# Hydroxychloroquine *Versus* COVID-19: A Rapid Systematic Review and Meta-Analysis

Amir Shamshirian[1,2], Amirhossein Hessami[3], Keyvan Heydari[2,3], Reza Alizadeh-Navaei[2*], Mohammad Ali Ebrahimzadeh[4], George W. YIP[5], Roya Ghasemian[6], Morteza Behnamfar[7], Hananeh Baradaran[8], Elham Aboufazeli[9], Hamed Jafarpour[3], Keyvan Karimifar[10], Aida Eftekhari[10], Danial Shamshirian[11*]

1. Department of Medical Laboratory Sciences, Student Research Committee, School of Allied Medical Science, Mazandaran University of Medical Sciences, Sari, Iran.

2. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

3. Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

4. Pharmaceutical Sciences Research Center, Department of Medicinal Chemistry, School of Pharmacy, Mazandaran University of Medical Science, Sari, Iran.

5. Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

6. Antimicrobial Resistance Research Center, Department of Infectious Diseases, Mazandaran University of Medical Sciences, Sari, Iran.

7. Student Research Committee, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.

8. Clinical Pharmacy Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

9. Department of Research and Development, IRAN NAJO Pharmaceutical Company, Tehran, Iran.

10. Student Research Committee, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

11. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Corresponding authors:
1. Danial Shamshirian, Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-Mail: shamshirian@sbmu.ac.ir
Tel: +98 912 640 6146
2. Reza Alizadeh-Navaei; Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
E-Mail: reza_nava@yahoo.com
Tel: +98 911 114 7563

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## Abstract

### Background:

Coronavirus Disease 2019 (COVID-19) has become a major global issue with rising the number of infected individuals and mortality in recent months. Among all therapeutic approaches, arguments have raised about hydroxychloroquine (HCQ) efficacy in the treatment of COVID-19. We aimed to overcome the controversies regarding the effectiveness of hydroxychloroquine in the treatment of COVID-19, using a systematic review and meta-analysis.

### Methods:

A systematic search was performed in PubMed, Scopus, Embase, Cochrane Library, Web of Science, Google Scholar and medRxiv pre-print database using all available MeSH terms for COVID-19 and hydroxychloroquine. Two authors selected and assessed the quality of studies independently using related checklists. Data have been extracted from included studies and analyzed using CMA v. 2.2.064. heterogeneity was also assessed using the *I*-squared test. We also conducted different sensitivity analyses to examine the effect of studies that greatly influence the results.

### Results:

Out of 14 studies entered into our systematic review, 12 studies including seven comparative studies with control group and five observational studies containing 3,428 participants have entered into the study. The results of the meta-analysis on comparative studies indicated no significant clinical effectiveness (negative in RT-PCR evaluation) for HCQ regimen in the treatment of COVID-19 in comparison to control group (RR: 1.04, 95% CI, 0.83-1.31). The same result was observed for the combination of HCQ+azithromycin (RR: 2.15, 95% CI, 0.31-14.77). Approximately 1.7 times higher mortality rate was observed among the HCQ regimen group in comparison to control group (RR: 1.73, 95% CI, 1.06-2.81), which was not related to the age differences according to meta-regression analysis ($P$=0.305). No substantial difference was observed for disease exacerbation (RR: 1.87, 95% CI, 0.28-12.36) between HCQ group and controls. Also, radiological findings significantly improved in the HCQ group (OR: 0.32, 95% CI, 0.11-0.98). Odds of known HCQ adverse effects (diarrhea, vomiting, blurred vision, rash, headache, etc.) occurred in the HCQ regimen group was approximately 3.5 times of control group (OR: 3.55, 95% CI, 1.61-7.82), but no substantial differences were found regarding intubation odds between HCQ group and control group (OR: 2.11, 95% CI, 0.31-14.03).

### Conclusion:

This systematic review and meta-analysis not only indicated no clinical benefits regarding HCQ treatment with/without azithromycin for COVID-19 patients, but the higher mortality rate and frequency of known HCQ adverse effects were observed for the HCQ regimen group. However, due to that most of the studies were non-randomized and results were not homogenous, selection bias was unavoidable and further large randomized clinical trials following comprehensive meta-analysis should be taken into account in order to achieve more reliable findings.

**Keywords:** Pandemic, 2019-nCoV, Coronavirus Outbreaks, SARS-CoV-2

## Introduction:

A novel coronavirus emerged from Wuhan, China, in December 2019 has named respiratory syndrome coronavirus 2 (SARS-CoV-2) and declared as a pandemic by World Health Organization (WHO) on March 26, 2020 [1]. According to Worldometer metrics, this novel virus has been responsible for approximately 3,794,890 infections and 262,641 death worldwide up to May 6, 2020.

Although a few months have passed since the onset of the new challenging disease, there is still no specific preventive and therapeutic approach in this regard. Therefore, the quarantine approach, personal hygiene, and social distancing are the basic protective measures against Coronavirus Disease 2019 (COVID-19) according to WHO advice for the public [2].

Moreover, according to a large amount of ongoing research regarding this pandemic issue, many controversies are arising daily among different fields of sciences, which has confronted a ***"pandemic"*** with an ***"infodemic"*** (e.g. *Is coronavirus an airborne? Is COVID-19 transmitted vertically in pregnancy? Should everyone wear a mask? How long can the virus survive on surfaces? Is it possible to get COVID-19 for a second time?* etc.).

In this regard, one of the hottest controversies is the hydroxychloroquine (HCQ) efficacy with/without azithromycin (AZM) for COVID-19 patients. While, several studies are talking about promising effects of HCQ regimen against SARS-CoV-2 infection for both prevention and treatment [3-5], others try to come up with neutral or even harmful effects of this drug for such patients when there is no ample evidence [6]. It is unavoidable that all these controversies affect the patient's outcome significantly.

Although there are some systematic reviews and meta-analysis in this regard [7,8], they suffer from several limitations and flaws that should be addressed with a more comprehensive study. Hence, due to the importance of the subject we carried out a rapid systematic review and meta-analysis, to report stronger evidence, which might help to overcome the controversies about the effectiveness of HCQ against COVID-19.

## Method:

### *Search Strategy*

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline was followed for study design, search protocol, screening, and reporting. A systematic search was performed *via* databases of PubMed, Scopus, Embase, Cochrane Library, Web of Science and Google Scholar (intitle) as well as preprint database of medRxiv up to May 6, 2020. Moreover, gray literature and references of eligible papers were considered for more available data in this case. The search strategy included all MeSH terms and free keywords found for COVID-19, SARS-CoV-2, and hydroxychloroquine. There was no time/location/ language limitation in this search.

### *Criteria study selection*

Two researchers (A.H and K.H) have screened and selected the papers independently and discussed to solve the disagreements with the third-party (R.A/D.Sh). Studies met the following criteria included into meta-analysis: 1) comparative or non-comparative clinical studies including observational/interventional studies with retrospective/prospective nature with/without control group as well as Randomized Clinical Trials (RCTs); and 2) studies reported the effect of HCQ with/without AZM in confirmed COVID-19 patients. Studies were excluded if they were: 1) animal studies, reviews, case reports, and *in vitro* studies; 2) duplicate publications; and 3) insufficient for calculating of desired parameters.

### *Data extraction & quality assessment*

Two researchers (A.Sh and A.H) have independently evaluated quality assessment of the studies and extracted data from selected papers. The supervisors (D.Sh/MA.E) resolved any disagreements in this step. Data extraction checklist included the name of the first author, publication year, region of study, number of patients, number of controls, mean age, treatment option, medication dosage, treatment duration, adverse effects, radiological results, nasopharyngeal culture status through Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and mortality.

The Jadad scale, ROBINS-*I* tool and Newcastle-Ottawa Scale (NOS) checklists were used to value the selected randomized controlled trials, non-randomized controlled trials and observational studies, respectively concerning various aspects of the methodology and study process. Risk-of-bias plots have been created through the *robvis* online tool [9].

### *Targeted outcomes*

1) Clinical effectiveness of HCQ with/without AZM in the treatment of COVID-19; 2) Mortality rate; 3) Disease exacerbation; 4) Frequency of known HCQ adverse effects occurred during treatment; 5) Intubation need; 6) Radiological improvement.

### *Comparisons*

1) HCQ in comparison to control group with standard treatment; 2) HCQ+AZM in comparison to control group with standard treatment.

### *Definitions*

- **Clinical effectiveness:** nasopharyngeal swab culture resulted negative in RT-PCR evaluation.
- **Disease exacerbation:** clinical symptoms of the disease are worsened.
- **Adverse effects:** occurrence of known symptoms related to HCQ such as diarrhea, vomiting, blurred vision, rash, headache, etc.
- **Group-A** in forest plots: the case groups who receive HCQ with/without AZM regimen.
- **Group-B** in forest plots: the control groups without HCQ/HCQ+AZM regimen.

### *Heterogeneity assessment*

I-square ($I^2$) statistic was used for heterogeneity evaluation. Following Cochrane Handbook for Systematic Reviews of Interventions [10], the $I^2$ was interpreted as follows: "*0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity. The importance of the observed value of $I^2$ depends on (i) magnitude and direction of effects and (ii) strength of evidence for heterogeneity (e.g. P-value from the chi-squared test, or a confidence interval for $I^2$).*"

In case of heterogeneity, DerSimonian and Laird random-effects model was applied to pool the outcomes; otherwise, the inverse variance fixed-effect model was used. Forest plots were presented to visualize the degree of variation between studies.

### *Data analysis*

Statistical analysis was performed using Comprehensive Meta-Analysis (CMA) *v*. 2.2.064 software. Risk Ratio (RR) or Odds Ratio (OR) were used for outcome estimation whenever appropriate with 95% Confident Interval (CI). Fixed/random-effects model was used according to heterogeneities. In case of zero frequency, the correction value of 0.1 was used. Meta-regression analysis was done to examine the impact of age difference on HCQ regimen group mortality RR. However, due to insufficient data we could not apply the meta-regression analysis on the other moderator variables such as sex, underlying disease, etc.

### *Publication bias & sensitivity analysis*

Begg's and Egger's tests as well as funnel plot was used for publication bias evaluation. *P*-value less than 0.05 was considered as statistically significant.

According to that the sensitivity analysis known as an essential part in systematic reviews with meta-analysis to determine the robustness of the obtained outcomes to the assumptions made in the data analysis [11], we conducted a sensitivity analysis to examine the effect of studies that greatly influence the result, especially by their weight through excluding them from the meta-analysis.

## Results

### *Study selection process*

The databases search resulted in 343 papers. Eighty-nine duplicated papers have been excluded and after first step screening, fifteen papers were assessed for eligibility. Finally, 14 papers entered into qualitative synthesis, of which 12 papers entered into the meta-analysis. PRISMA flow diagram for the study selection process presented in Figure 1.

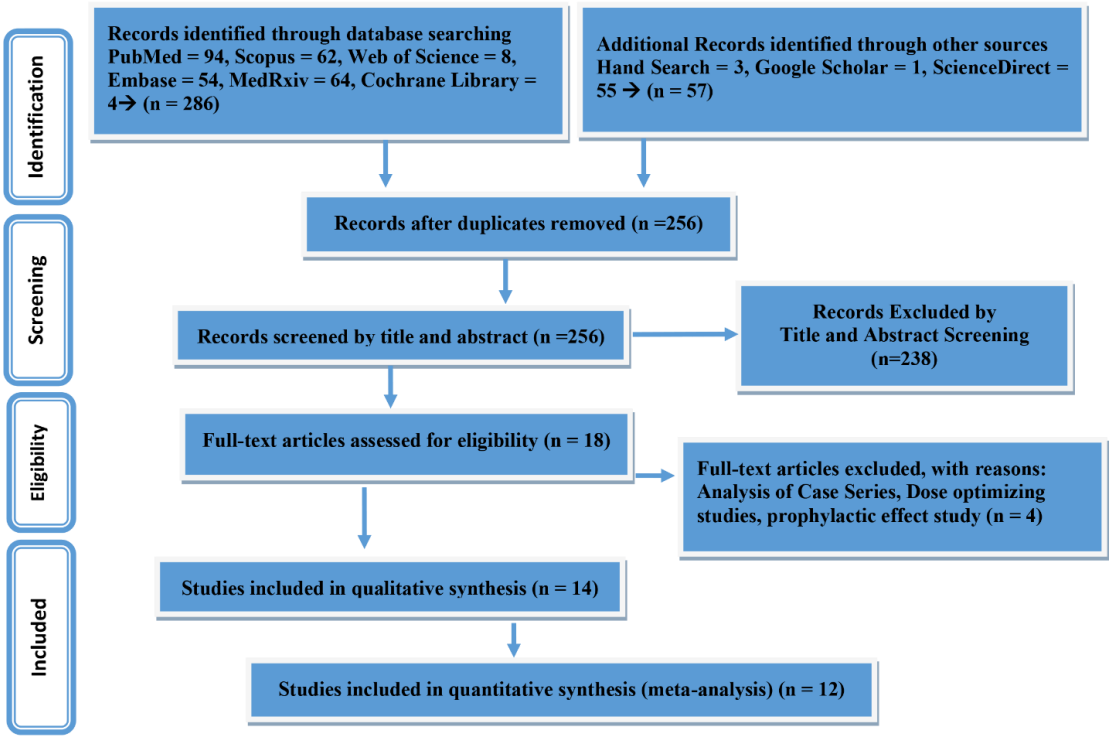


*Figure 1.PRISMA flow diagram for the study selection process*

### *Study characteristics*

Out of 12 studies entered into the meta-analysis, seven studies were comparative randomized and non-randomized (only two studies were randomized) and five were observational, of which, HCQ arms of the comparative studies has been combined with observational studies for effect size meta-analysis. The studies' sample size ranged from 10 to 1061 including 3,428 participants. Characteristics of studies entered into the systematic review presented in Table 1.

*Table 1. Characteristics of studies entered into the systematic review*

| Study | Country | Quality Score/ Risk of Bias | Patients type | No. Patients | Cases | Controls | Treatment regimen | Duration (days) | Mean age (± SD) |
|---|---|---|---|---|---|---|---|---|---|
| Chen *et al.* 2020 [12] | China | 5/8* | Nonsevere | 62 | 31 | 31 | HCQ 400 mg/d | 5 | 44.7 (± 15.3) |
| Jun *et al.* 2020 [13] | China | 5/8* | - | 30 | 15 | 15 | HCQ 400 mg/d | 5 | - |
| Mahévas *et al.* 2020 [14] | France | 5/8* | Nonsevere | 181 | 84 | 97 | HCQ 600 mg/d | 7 | - |
| Tang *et al.* 2020 [15] | China | 6/8* | Mild, Moderate, Severe | 150 | 70 | 80 | HCQ 400-800 mg/d | 14-21 | 46.1 (±14.7) |
| Gautret (A) *et al.* 2020 [16] | France | 8/9** | - | 80 | 80 | - | HCQ 400 mg/d + AZM | 10 | 52.1 (± 14.8) |
| Gautret (B) *et al.* 2020 [17] | France | Moderate*** | - | 36 | 14 | 16 | HCQ 600 mg/d | 6 | 45.1 (± 22) |
| | | | - | | 6 | 16 | HCQ 600 mg/d + AZM # | | |
| Magagnoli *et al.* 2020 [18] | USA | 8/9** | - | 368 | 97 | 158 | HCQ | - | - |
| | | | - | | 113 | 158 | HCQ+AZM | | |
| Molina *et al.* 2020 [19] | France | Moderate*** | Severe | 11 | 11 | - | HCQ 600 mg/d + AZM | 6 | 58.7 (± 14.3) |
| Chorin *et al.* 2020 [20] | USA | 8/9** | - | 84 | 84 | - | HCQ + AZM | - | 63 (± 15) |
| Joshua *et al.* 2020 [21] | USA | Moderate*** | - | 63 | 32 | 31 | HCQ 200-400 mg/d | 5 | 62.7 (± 15) |
| Million *et al.* 2020 [22] | France | 6/9** | - | 1061 | 1061 | - | HCQ + AZM | 3< | 43.6 |
| Bo Yu *et al.* 2020 [23] | China | 6/9** | Critically ill | 568 | 48 | 520 | HCQ 400 mg/d | 7-10 | 67 (± 14.0) |
| Ramireddy *et al.* 2020 [24] | USA | - | - | 98 | 10 | - | HCQ | - | 62.3 (± 17) |
| | | | - | | 61 | - | HCQ + AZM | | |
| Barbosa *et al.* 2020 [25] | Brazil | Moderate*** | - | 636 | 412 | 224 | HCQ + AZM ¶ | 6 | 62.5 (± 15.5) |

*Quality assessed using Jadad Checklist
** Quality assessed using the Newcastle-Ottawa Scale Checklist
*** Risk of Bias assessed using ROBINS I tool

HCQ: hydroxychloroquine, AZM: azithromycin
# 500mg on day1 followed by 250mg per day, the next four days
¶ Hydroxychloroquine 800mg on the first day and 400mg for another 6 days and azithromycin 500mg once daily for five days.

### *Quality assessment*

Results of quality assessment for studies entered into meta-analysis using Jadad, ROBINS-*I* and NOS checklists were reported in Table 1 and summary of risk of bias has presented in Fig. 2.

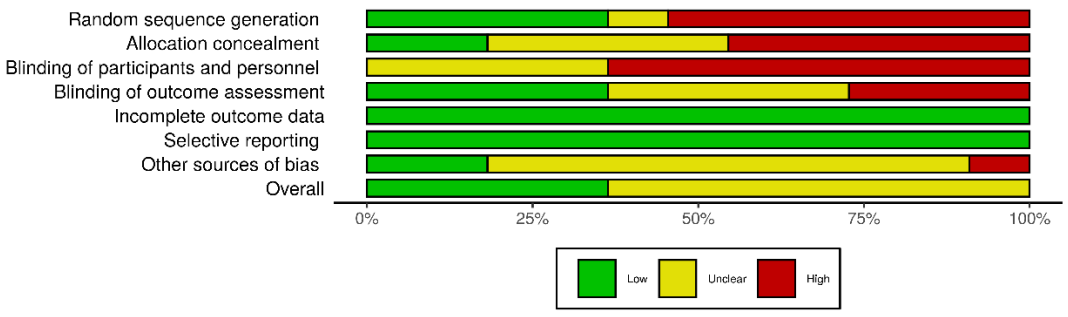


*Figure 2. Summary of risk of bias for studies entered into meta-analysis*

### *Publication bias*

Results of Begg's and Egger's tests for every performed analysis were insignificant as follows: hydroxychloroquine regimen effectiveness ($P_B$=0.22; $P_E$=0.15), association between hydroxychloroquine regimen and mortality rate in controlled randomized and non-randomized studies ($P_B$= 0.75; $P_E$=0.51) and overall mortality in all studies ($P_B$= 0.36; $P_E$=0.09). The funnel plot for publication bias of studies presented in Supplementary Fig. 1.

### *Meta-analysis findings*

### *Treatment outcome*

### *Hydroxychloroquine regimen effectiveness*

The meta-analysis of risk ratios (RR) for HCQ effectiveness in all of the comparative randomized and non-randomized studies showed that there were no significant differences between case group, who received the standard treatment with HCQ regimen and the control group that received the standard treatment without HCQ (RR: 1.04, 95% CI, 0.83-1.31). We also found no significant risk difference (RD) between two groups regarding effectiveness of HCQ in COVID-19 patients (RD: 0.07, 95% CI, -0.11-0.25) (Fig. 3). Also, no substantial effectiveness for HCQ was found by meta-analysis of only controlled randomized studies as well (RR: 1.19, 95% CI, 0.87-1.63; RD: 0.12, 95% CI, -0.07-0.33) (Fig. 4).

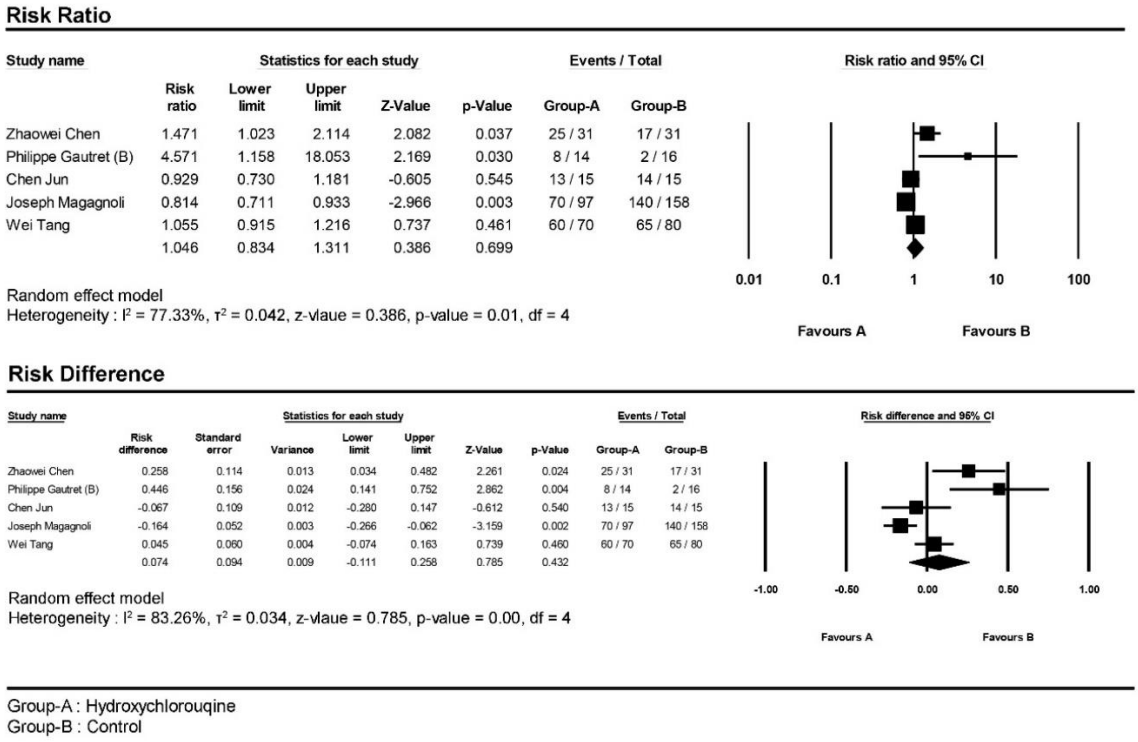


*Figure 3. Forest plot for pooling risk ratios and risk differences regarding hydroxychloroquine regimen in comparative randomized and non-randomized studies*

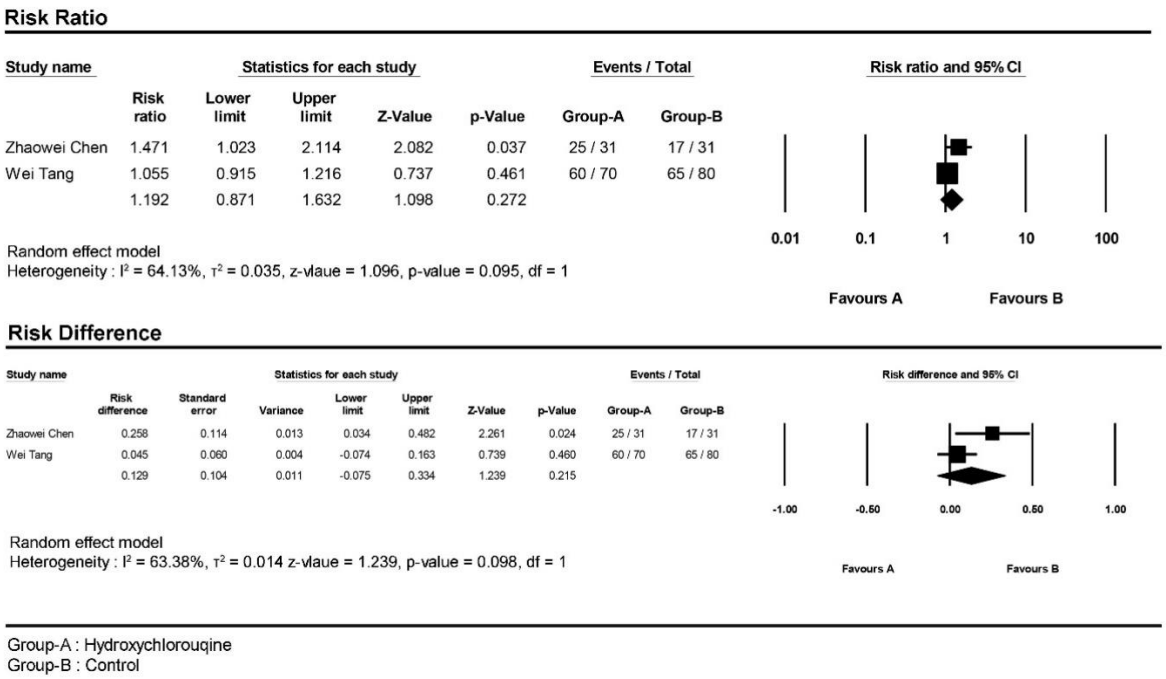


*Figure 4. Forest plot for pooling risk ratios and risk differences regarding hydroxychloroquine regimen in only controlled randomized studies*

### *Sensitivity analysis for hydroxychloroquine regimen effectiveness*

Regardless of separate analysis only on controlled randomized studies (Fig. 4), to evaluate the impact of inverse RRs as well as study weigh on the meta-analysis results, we conducted two sensitivity analyses as follows: 1) according to the substantial impact of Gautret (B) *et al.* study (RR: 4.57, 95% CI, 1.15-18.05) with very small sample size on the meta-analysis, by excluding this study, no significant changes was observed (RR: 1.00, 95% CI, 0.81-1.22; RD: 0.004, 95% CI, -0.16-0.17) (Supplementary Fig. 2); 2) considering three studies with *P*-value less than 0.05; two have a $P<0.05$ in favour of Group-A and one has a $P<0.05$ in favour of Group-B; this is the Magagnoli *et al.* study, for which the 95% CI of RR has no intersection with the Chen *et al.* and Gautret (B) *et al.*, thus, the new sensitivity analysis by excluding study of Magagnoli *et al.*, resulted in no difference as well (RR: 1.15, 95% CI, 0.88-1.49; RD: 0.14, 95% CI, -0.04-0.33) (Supplementary Fig. 3); and 3) by excluding both studies, meta-analysis showed no significant difference similarly (RR: 1.08, 95% CI, 0.88-1.31; RD: 0.07, 95% CI, -0.08-0.22) (Supplementary Fig. 4).

### *Hydroxychloroquine + azithromycin regimen*

No significant difference was found for effectiveness of HCQ+AZM combination regimen in comparison to control group in comparative studies entered into meta-analysis (RR: 2.15, 95% CI, 0.31-14.77; RD: 0.37, 95% CI, -0.58-1.33) (Fig. 5).

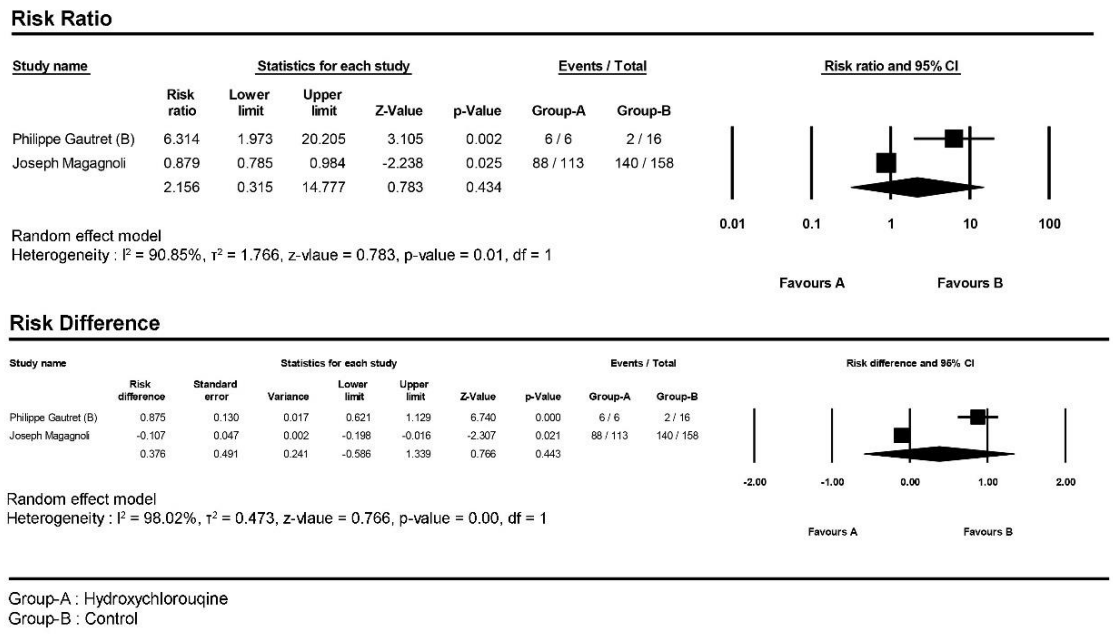


*Figure 5. Forest plot for pooling risk ratios and risk differences regarding hydroxychloroquine + azithromycin regimen*

### *Hydroxychloroquine regimen & mortality rate*

The meta-analysis of death outcome in comparative randomized and non-randomized studies showed approximately 1.7 times higher mortality in HCQ regimen group with/without AZM in comparison to standard treatment group (RR: 1.73, 95% CI, 1.06-2.81) (Fig. 6).

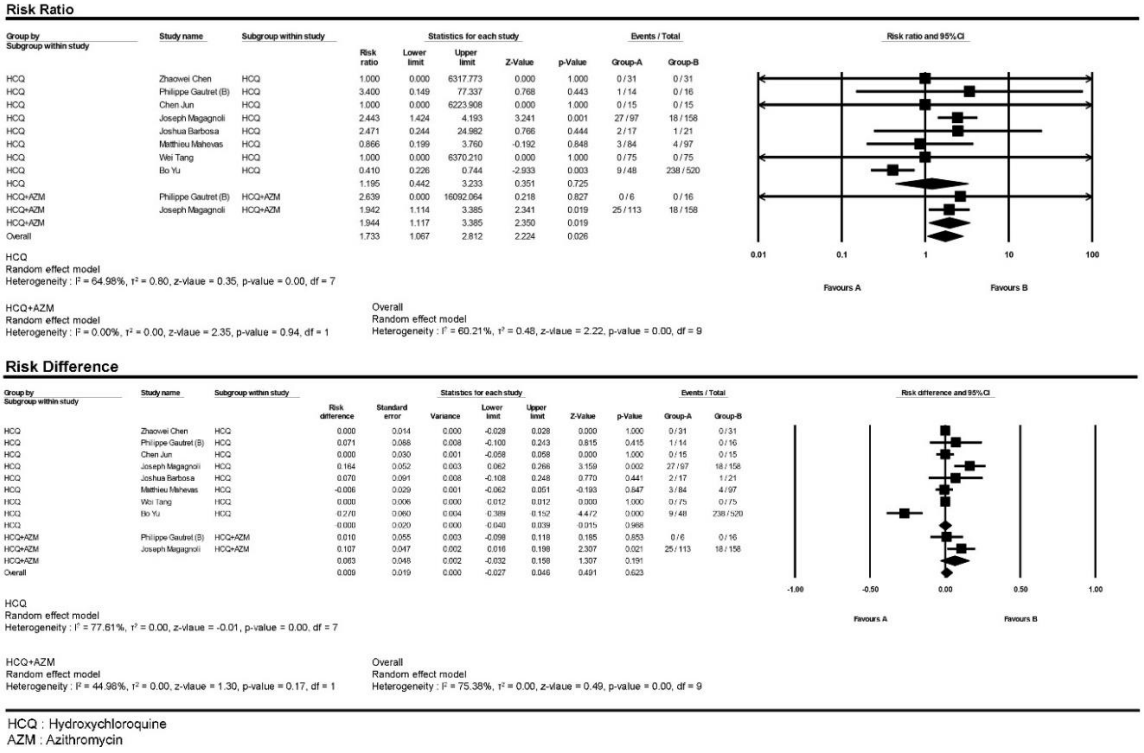


*Figure 6. Forest plot for pooling risk ratios and risk differences regarding mortality rate in randomized and non-randomized studies*

### *Sensitivity analysis for hydroxychloroquine regimen mortality*

Due to the surprising mortality rate in the control group of the Yu *et al.* study, which led to the inverse RR in comparison to other studies and greatest weight in final estimation in our analysis, we have conducted a sensitivity analysis by excluding this study. The sensitivity analysis resulted in approximately two times higher mortality in HCQ/HCQ+AZM regimen arm in comparison to control group (RR: 2.07, 95% CI, 1.43-2.99; RD: 0.01, 95% CI, -0.01-0.03) (Supplementary Fig. 5). In addition, concerning substantial influence of Magagnoli *et al.* study weigh on mortality, we have performed another sensitivity analysis by excluding both studies, which indicated an insignificant association between mortality rate in HCQ regimen in comparison to control group in randomized and non-randomized studies (RR: 1.34, 95% CI, 0.44-4.10; RD: 0.00, 95% CI, -0.01-0.01) (Supplementary Fig. 6).

### *Meta-regression analysis on effects of age differences on mortality*

Meta-regression findings indicated that age difference of studies had no substantial effects on risk ratios related to the HCQ regimen group mortality rate (*P*=0.305) (Fig. 7).

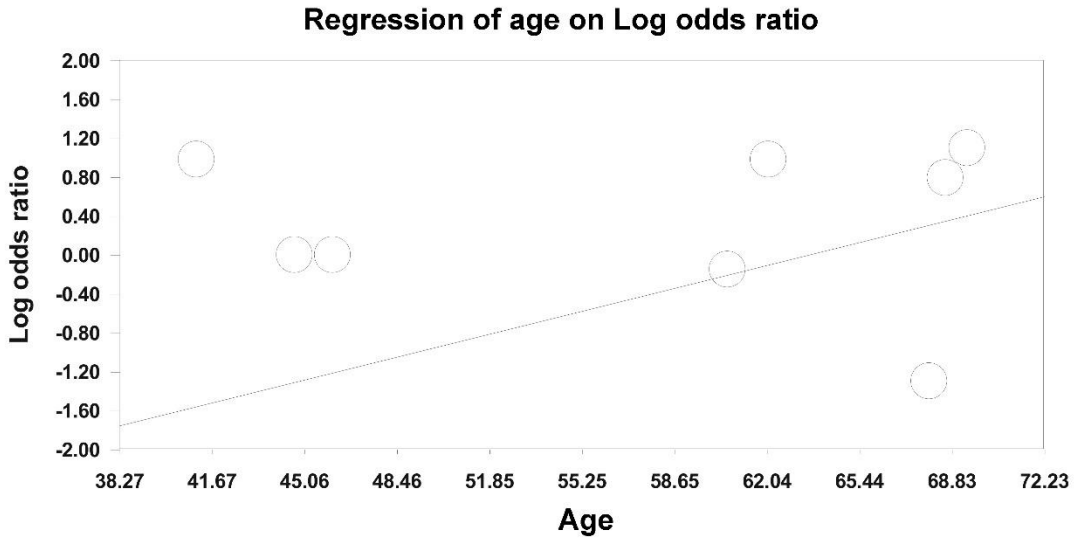


*Figure 7. Meta-regression plot for effect of age difference on hydroxychloroquine regimen mortality Risk Ratio*

### *Overall mortality*

For overall mortality we considered the treatment arms of the all comparative studies as observational studies and combined all types together as observational. Pooling mortality rate of studies resulted in overall mortality of 6.7% (95% CI, 3%-14%) for both HCQ and HCQ+AZM regimen (Fig. 8).

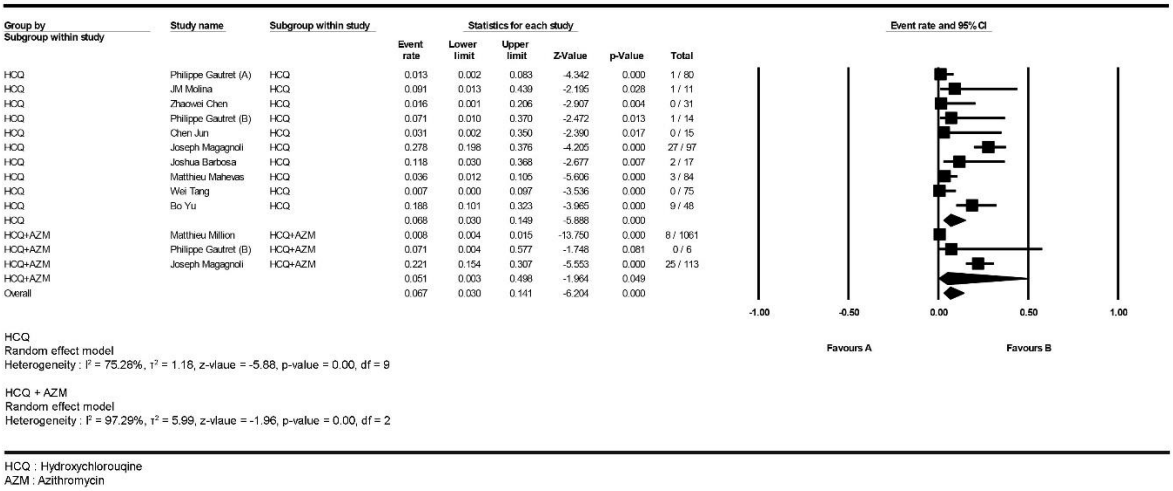


*Figure 8. Forest plot for pooling mortality rates*

### *Disease exacerbation*

Meta-analysis of all comparative studies showed that the disease exacerbation was not significantly different between HCQ group and control group (RR: 1.87, 95% CI, 0.28-12.36; RD: 0.05, 95% CI, -0.12-0.23) (Fig. 9). Also, doing meta-analysis only on controlled randomized studies indicated no considerable disease exacerbation differences between two groups (RR: 0.59, 95% CI, 0.04-7.79; RD: -0.08, 95% CI, -0.32-0.14) (Fig. 10).

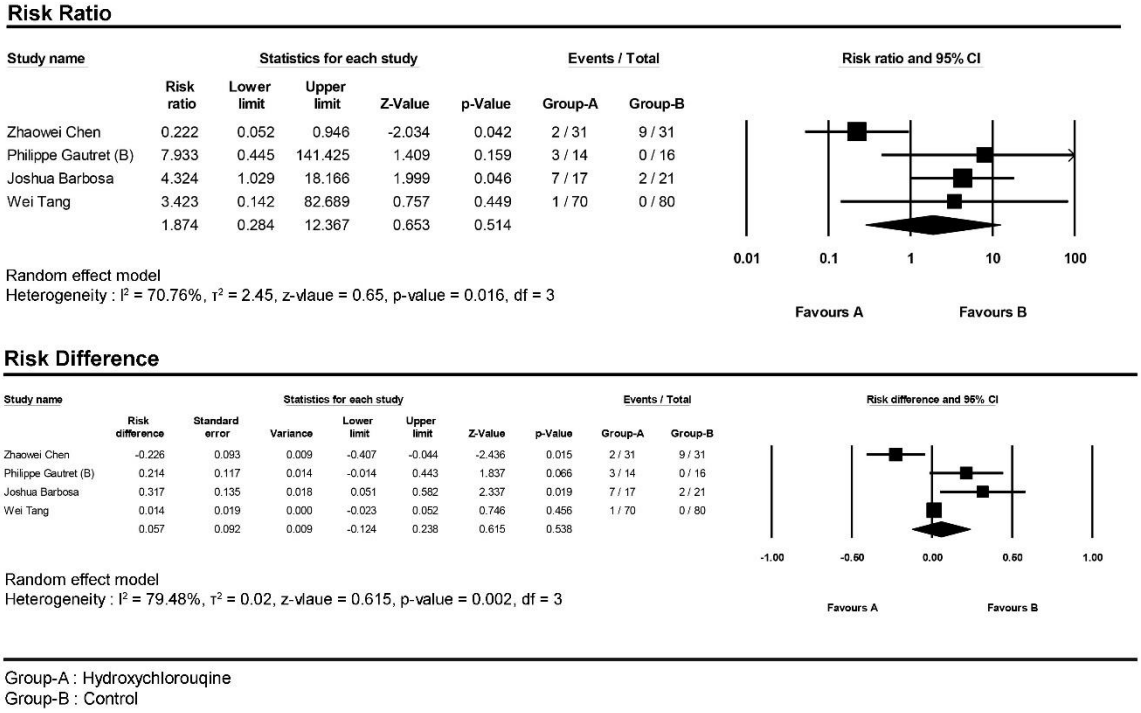


*Figure 9. Forest plot for pooling risk ratios and risk differences regarding disease exacerbation in comparative randomized and non-randomized studies*

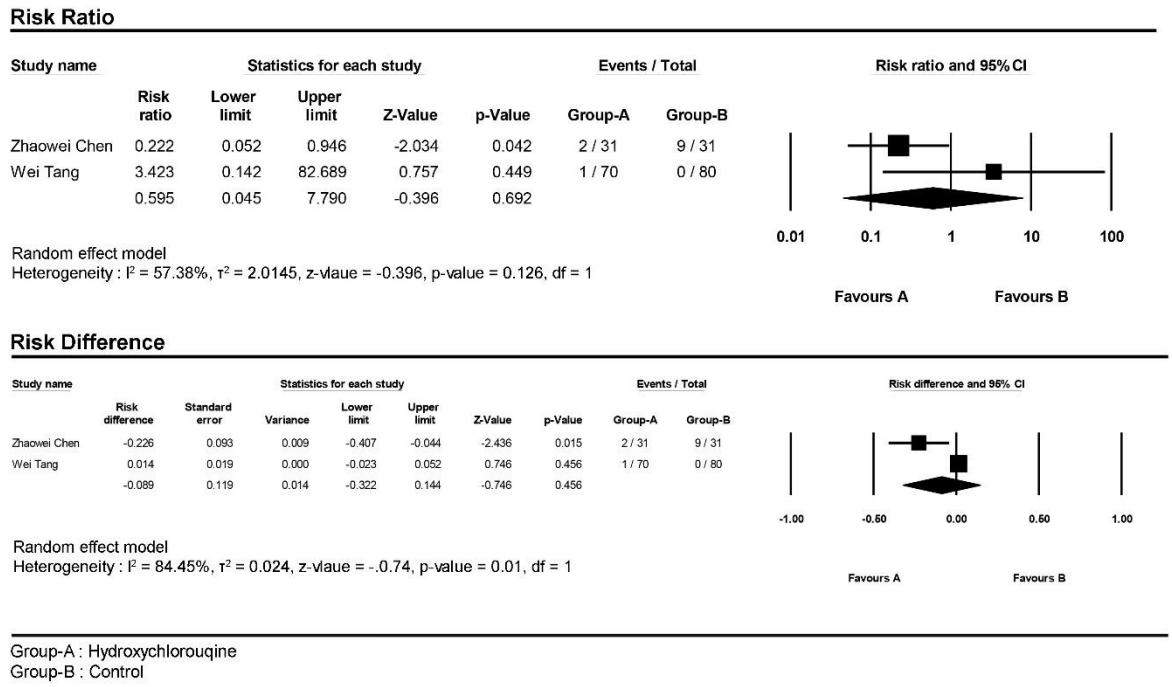


*Figure 10. Forest plot for pooling risk ratios and risk differences regarding disease exacerbation in only controlled randomized studies*

## *Intubation*

Meta-analysis of comparative randomized and non-randomized studies indicated that there were no significant differences between HCQ group and control group about odds of intubation during treatment (OR: 2.11, 95% CI, 0.31-14.03) (Fig. 11).

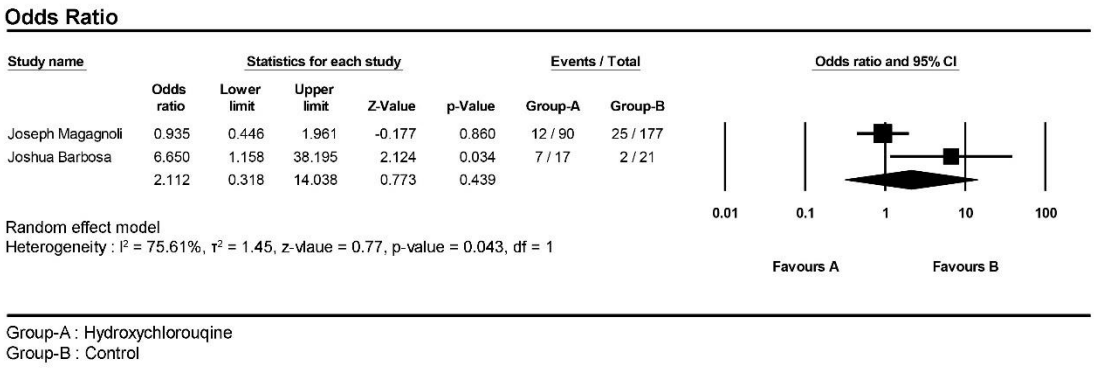


*Figure 11. Forest plot for pooling odds ratios regarding intubation status*

### *Adverse effects*

The meta-analysis of comparative randomized and non-randomized studies showed that the odds of adverse effects occurrence in patients who received HCQ regimen was approximately 3.5 times higher than control group without HCQ regimen (OR: 3.55, 95% CI, 1.61-7.82) (Fig. 12). Likewise, doing meta-analysis only on controlled randomized studies indicated 4.5 times higher odds of experiencing adverse effects in patients who receive HCQ regimen in comparison to control group (OR: 4.53, 95% CI, 1.86-11.03) (Fig. 13).

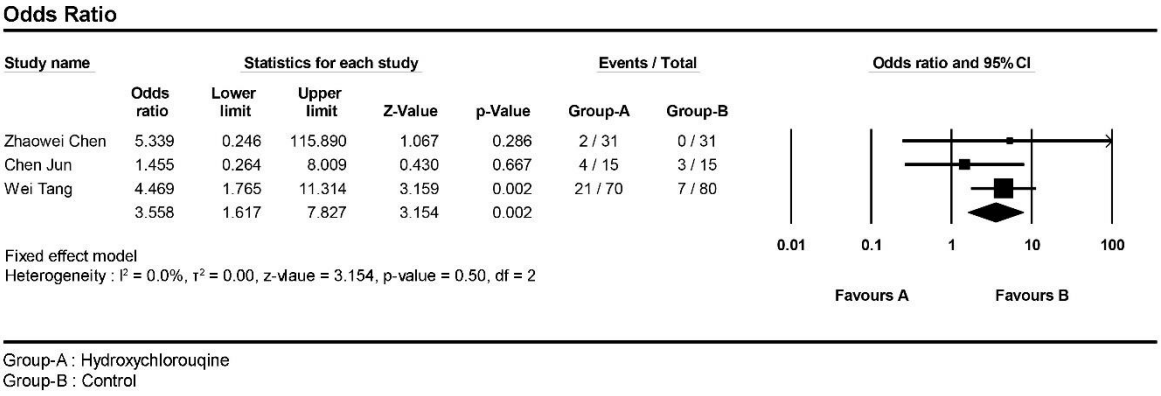


*Figure 12. Forest plot for pooling odds ratios regarding adverse effects in comparative randomized and non-randomized*

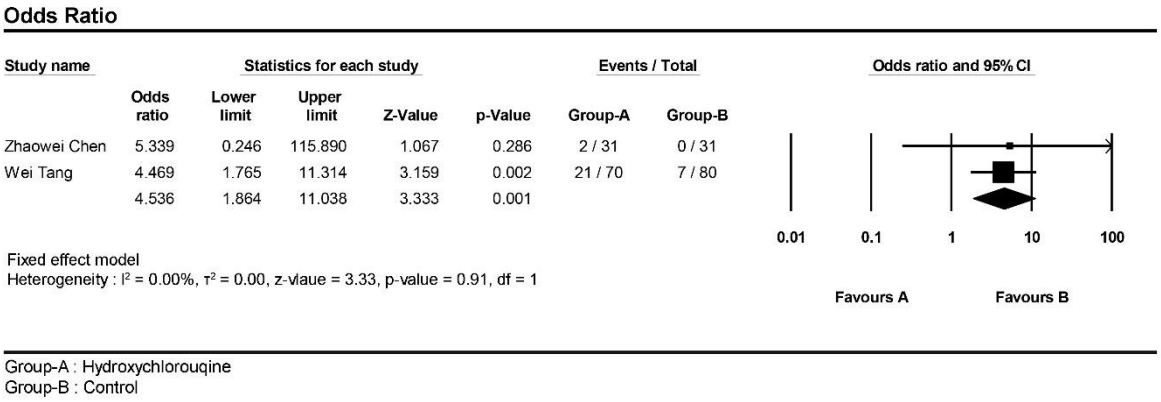


*Figure 13. Forest plot for pooling odds ratios regarding adverse effects in only controlled randomized studies*

### *Radiological improvement*

A considerable Computed Tomography-Scan (CT-Scan) improvement was observed in HCQ group with odds ratio of 0.32 (95% CI, 0.11-0.98) (Fig. 14).

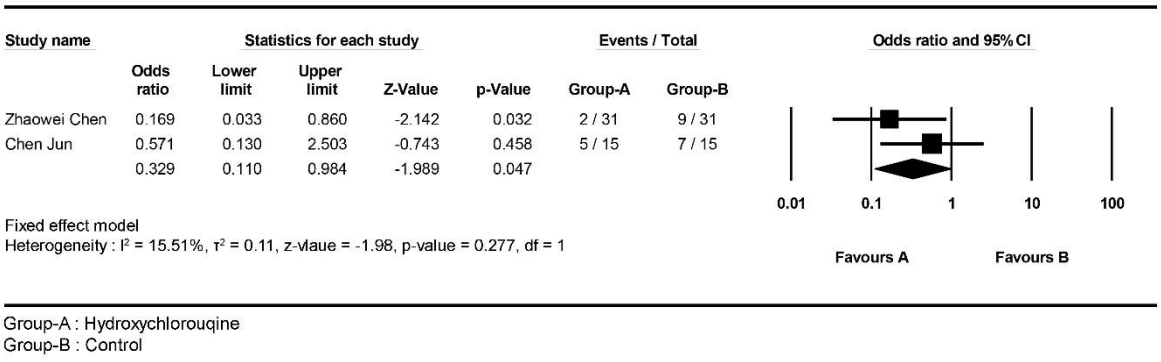


Figure 14. Forest plot for pooling odds ratios regarding radiological improvement

### *Meta-analysis of observational studies (Fig. 15)*

We considered the treatment arms of the comparative studies as observational studies for this section. Hence, the meta-analysis of the other events rate showed the following results: 14% of patients suffered from known HCQ adverse effects (95% CI, 3%-46%); CT-Scan improvement has been observed in 16% of COVID-19 patients (95% CI, 2%-57%); 74% (95% CI, 62%-83%) of patients were discharged from the hospital or their nasopharyngeal culture resulted negative in RT-PCR evaluation, whereas 12% (95% CI, 2%-38%) of patients have been exacerbated, 7% (95% CI, 2%-17%) were admitted to the intensive care unit (ICU) and 23% (95% CI, 6%-57%) underwent intubation.

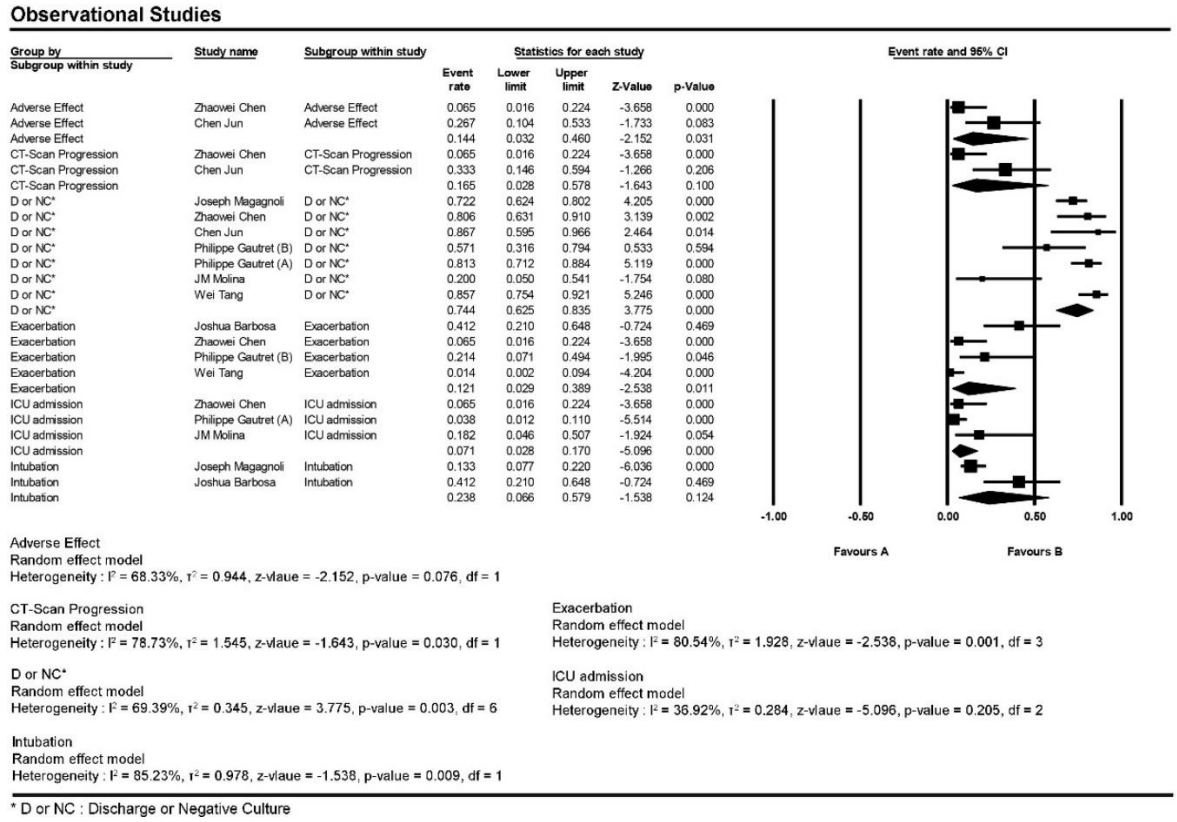


*Figure 15. Forest plot for pooling events of observational studies*

## Discussion

More than three months after the closure of the Hubei region, there is still very little high-quality data on every treatment's regimen, which raises questions about gaps in scientific works. In this context, one can only be surprised to see observational data without a control group covering a large number of patients when there is an essential need for comparative randomized data. In fact, Analysis of current data is difficult because at least 90% of spontaneous recovery are observed at the base. Thus, it takes well over 90% of recovery to consider that any treatment really brings something. The HCQ was no exception to this rule with a result greater than 90%. On the other hand, in a few percent, the disease becomes serious with Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure. This is where we would like to know what the treatments bring. Concerning all of the limitations and analyze difficulties, we have conducted this systematic review and meta-analysis with great caution and sensitivity in performing analyzes in order to try for overcoming the current controversies regarding effectiveness of HCQ in the treatment of COVID-19, at least at the base.

Considering the matter, recent investigations indicated that a high concentration of cytokines in the plasma called cytokine storm would be related to severe COVID-19 patients. In this situation, medications transposition is a critical need to find effective anti-inflammatory agents to decrease the cytokines and pro-inflammatory factors production [26]. In this regard, HCQ has been known as an effective anti-inflammatory agent for a long time (since the 1950s), especially in autoimmune disorders [27]. Besides, the outcome of a new experimental study conducted by Liu *et al.* has been mentioned in the title of their publication as follows: "*Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro*". This also has been investigated and resulted same as the *in vitro* study of Yao *et al.* [28].

In addition, Pagliano *et al.* in their letter to the editor of *Clinical Infectious Diseases (CID)* journal, have been recommended the use of HCQ as pre/post-exposure prophylaxis against SARS-CoV-2 infection for health care staffs exposed to the virus in contaminated environments [29].

In contrast, Maurizio Guastalegname and Alfredo Vallone are claiming about the uselessness and even harmful effects of HCQ against COVID-19 in their letter to the editor in the above journal [6]. They believe that, while the pathogenicity of the SARS-CoV-2 is still unknown, we should be cautious about the treatment decision, which has been proved through *in vitro* base studies in order to avoid dire paradoxical consequences like what has happened in treatment of *Chikungunya Virus* infection with chloroquine [30]. Moreover, Molina *et al.* have followed 11 patients with HCQ + azithromycin regimen and concluded no clinical benefit and reasonable anti-viral activity [19]. In addition, the pre-print of a Quasi-Randomized Comparative Study conducted in Detroit, Michigan, has been indicated not only any clinical benefits for HCQ but even increased need for urgent respiratory support ($p$=0.013) [21].

Also, H.J. Kim *et al.* in their opinion publication *for the COVID-19 Global Rheumatology Alliance* pointed at the shortage of HCQ following a sudden high demand after Gautret and colleagues' publication on 20 March 2020 [17]. They also referred to that HCQ is a crucial treatment choice for patients with systemic lupus erythematosus and rheumatoid arthritis disorders, who get into trouble in finding HCQ in this critical time [31]. Authors recommend that scientific communities have to be very cautious and do not rush in the decision when there is no ample evidence for the subject, especially in such critical situations, which can lead to irreparable consequences. In fact, even if the efficacy of HCQ is confirmed, the world will be

facing a new issue for both COVID-19 and rheumatic disorders patients: "*Shortage of Hydroxychloroquine*".

More recently, the preprinted study of Magagnoli *et al.* [18] on 368 United States veterans reported not only any clinical benefits for HCQ/HCQ+AZM regimen in COVID-19 patients but even association between higher mortality rate and HCQ group (hazard ratio: 2.61, 95% CI, 1.10-6.17; *P*=0.03). Also, the target trial emulation conducted by Mahévas *et al.* [14], did not support the effectiveness of the HCQ regimen, which has performed on 181 patients with SARS-CoV-2 hypoxic pneumonia.

In this case, we carried out the present systematic review in order to reach a clear result regarding taking or not-taking HCQ. In this study, although the risk ratio was higher than 1, there was no significant difference between HCQ arm who received 400-600 mg HCQ daily regimen and standard treatment arm, which was the same for the HCQ+AZM regimen as well. Surprisingly, not only no clinical benefits were found regarding HCQ treatment with/without AZM for COVID-19 patients, but the higher mortality rates were observed for the HCQ regimen group. It is noteworthy that the main weight of mortality was related to the study of Magagnoli *et al.* with the highest rate of death [18]. In addition, more cases in the HCQ group presented the improvement in CT-Scan results in comparison to the control group. Moreover, the considerable higher frequency of known HCQ adverse effects such as diarrhea, vomiting, blurred vision, rash, headache, etc. was observed in HCQ groups.

It is remarkable that, Sarma *et al.* [8] conducted a meta-analysis on three studies and has been concluded the promising effects for HCQ clinical cure, which is inconsistent with our overall conclusion. Additionally, more recently, Million *et al.* [7] carried out "*a meta-analysis based on the first available reports*" released in *IHU Méditerranée Infection* (*COVID-IHU #10*), in which they have claimed a promising trend toward the chloroquine derivatives benefits against COVID-19 and suggested it to prescribe as a grade *I* recommendation. Going deeper into the content, the study suffers from some flaws such as ignoring heterogeneity as well as the pattern of dispersion in the results, combining different outcomes in an unusual way, using only odds ratios, when the risk ratios are the priority and preferred in such analysis, talking about four RCTs, whereas some are non-randomized, etc. Also, concerning the importance of mortality, Magagnoli *et al.* [18] findings, which go against the expected by authors, is ignored without any sensitivity analysis.

As a matter of fact, "*statistics are only a tool for understanding and not an end in itself. If it is easy to say anything with statistics, it is also easy to say that you can make them say anything. However, using it well, with a strong consideration of the clinical sciences and an understanding of the possible sense of bias can go very far*", Dr. Rauss says.

While our analysis did not indicate a considerable effectiveness for the HCQ/HCQ+AZM regimen, due to the small number of studies with a small sample size, it is too early to reach a reliable decision. In fact, the possible influence of biases on the results in analysis of such data is inevitable, but what is more important is to know in which direction, the biases can play the role on the results. Thus, as we know, observational studies give a vision that overestimates the real result. Under these conditions, when we incorporate such data, and the result is not good, it means that we have a minimized vision of the result. Hence, the question arises whether the result might be even worse than what is observed?! If yes, then it would be understood that *"impact of this meta-analysis finings with strong consideration of the clinic and an understanding of the possible sense of bias is much greater than what is observed and presented"*; Dr. Rauss says. Hence, it seems that we are very far from an effective treatment

and the urgent need arises for comprehensive randomized controlled trials in order to investigate the efficacy and safety of HCQ consumption and other treatment regimen and approaches in COVID-19 patients. In point of fact, the studies to come should completely reverse what has already been done and just this point is already an important information because the probability is, in fact, extremely low.

In this regard, searching clinical trials registry databases such as WHO International Clinical Trials Registry Platform (ICTRP), Clinicaltrials.gov, Center Watch, Chinese Clinical Trials Registry, International Standard Randomised Controlled Trial Number (ISRCTN), EU Clinical Trials Register, OpenTrials and Iranian Registry of Clinical Trials, resulted in hundreds registered trials on the subject of efficacy, safety and prophylaxis potency of HCQ in COVID-19 patients. Hence, in the near future, the results of these trials will help the medical communities to reach a general opinion regarding the utilization of HCQ as a pre/post-exposure as well as a treatment option for patients infected with SARS-CoV-2.

To our knowledge, this is the most updated systematic review that carried out a meta-analysis for investigating the role of HCQ with/without AZM in COVID-19 patients. However, after releasing outcomes of underway clinical trials, especially "*Solidarity*" clinical trial for COVID-19 treatment by WHO, an updated systematic review and meta-analysis on this subject could be more conclusive and reliable.

It is worth noting that the current meta-analysis includes the following limitations: 1) due to that most of the studies were non-randomized and results were not homogenous, selection bias was unavoidable; 2) various treatment plan regarding medication dosage and treatment duration; 3) insufficient moderator variables distribution by group such as sex, underlying disease, etc.; 4) significant mortality rate was dependent on one study (Magagnoli *et al.*); and 5) inclusion of studies with small sample size, which leads to type *II* statistical errors. To overcome the limitations and bias, results of the study should be confirmed by robustly randomized studies.

## Conclusion

This systematic review and meta-analysis not only indicated no clinical benefits regarding hydroxychloroquine treatment with/without azithromycin for COVID-19 patients, but the higher mortality rates and frequency of known HCQ adverse effects were observed for the HCQ regimen group. However, due to that most of the studies were non-randomized and results were not homogenous, selection bias was unavoidable, further large clinical trials following comprehensive meta-analysis should be taken into account in order to achieve more reliable findings.

## Conflict of interests

The authors declare that they have no conflict of interests.

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## References:


1. Organization WH. Coronavirus disease (COVID-2019) situation reports. Geneva: WHO; 2020.
2. Organization WH. Coronavirus disease (COVID-19) advice for the public. 2020.
3. Pourdowlat G, Panahi P, Pooransari P, Ghorbani F. Prophylactic Recommendation for Healthcare Workers in COVID-19 Pandemic. J Advanced Journal of Emergency Medicine 2020.
4. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents 2020:105932.
5. Zhou D, Dai SM, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. The Journal of antimicrobial chemotherapy 2020.
6. Guastalegname M, Vallone A. Could chloroquine /hydroxychloroquine be harmful in Coronavirus Disease 2019 (COVID-19) treatment? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2020.
7. Million M, Gautret Ph, Raoult D. The efficacy of Chloroquine derivatives in COVID-19: a meta-analysis based on the first available reports. In: Infection IM, ed. IHU Méditerranée Infection2020.
8. Sarma P, Kaur H, Kumar H, et al. Virological and Clinical Cure in Covid-19 Patients Treated with Hydroxychloroquine: A Systematic Review and Meta-Analysis. J Med Virol 2020;n/a.
9. McGuinness LA. robvis: An R package and web application for visualising risk-of-bias assessments. 2019.
10. Higgins JPT, Thomas J, Chandler J, et al. Cochrane handbook for systematic reviews of interventions version 6.0. 2nd Edition ed: John Wiley & Sons; 2019.
11. Bown MJ, Sutton AJ. Quality Control in Systematic Reviews and Meta-analyses. European Journal of Vascular and Endovascular Surgery 2010;40:669-77.
12. CHEN Jun LD, LIU Li,LIU Ping,XU Qingnian,XIA Lu,LING Yun,HUANG Dan,SONG Shuli,ZHANG Dandan,QIAN Zhiping,LI Tao,SHEN Yinzhong,LU Hongzhou. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). 2020;49:0-.
13. Chen Z, Hu J, Zhang Z, et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. 2020:2020.03.22.20040758.
14. Mahevas M, Tran V-T, Roumier M, et al. No evidence of clinical efficacy of hydroxychloroquine in patients hospitalized for COVID-19 infection with oxygen requirement: results of a study using routinely collected data to emulate a target trial. 2020:2020.04.10.20060699.
15. Tang W, Cao Z, Han M, et al. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. 2020:2020.04.10.20060558.
16. Philippe Gautret, Jean-Christophe Lagier, Philippe Parola, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: an observational study. IHU Méditerranée Infection 2020;27.
17. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020:105949.
18. Magagnoli J, Narendran S, Pereira F, et al. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. 2020:2020.04.16.20065920.
19. Molina JM, Delaugerre C, Goff JL, et al. No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19 Infection. Médecine et Maladies Infectieuses 2020.
20. Chorin E, Dai M, Shulman E, et al. The QT Interval in Patients with SARS-CoV-2 Infection Treated with Hydroxychloroquine/Azithromycin. 2020:2020.04.02.20047050.
21. Barbosa J, Kaitis D, Freedman R, Le K, Lin X. Clinical outcomes of hydroxychloroquine in hospitalized patients with COVID-19 : a quasi-randomized comparative study. Bibliovid 2020.
22. al. MMe. Early treatment of 1061 COVID-19 patients with hydroxychloroquine and azithromycin, Marseille, France. Bibliovid 2020.
23. Yu B, Wang DW, Li C. Hydroxychloroquine application is associated with a decreased mortality in critically ill patients with COVID-19. 2020:2020.04.27.20073379.
24. Ramireddy A, Chugh HS, Reinier K, et al. Experience with Hydroxychloroquine and Azithromycin in the COVID-19 Pandemic: Implications for QT Interval Monitoring. 2020:2020.04.22.20075671.
25. Barbosa Esper R, Souza da Silva R, Costa Oikawa FT, et al. Empirical treatment with hydroxychloroquine and azithromycin for suspected cases of COVID-19 followed-up by telemedicine. Dropbox 2020.

26. Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. Int J Antimicrob Agents 2007;30:297-308.
27. Detert J, Klaus P, Listing J, et al. Hydroxychloroquine in patients with inflammatory and erosive osteoarthritis of the hands (OA TREAT): study protocol for a randomized controlled trial. Trials 2014;15:412.
28. Yao X, Ye F, Zhang M, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020.
29. Pagliano P, Piazza O, De Caro F, Ascione T, Filippelli A. Is Hydroxychloroquine a possible post-exposure prophylaxis drug to limit the transmission to health care workers exposed to COVID19? Clin Infect Dis 2020.
30. Roques P, Thiberville SD, Dupuis-Maguiraga L, et al. Paradoxical Effect of Chloroquine Treatment in Enhancing Chikungunya Virus Infection. Viruses 2018;10.
31. Kim AHJ, Sparks JA, Liew JW, et al. A Rush to Judgment? Rapid Reporting and Dissemination of Results and Its Consequences Regarding the Use of Hydroxychloroquine for COVID-19. Annals of internal medicine 2020.