Hydroxychloroquine and Azithromycin as a Treatment of COVID-19: Results of an Open-Label Non-Randomized Clinical Trial: Response to David Spencer (Elsevier)

Didier Raoult[1,2], Matthieu Million[1,2], Philippe Gautret[1,3], Jean-Christophe Lagier[1,2], Philippe Colson[1,2], Philippe Parola[1,3], and Jean-Marc Rolain[1,2]

Affiliations :

[1] IHU-Méditerranée Infection, Marseille, France

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

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

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

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

We thank the authors for the comments provided for our article (1-3), but we would like to clarify key points for the story of this manuscript (4) that are critical in the context of COVID-19 outbreak and for the perspective of this work. When COVID-19 starts around the world the Editor-In-Chief of the Journal International Journal of Antimicrobial Agents (JM. Rolain) asked colleagues (D. Raoult, PR. Hsueh, and S. Stefani) to launch a special issue in the journal to create a real-time rapid debate around this emerging disease with special regards to therapeutic options (5). Our preliminary paper (4) in this way was relatively trivial i.e reported, in an emergency situation, a comparative analysis between a small group treated with hydroxychloroquine and another small group not treated with hydroxychloroquine showing a significant decrease of viral shedding after 6 days of therapy.

Surprisingly, despite the very small size of the group, the addition of azithromycin made a difference on the endpoint we chose, which is the disappearance of the viral load in the pharynx that is the only data that can be analyzed on a small group. Indeed, neither mortality, nor the passage in intensive care unit, nor the duration of the treatment can be evaluated on such a small group. This preliminary information was essential in our opinion especially as it confirmed the preliminary *in vitro* and *in vivo* results against SARS-CoV-2 announced by the Chinese (6-8), also confirming previous *in vitro* reports on the anti-SARS-CoV-1 coronavirus activity dating back to 2004 (9-12). This preliminary report paved the way for work testing its reproducibility.

On the therapeutic level, the hydroxychloroquine + azithromycin combination was found to be the most effective (4) consistent with *in vitro* synergistic antiviral activity reported in our laboratory (13). Azithromycin had already, contrary to what one of the authors says, been tested effectively on Zika (14,15), so we knew that it had an antiviral action. With regard to our seminal paper on *in vivo* anti-SARS-CoV-2 activity of hydroxychloroquine (4), we were subjected to unprecedented violence. I (DR) was asked to confess that I had a

relationship and a conflict of interest with Sanofi, which is laughable when you use generics and you have had no relationship with the pharmaceutical industry at all at IHU (our center) for 5 years. At the same time, the authors who published on remdesivir, for those we know, the French, did not declare any conflict of interest in the New England Journal of Medicine (16). In fact, it was much more credible to look for conflicts of interest relating to Gilead than to Sanofi (17). The second thing is that I (DR) was harassed to give all the evidence to show that this was done after the agreement of our government, the evaluation by the Committee for the Protection of Individuals, and that it was done in all regularity (validated by ANSM, the French FDA, available online in the EU Clinial Trial Register Page, EudraCT number: 2020-000890-25). Subsequently, we were threatened for retractation of this article, with no justification other than the opinion of people who were fiercely hostile to the use of hydroxychloroquine. It should be noted that this paper is now by far the most cited paper in the literature on the treatment of COVID-19, exceeding 1600 citations in Google Scholar.

As a result of this paper, half of the world's population now benefits from a recommendation of hydroxychloroquine with or without azithromycin, this currently concerns more than 4.5 billion people (18). On the other hand, methodological problems and problems of scientific misconduct with non-declaration of conflict of interest have multiplied for therapeutics including remdesivir in the best journals, including those of Elsevier, which ended up with the retraction of a paper that had probably been completely invented (19).

Finally, we have recently carried out a meta-analysis of all the work done on hydroxychloroquine (20) that is upgraded in this response. Here, we specifically focused on mortality and viral shedding persistence, including a new randomized controlled trial reporting a favorable effect on mortality (21) (Figure 1). Importantly, while the conflict has been particularly violent in France and the United States, 5 studies from both these countries has just shown that hydroxychloroquine reduces rate of hospitalization, length of

hospitalization, mortality, and viral shedding in 4,642 (22), 3,737 (23), 2,820 (24), 2,541 (25) and 518 (26) patients.

This new meta-analysis (Figure 1) included 18,211 patients (10,409 treated by a chloroquine derivative) from 12 studies and assessed mortality in 4 countries (China (27), France (22,23,28-30), Spain (31), and USA (24-26,32,33). A two-fold decrease of the risk of death was confirmed in clinical studies (number of comparisons (n) = 8, odds ratio 0.53, 95% confidence interval (95%CI) 0.40 – 0.71, p = .00003) but not among big data studies (n = 6, OR = 0.92, 95%CI 0.76 - 1.10, p = .36 – Figure 1A). Heterogeneity was significant between clinical and big data studies (Q-value 9.45, p = .002). Effect size was consistent among clinical studies (I^2 = 31%, p = 0.18) but not among big data studies (I^2 = 69%, p = .006). Indeed, a new big data study (24) recently reported a very significant two-fold decrease in mortality in 2,820 patients from the 8 hospitals of the Mount Sinai Health System (New York, USA). This result contrasted with other big data studies (22,32,33). Despite substantial heterogeneity, a significant summary effect was observed when including all comparisons from all included studies (n = 14, OR 0.79, 95%CI 0.67 - 0.92, p = .003). Exclusion of the study from our center (23) did not modify the overall effect (n = 13, OR = 0.82, 95%CI 0.70 – 0.97, p = .023) nor the two-fold decrease in the risk of death among the 7 clinical studies from other centers (n = 7, OR 0.48, 95%CI 0.32-0.72, p = .0004).

Regarding persistent viral shedding, a total of 4,540 patients (3,544 treated by a chloroquine derivative) from 8 studies from only 4 countries were included (5 from China (21,34-37)), 1 from France (23), 1 from Saudi Arabia (38) and 1 from South Korea (39)) with a significant two-fold decrease of the risk of viral persistence (11 comparisons, OR 0.47, 95%CI 0.28 – 0.79, p = .005, Figure 1B). Exclusion of our study (23) did not change the effect size (n = 10, OR = 0.45, 95%CI 0.23 – 0.88, p = .02). Strikingly, none of the big data studies and none of the studies from USA assessed the virus persistence.

This new meta-analysis shows that, apart from the unverifiable work that did not assess virological outcome and carried out by people who had conflicts of interest with Gilead (17), the body of publications shows that hydroxychloroquine therapy is significantly and reproducibly correlated with a two-fold decrease in both mortality and viral shedding.

In practice, our seminal work (4) has benefited from a massive diffusion despite a profusion of papers that have not been verified but accepted each time they had a negative position towards hydroxychloroquine (40). However, the facts being stubborn, the accumulation of publications showing that hydroxychloroquine is effective following our paper, or at the same time by Chinese authors, leaves no doubt that this preliminary study did indeed paved the way for a therapeutic strategy that is now being generalized throughout the world, and whose favorable results have been replicated several times.

References

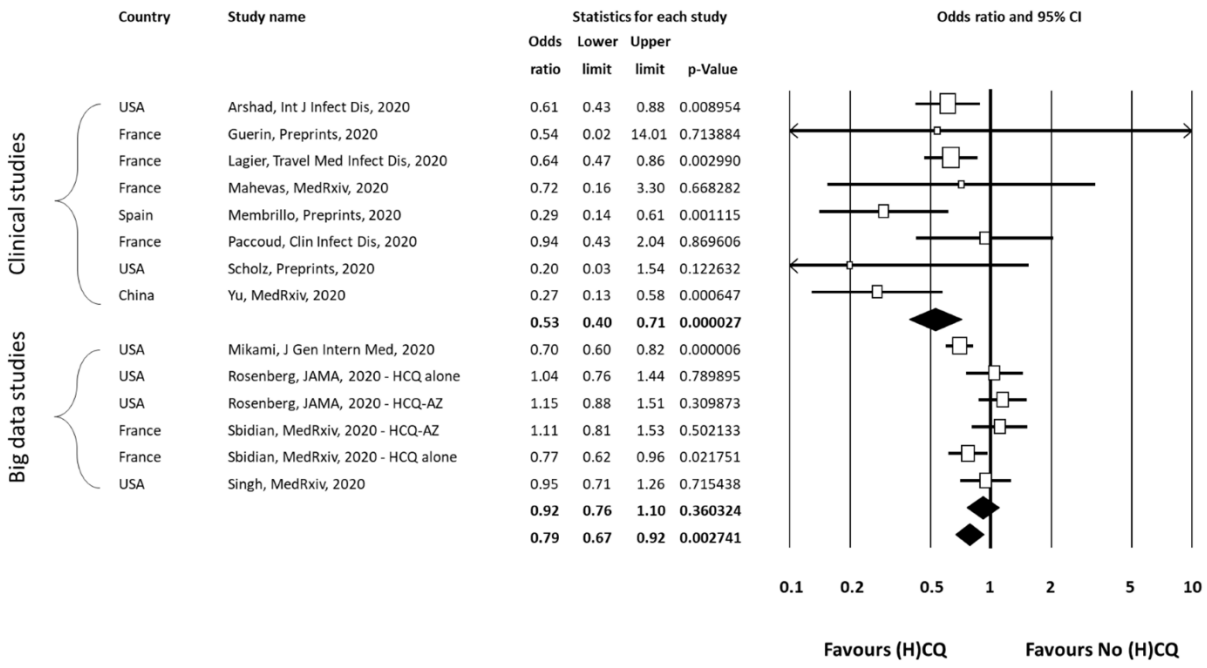
1. Machiels JD, Bleeker-Rovers CP, ter Heine R, et al. Reply to Gautret et al. Hydroxychloroquine sulfate and azithromycin for COVID-19: what is evidence and what are the risks ? Int J Antimicrob Agents. 2020; In press. doi : 10.1016/j/ijantmicag.2020.106056.
2. Rosendaal FR. Review of: "Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial Gautret et al. Int J Antimicrob Agents. 2020; In press 2010; doi: 10.1016/j.ijantimicag.2020.105949
3. Voss A, Coombs G, Unal S, Saginur R, Hsueh PR. Publishing in face of the COVID-19 pandemic. Int J Antimicrob Agents. 2020; In press. doi: 10.1016/j.ijantimicag.2020.106081
4. 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 [published online ahead of print, 2020 Mar 20]. Int J Antimicrob Agents. 2020;105949. doi: 10.1016/j.ijantimicag.2020.105949
5. Raoult D, Hsueh PR, Stefani S, Rolain J.M. COVID-19 Therapeutic and Prevention. Int J Antimicrob Agents. 2020 apr; 55(4):105937. Epub 2020 Mar 7. doi: 10.1016/j.ijantimicag.2020.105937.
6. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020 Mar 16;14(1):72-73. Epub 2020 Feb 19.doi: 10.5582/bst.2020.01047
7. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;10-0282.
8. 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) [published online ahead of print, 2020 Mar 9]. Clin Infect Dis. 2020;ciaa237. doi: 10.1093/cid/ciaa237.
9. Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun. 2004;323(1):264-268. doi: 10.1016/j.bbrc.2004.08.085
10. 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(4):297-308. doi: 10.1016/j.ijantimicag.2007.05.015
11. Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. Lancet Infect Dis. 2006;6(2):67-69. doi: 10.1016/S1473-3099(06)70361-9
12. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005;2:69. Published 2005 Aug 22. doi: 10.1186/1743-422X-2-69
13. Andreani J, Le Bideau M, Duflot I, et al. In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. Microb Pathog. 2020;145:104228. doi: 10.1016/j.micpath.2020.104228
14. Retallack H, Di Lullo E, Arias C, et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proc Natl Acad Sci U S A. 2016;113(50):14408-14413. doi: 10.1073/pnas.1618029113

15. Li C, Zu S, Deng YQ, et al. Azithromycin Protects against Zika virus Infection by Upregulating virus-induced Type I and III Interferon Responses [published online ahead of print, 2019 Sep 16]. Antimicrob Agents Chemother. 2019;63(12):e00394-19. doi : 10.1128/AAC.00394-19
16. Grein J, Ohmagari N, Shin D, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med. 2020;382(24):2327-2336. doi: 10.1056/NEJMoa2007016
17. Roussel Y, Raoult D. Influence of conflicts of interest on public positions in the COVID-19 era, the case of Gilead sciences. New Microbes New Infect. 2020; 100710. In press. doi: 10.1016/j.nmni.2020.100710
18. Roussel Y, Raoult D. Hydroxychloroquine recommendations toward the world: first evaluations. IHU preprints 2020. doi: 10.35088/wjzn-1x68
19. Mehra MR, Ruschitzka F, Patel AN. Retraction-Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis [retraction of: Lancet. 2020 May 22;:]. Lancet. 2020;395(10240):1820. doi: 10.1016/S0140-6736(20)31324-6
20. Million M, Gautret P, Colson P, et al. Clinical efficacy of chloroquine derivatives in COVID-19 infection: Comparative meta-analysis between the big data and the real world. New Microbes New Infections 2020; 100709. In press. doi: 10.1016/j;nmni.2020.100709
21. Chen L, Zhang ZY, Fu JG. et al. Efficacy and safety of chloroquine or hydroxychloroquine in moderate type of COVID-19: a prospective open-label randomized controlled study medRxiv 2020.06.19.20136093. doi: 10.1101/2020.06.19.20136093
22. Sbidian E, Josse J, Lemaitre G, et al. 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; doi: 10.1101/2020.06.16.20132597
23. Lagier JC, Million M, Gautret P, et al. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis [published online ahead of print, 2020 Jun 25]. Travel Med Infect Dis. 2020;101791. doi: 10.1016/j.tmaid.2020.101791
24. Mikami T, Miyashita H, Yamada T, et al. Risk Factors for Mortality in Patients with COVID-19 in New York City [published online ahead of print, 2020 Jun 30]. J Gen Intern Med. 2020;1-10. doi: 10.1007/s11606-020-05983-z
25. Arshad S, Kilgore P, Chaudhry ZS, et al. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. Int J Infect Dis. 2020. doi : https://doi.org/10.1016/j.ijid.2020.06.099
26. Scholz, M. Derwand, R. Zelenko, V. COVID-19 Outpatients – Early Risk-Stratified Treatment with Zinc Plus Low Dose Hydroxychloroquine and Azithromycin: A Retrospective Case Series Study. Preprints 2020 ; 2020070025. doi : 10.20944/preprints202007.0025.v1.
27. Yu B, Li C, Chen P, et al. Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19 [published online ahead of print, 2020 May 15] [published correction appears in Sci China Life Sci. 2020 Jun 18;:]. Sci China Life Sci. 2020; 1-7. doi:10.1007/s11427-020-1732-2

28. Guérin V, Lévy P, Thomas, J, Lardenois T, Lacrosse P, Sarrazin E, Regensberg de Andreis N, Wonner M. Azithromycin and Hydroxychloroquine Accelerate Recovery of Outpatients with Mild/Moderate COVID-19. Preprints 2020; 2020050486 doi: 10.20944/preprints202005.0486.v1
29. Mahévas M, Tran VT, Roumier M, et al. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data [published correction appears in BMJ. 2020 Jun 18;369:m2328]. BMJ. 2020;369:m1844. Published 2020 May 14. doi: https://doi.org/10.1136/bmj.m1844
30. Paccoud O, Tubach F, Baptiste A, et al. Compassionate use of hydroxychloroquine in clinical practice for patients with mild to severe Covid-19 in a French university hospital [published online ahead of print, 2020 Jun 18]. Clin Infect Dis. 2020;ciaa791. doi: 10.1093/cid/ciaa791
31. Membrillo de Novales FJ, Ramírez-Olivencia G, M Estébanez, Begoña de Dios , Dolores Herrero M , Mata T , Alberto M. Borobia , Gutiérrez C, Simón M, Ochoa A , Martínez Y , Aguirre A , Francisco de Asís Alcántara , Fernández-GonzálezP , López E, Sergio Campos S , Navarro M , Ballester L.E.Early Hydroxychloroquine Is Associated with an Increase of Survival in COVID-19 Patients: An Observational Study. Preprints. 2020; 2020050057. doi: 10.20944/preprints202005.0057.v1.
32. Rosenberg ES, Dufort EM, Udo T, et al. Association of Treatment With Hydroxychloroquine or Azithromycin With In-Hospital Mortality in Patients With COVID-19 in New York State [published online ahead of print, 2020 May 11]. JAMA. 2020;323(24):2493-2502. doi: 10.1001/jama.2020.8630
33. Singh S, Khan A, Chowdhry M, Chatterjee A. Outcomes of Hydroxychloroquine Treatment Among Hospitalized COVID-19 Patients in the United States- Real-World Evidence From a Federated Electronic Medical Record Network. medRxiv. 2020.05.12;20099028; doi: 10.1101/2020.05.12.20099028
34. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). J Zhejiang Unniv. Med Sci. 2020;49:0.
35. 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. Treating COVID-19 with Chloroquine. 2020. J Mol Cell Biol. In press. doi: 10.1093/jmcb/mjaa014.
36. Huang M, Li M, Xiao F, Liang J, Pang P, Tang T, Liu S, Chen B, Shu J, You Y, Li Y, Tang M, Zhou J, Jiang G, Xiang J, Hong W, He S, Wang Z, Feng J, Lin C, Ye Y, Wu Z, Li Y, Zhong B, Sun R, Hong Z, Liu J, Chen H, Xiaohua Wang, Zhonghe Li, Pei D, Tian L, Xia J, Jiang S, Zhong N, Shan H. 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. doi: 10.1101/2020.04.26.20081059
37. 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. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. medRxiv . 2020.04.10;20060558. doi: 10.1101/2020.04.10.20060558 (version 1).

38. Shabrawishi MH, Naser AY, Aldobyany AM, Negative nasopharyngeal SARS-CoV-2 PCR conversion in Response to different therapeutic interventions. medRxiv. Posted May 11, 2020. doi: 10.1101/2020.05.08.20095679
39. Kim MS, Jang SW, Park YK et al, Treatment Response to Hydroxychloroquine, Lopinavir/Ritonavir, and Antibiotics for Moderate COVID 19: A First Report on the Pharmacological Outcomes from South Korea. medRxiv. 2020.05.13;20094193. doi: 10.1101/2020.05.13.20094193.
40. Million M, Roussel Y, Raoult D. Chloroquine and COVID-19: A western medical and scientific drift? Eur J Intern Med. 2020b;S0953-6205(20)30266-1. doi: 10.1016/j.ejim.2020.06.020

A. Mortality



B. Persistent viral shedding

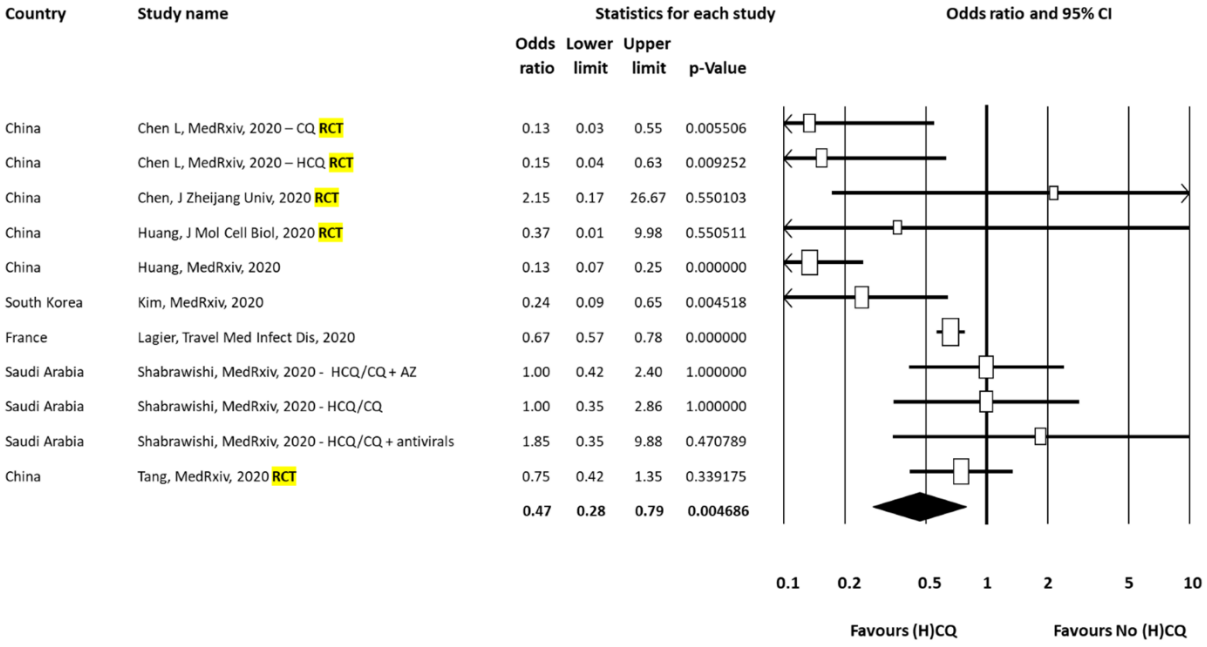


Figure 1. Meta-analysis on chloroquine derivatives for COVID-19

CI: confidence interval, HCQ: hydroxychloroquine, CQ: Chloroquine, RCT: randomized controlled trial, (H)CQ: chloroquine derivatives (hydroxychloroquine (HCQ) or chloroquine (CQ)). This meta-analysis was performed with a random-effects model using Comprehensive Meta-Analysis v3 (Biostat, Englewood, NJ, USA).