu, Z., Zhong, W., Wang, M., 2020. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov.. https://doi.org/10.1038/s41421-020-0156-0.

Liu, W., Li, H., 2020. COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism. ChemRxiv. https://doi.org/10.26434/chemrxiv.11938173.v6.

Magagnoli, J., Narendran, S., Pereira, F., Cummings, T., Hardin, J.W., Sutton, S.S., Ambati, J., 2020. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. medRxiv. https://doi.org/10.1101/2020.04.16.20065920..

Mahallawi, W.H., Khabour, O.F., Zhang, Q., Makhdoum, H.M., Suliman, B.A., 2018. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine. https://doi.org/10.1016/j.cyto.2018.01.025.

Mahévas, M., Tran, V.-T., Roumier, M., Chabrol, A., Paule, R., Guillaud, C., Fois, E., Lepeule, R., Szwebel, T.-A., Lescure, F.-X., 2020. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data. bmj 369..

Malaviya, R., Laskin, J.D., Laskin, D.L., 2017. Anti-TNFα therapy in inflammatory lung diseases. Pharmacol. Ther. https://doi.org/10.1016/j.pharmthera.2017.06.008..

Mallat, J., Hamed, F., Balkis, M., Mohamed, M.A., Mooty, M., Malik, A., Nusair, A., Bonilla, F., 2020. Hydroxychloroquine is associated with slower viral clearance in clinical COVID-19 patients with mild to moderate disease: A retrospective study. medRxiv..

Marra, M.A., Jones, S.J.M., Astell, C.R., Holt, R.A., Brooks-Wilson, A., Butterfield, Y.S.N., Khattra, J., Asano, J.K., Barber, S.A., Chan, S.Y., Cloutier, A., Coughlin, S.M., Freeman, D., Girn, N., Griffith, O.L., Leach, S.R., Mayo, M., McDonald, H., Montgomery, S.B., Pandoh, P.K., Petrescu, A.S., Robertson, A.G., Schein, J.E., Siddiqui, A., Smailus, D.E., Stott, J.M., Yang, G.S., Plummer, F., Andonov, A., Artsob, H., Bastien, N., Bernard, K., Booth, T.F., Bowness, D., Czub, M., Drebot, M., Fernando, L., Flick, R., Garbutt, M., Gray, M., Grolla, A., Jones, S., Feldmann, H., Meyers, A., Kabani, A., Li, Y., Normand, S., Stroher, U., Tipples, G.A., Tyler, S., Vogrig, R., Ward, D., Watson, B., Brunham, R.C., Krajden, M., Petric, M., Skowronski, D.M., Upton, C., Roper, R.L., 2003. The genome sequence of the SARS-associated coronavirus. Science (80.-). https://doi.org/10.1126/science.1085953.

McGonagle, D., Sharif, K., O'Regan, A., Bridgewood, C., 2020. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmun. Rev. https://doi.org/10.1016/j.autrev.2020.102537..

Mehra, M.R., Desai, S.S., Ruschitzka, F., Patel, A.N., 2020. Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet.

Mehra, M.R., Ruschitzka, F., Patel, A.N., n.d. Retraction—Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet 0. https://doi.org/10.1016/S0140-6736(20)31324-6..

Mercuro, N.J., Yen, C.F., Shim, D.J., Maher, T.R., McCoy, C.M., Zimetbaum, P.J., Gold, H.S., 2020. Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for coronavirus disease 2019 (COVID-19). JAMA Cardiol..

Mfeukeu Kuate, L., Ngatchou, W., Temgoua, M.N., Kouanfack, C., Lemogoum, D., Noutakdie Tochie, J., Zemsi, A., Fomete, L., Lekelem, S., Zemsi, S., Tambekou Sobngwi, J., Ntandzi, T., Ngongang Ouankou, C., Wasnyo, Y., Ntsama Assiga, A., Nkeck, J.R., Musa Jingi, A., Guewo, M., Pefura Yone, E.W., Omgba Moussi, C., Owono Etoundi, P., Yombi, J.C., Menanga, A.P., Kingue, S., Ze Minkande, J., Mbanya, J.C., Ongolo Zogo, P.C., Fouda, P.J., Sobngwi, E., 2020. Electrocardiographic safety of daily Hydroxychloroquine 400mg plus Azithromycin 250mg as an ambulatory treatment for COVID-19 patients in Cameroon. medRxiv 2020.06.24.20139386. https://doi.org/10.1101/2020.06.24.20139386.

Mitja, O., Ubals, M., Corbacho, M., Alemany, A., Suner, C., Tebe, C., Tobias, A., Penafiel, J., Ballana, E., Perez, C.A., Admella, P., Riera-Marti, N., Laporte, P., Mitja, J., Clua, M., Bertran, L., Gavilan, S., Ara, J., Sarquella, M., Argimon, J.M., Cuatrecasas, G., Canadas, P., Elizalde-Torrent, A., Fabregat, R., Farre, M., Forcada, A., Flores-Mateo, G., Lopez, C., Muntada, E., Nadal, N., Narejos, S., Gil-Ortega, A.N., Prat, N., Puig, J., Quinones, C., Ramirez-Viaplana, F., Reyes-Uruena, J., Riveira-Munoz, E., Ruiz, L., Sanz, S., Sentis, A., Sierra, A., Velasco, C., Vivanco-Hidalgo, R.M., Zamora, J., Casabona, J., Vall-Mayans, M., G-Beiras, C., Clotet, B., 2020. A Cluster-Randomized Trial of Hydroxychloroquine as Prevention of Covid-19 Transmission and Disease. medRxiv 2020.07.20.20157651. https://doi.org/10.1101/2020.07.20.20157651..

Mlinarić, A., Horvat, M., Smolčić, V.Š., 2017. Dealing with the positive publication bias: Why you should really publish your negative results. Biochem. Medica. https://doi.org/10.11613/BM.2017.030201..

Molina, J.M., Delaugerre, C., Le Goff, J., Mela-Lima, B., Ponscarme, D., Goldwirt, L., de Castro, N., 2020. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Med. Mal. Infect. https://doi.org/10.1016/j.medmal.2020.03.006..

Negoescu, A., Thornback, A., Wong E., Ostor, A.J., 2013. Long QT and hydroxychloroquine; a poorly recognised problem in rheumatology patients. Arthritis Rheum..

Noble, D., 1962. A modification of the Hodgkin—Huxley equations applicable to Purkinje fibre action and pacemaker potentials. J. Physiol.. https://doi.org/10.1113/jphysiol.1962.sp006849.

Nosál', R., Jančinová, V., 2002. Cationic amphiphilic drugs and platelet phospholipase A2 (cPLA2). Thromb. Res. https://doi.org/10.1016/S0049-3848(02)00036-1..

Oteo, J.A., Marco, P., Ponce de León, L., Roncero, A., Lobera, T., Lisa, V., 2020. A short therapeutic regimen based on hydroxychloroquine plus azithromycin for the treatment of COVID-19 in patients with non-severe disease. A strategy associated with a reduction in hospital admissions and complications. medRxiv 2020.06.10.20101105. https://doi.org/10.1101/2020.06.10.20101105..

Ozden, S., Lucas-Hourani, M., Ceccaldi, P.E., Basak, A., Valentine, M., Benjannet, S., Hamelin, J., Jacob, Y., Mamchaoui, K., Mouly, V., Desprès, P., Gessain, A., Butler-Browne, G., Chrétien, M., Tangy, F., Vidalain, P.O., Seidah, N.G., 2008. Inhibition of Chikungunya virus infection in cultured human muscle cells by furin inhibitors: Impairment of the maturation of the E2 surface glycoprotein. J. Biol. Chem.. https://doi.org/10.1074/jbc.M802444200.

Pilcher, D.B., 1975. Hydroxychloroquine sulfate in prevention of thromboembolic phenomena in surgical patients. Am. Surg..

Piller, C., 2020. Former FDA leaders decry emergency authorization of malaria drugs for coronavirus. Science (80-). https://doi.org/10.1126/science.abc1337.

Poissy, J., Goutay, J., Caplan, M., Parmentier, E., Duburcq, T., Lassalle, F., Jeanpierre, E., Rauch, A., Labreuche, J., Susen, S., 2020. Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence. Circulation. https://doi.org/10.1161/circulationaha.120.047430.

Raines, M.F., Bhargava, S.K., Rosen, E.S., 1989. The blood-retinal barrier in chloroquine retinopathy. Investig. Ophthalmol. Vis. Sci..

Rand, J., Wu, X.-X., Ashton, A., 2008. Hydroxychloroquine Protects the Annexin A5 Anticoagulant Shield from Disruption by Antiphospholipid Antibodies. Blood. https://doi.org/10.1182/blood.v112.11.404.404.

Reardon, M., Malik, M., 1996. QT interval change with age in an overtly healthy older population. Clin. Cardiol. https://doi.org/10.1002/clc.4960191209.

Richardson, S., Hirsch, J.S., Narasimhan, M., Crawford, J.M., McGinn, T., Davidson, K.W., Barnaby, D.P., Barnaby, D.P., Becker, L.B., Chelico, J.D., Cohen, S.L., Cookingham, J., Coppa, K., Diefenbach, M.A., Dominello, A.J., Duer-Hefele, J., Falzon, L., Gitlin, J., Hajizadeh, N., Harvin, T.G., Hirschwerk, D.A., Kim, E.J., Kozel, Z.M., Marrast, L.M., Mogavero, J.N., Osorio, G.A., Qiu, M., Zanos, T.P., 2020. Presenting Characteristics, Comorbidities, and Outcomes among 5700 Patients Hospitalized with COVID-19 in the New York City Area. JAMA - J. Am. Med. Assoc. https://doi.org/10.1001/jama.2020.6775.

Riva, J.J., Malik, K.M.P., Burnie, S.J., Endicott, A.R., Busse, J.W., 2012. What is your research question? An introduction to the PICOT format for clinicians. J. Can. Chiropr. Assoc..

Roche, P.A., Furuta, K., 2015. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat. Rev. Immunol. https://doi.org/10.1038/nri3818.

Saitoh, S.I., Miyake, K., 2009. Regulatory molecules required for nucleotide-sensing Toll-like receptors. Immunol. Rev. https://doi.org/10.1111/j.1600-065X.2008.00729.x.

Saleh, M., Gabriels, J., Chang, D., Kim, B.S., Mansoor, A., Mahmood, E., Makker, P., Ismail, H., Goldner, B., Willner, J., Beldner, S., Mitra, R., John, R., Chinitz, J., Skipitaris, N., Mountantonakis, S., Epstein, L.M., 2020. The Effect of Chloroquine, Hydroxychloroquine and Azithromycin on the Corrected QT Interval in Patients with SARS-CoV-2 Infection. Circ. Arrhythmia Electrophysiol. https://doi.org/10.1161/CIRCEP.120.008662.

Sánchez-Chapula, J.A., Salinas-Stefanon, E., Torres-Jácome, J., Benavides-Haro, D.E., Navarro-Polanco, R.A., 2001. Blockade of currents by the antimalarial drug chloroquine in feline ventricular myocytes. J. Pharmacol. Exp. Ther..

Sbidian, E., Josse, J., Lemaitre, G., Mayer, I., Bernaux, M., Gramfort, A., Lapidus, N., Paris, N., Neuraz, A., Lerner, I., Garcelon, N., Rance, B., Grisel, O., Moreau, T., Bellamine, A., Wolkenstein, P., Varoquaux, G., Caumes, E., Lavielle, M., Mekontso Dessap, A., Audureau, E., 2020. Hydroxychloroquine with or without azithromycin and in-hospital mortality or discharge in patients hospitalized for COVID-19 infection: a cohort study of 4,642 in-patients in France. medRxiv 2020.06.16.20132597. https://doi.org/10.1101/2020.06.16.20132597..

Singh, A.K., Singh, A., Shaikh, A., Singh, R., Misra, A., 2020a. Chloroquine and hydroxychloroquine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries. Diabetes Metab. Syndr. 14, 241–246. https://doi.org/10.1016/j.dsx.2020.03.011.

Singh, S., Khan, A., Chowdhry, M., Chatterjee, A., 2020. Outcomes of hydroxychloroquine treatment among hospitalized COVID-19 patients in the United States-real-world evidence from a federated electronic medical record network. medRxiv..

Tang, W., Cao, Z., Han, M., Wang, Z., Chen, J., Sun, W., Wu, Y., Xiao, W., Liu, S., Chen, E., Chen, W., Wang, X., Yang, J., Lin, J., Zhao, Q., Yan, Y., Xie, Z., Li, D., Yang, Y., Liu, L., Qu, J., Ning, G., Shi, G., Xie, Q., 2020. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. medRxiv. https://doi.org/10.1101/2020.04.10.20060558.

Tang, X., Wu, C., Li, X., Song, Y., Yao, X., Wu, X., Duan, Y., Zhang, H., Wang, Y., Qian, Z., Cui, J., Lu, J., 2020. On the origin and continuing evolution of SARS-CoV-2. Natl. Sci. Rev. https://doi.org/10.1093/nsr/nwaa036..

The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China, 2020. Zhonghua Liu Xing Bing Xue Za Zhi. https://doi.org/10.3760/cma.j.issn.0254-6450.2020.02.003..

Vincent, M.J., Bergeron, E., Benjannet, S., Erickson, B.R., Rollin, P.E., Ksiazek, T.G., Seidah, N.G., Nichol, S.T., 2005. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol. J.. https://doi.org/10.1186/1743-422X-2-69.

Wang, H., Yang, P., Liu, K., Guo, F., Zhang, Y., Zhang, G., Jiang, C., 2008. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. Cell Res. https://doi.org/10.1038/cr.2008.15.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., Xiao, G., 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. https://doi.org/10.1038/s41422-020-0282-0.

Wang, S.F., Tseng, S.P., Yen, C.H., Yang, J.Y., Tsao, C.H., Shen, C.W., Chen, K.H., Liu, F.T., Liu, W.T., Chen, Y.M.A., Huang, J.C., 2014. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. Biochem. Biophys. Res. Commun.. https://doi.org/10.1016/j.bbrc.2014.07.090.

Wolfram, J., Nizzero, S., Liu, H., Li, F., Zhang, G., Li, Z., Shen, H., Blanco, E., Ferrari, M., 2017. A chloroquine-induced macrophage-preconditioning strategy for improved nanodelivery. Sci. Rep. https://doi.org/10.1038/s41598-017-14221-2..

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B. S., McLellan, J.S., 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science (80-). https://doi.org/10.1126/science.aax0902.

Wu, Z., McGoogan, J.M., 2020. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China. JAMA. https://doi.org/10.1001/jama.2020.2648.

Yam, J.C.S., Kwok, A.K.H., 2006. Ocular toxicity of hydroxychloroquine. Hong Kong. Med. J..

Yang, Ji, Yang, X., Yang, Jie, Li, M., 2018. Hydroxychloroquine inhibits the differentiation of Th17 cells in systemic lupus erythematosus. J. Rheumatol.. https://doi.org/10.3899/jrheum.170737.

Yang, Y., Shen, C., Li, J., Yuan, J., Yang, M., Wang, F., Li, G., Li, Y., Xing, L., Peng, L., Wei, J., Cao, M., Zheng, H., Wu, W., Zou, R., Li, D., Xu, Z., Wang, H., Zhang, M., Zhang, Z., Liu, L., Liu, Y., 2020. Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. medRxiv. https://doi.org/10.1101/2020.03.02.20029975.

Yao, X., Ye, F., Zhang, M., Cui, C., Huang, B., Niu, P., Liu, X., Zhao, L., Dong, E., Song, C., Zhan, S., Lu, R., Li, H., Tan, W., Liu, D., 2020. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin. Infect. Dis. https://doi.org/10.1093/cid/ciaa237..

Yu, B., Li, C., Chen, P., Zhou, N., Wang, L., Li, J., Jiang, H., Wang, D.-W., 2020a. Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19. Sci. China. Life Sci. 1.

Yu, B., Li, C., Chen, P., Zhou, N., Wang, L., Li, J., Jiang, H., Wang, D.-W., Bo, Y., Chenze, L., Peng, C., Ning, Z., Luyun, W., Jia, L., Hualiang, J., Dao-Wen, W., 2020b. Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19. Sci. CHINA-LIFE Sci. 1. https://doi.org/10.1007/s11427-020-1732-2.

Zhang, L., Yan, X., Fan, Q., Liu, H., Liu, X., Liu, Z., Zhang, Z., 2020. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. J. Thromb. Haemost.. https://doi.org/10.1111/jth.14859.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., Cao, B., 2020a. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. https://doi.org/10.1016/S0140-6736(20)30566-3.

Zhou, P., Yang, X. Lou, Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., Chen, H.D., Chen, J., Luo, Y., Guo, H., Jiang, R. Di, Liu, M.Q., Chen, Y., Shen, X.R., Wang, X., Zheng, X.S., Zhao, K., Chen, Q.J., Deng, F., Liu, L.L., Yan, B., Zhan, F.X., Wang, Y.Y., Xiao, G.F., Shi, Z.L., 2020b. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. https://doi.org/10.1038/s41586-020-2012-7.