Original Article

Revisiting a Meta-analysis Shows that Hydroxychloroquine with Azithromycin may be Efficient in Covid-19 patients

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Abstract

Objective: To analyze the impact of study selection on the results of a recently published meta-analysis of the efficacy of hydroxychloroquine (HCQ) and hydroxychloroquine plus azithromycin (AZI) in Covid-19 patients.

Methods: 31 studies were reviewed looking for critical bias. Combined hazard ratios and confidence intervals were calculated for both treatments using a fixed effects size model and a random effects model. Quantitative analysis regarding the toxicity of the association HCQ plus AZI is made.

Results: Meta-analyses performed on the 11 studies we deem critically unbiased show a mortality reduction of 55% for HCQ and 66% for HCQ plus AZI.

For both treatments, our meta-analysis indicates a significant efficacy in reducing mortality in hospitalized Covid-19 patients.

Keywords: Covid-19; Meta-analysis; Critical bias; Clinical studies; Hydroxychloroquine; Azithromycin

1. Introduction

The article [1]: "Effect of hydroxychloroquine (HCQ) with or without azithromycin (AZI) on the mortality of coronavirus disease 2019 (Covid-19) patients: a systematic review and meta-analysis", published on August 26, 2020 in Clinical Microbiology and Infection, concludes to the inefficacy of hydroxychloroquine in the treatment of hospitalized Covid-19 patients. However, this study presents many weak points and inconsistencies. Firstly, the statistical methodology, which raises concerns, provides results which fuel controversies among clinicians. Secondly, and more importantly, crucial information were neglected to the sole profit of a statistical approach. Neglected data were: patients clinical status, disease stage, study conditions, posology indications on the treatments under investigation (HCQ or HCQ plus AZI). This, in a meta-analysis, does not allow to draw conclusions on clinical practice with Covid-19 patients. Despite the authors claim of having followed a well established methodology to identify critical bias, their article is astonishingly lacking explicit explanation on why they have specifically retained 17 studies among 31 preselected ones. Despite the meta-analysis authors list a number of hard limitations in their discussion, they rely blindly on their calculations to firmly suggest that: (1) HCQ alone does not show efficacy against Covid-19; and (2) any patient treated by HCQ and AZI, at any stage of the disease, would develop a high risk of cardiac failure subsequent to treatment intake.

2. Methods

We have reviewed the 31 preselected studies [2-32] looking for critical bias not allowing some of these studies to be retained in the meta-analysis calculation. Following Fiolet et al., we have excluded the study of Kuderer [15] because it was performed on the same cancer registry (CCC19) as the study of Rivera [24].

2.1 Efficacy of HCQ with or without AZI

In our meta-analysis, we calculated the combined hazard ratio (HR) using both a fixed effects size model and a random effects model, according to Borenstein [33] (Introduction to Meta-Analysis, 2007). The variance of each study was retro-calculated using the published adjusted hazard ratio (aHR) and 95% confidence interval (95% CI). Contrary to the logrank method, this approach does not require a hypothesis on the expected mortality in the treatment and control arms. Details of the calculations are provided in Figures 1a and 1b. We have also used the logrank method to calculate the variances of an expected mortality of 26% [1,34].

Figure 1a Meta-analysis: fixed effect model calculations for *n* studies

$$\overline{HR}_{\bullet} = \frac{\sum_{i=1}^{n} w_i HR_i}{\sum_{i=1}^{n} w_i}$$ combined hazard ratio

$$w_i = \frac{1}{v_i}$$ statistical weight

$$v_{\bullet} = \frac{1}{\sum_{i=1}^{n} w_i}$$ variance of the fixed model

$$v_i = \left[\frac{1.96}{\ln(HR_i) - \ln(HR_i^{inf})}\right]^2$$

or

$$v_i = \left[\frac{1.96}{\ln(HR_i^{sup}) - \ln(HR_i)}\right]^2$$

retro-calculated variance for study i

$$SE(\overline{HR}_{\bullet}) = \sqrt{v_{\bullet}}$$ standard error of the model

$$p = 2x\left[1 - \phi(|Z|)\right] \text{ avec } Z = \frac{\overline{HR}_{\bullet}}{SE(\overline{HR}_{\bullet})}$$

ϕ : standard normal cumulative distribution function

$$\overline{HR}_{inf} = \overline{HR}_{\bullet} - 1.96 * SE(\overline{HR}_{\bullet})$$

$$\overline{HR}_{sup} = \overline{HR}_{\bullet} + 1.96 * SE(\overline{HR}_{\bullet})$$

95% confidence interval

Figure 1b

Variance calculation in the case of a random effects model

$$Q = \sum_{i=1}^{k} w_i \left(HR_i - \overline{HR}_{\bullet}\right)^2$$

$$C = \sum w_i - \frac{\sum w_i^2}{\sum w_i}$$

$$\tau^2 = \begin{cases} \frac{Q - df}{C} & \text{if } Q > df \\ 0 & \text{if } Q \leq df \end{cases}$$

τ^2 *variance between studies*

$df = n$ *number of studies or number of degres of freedom*

$$v_i = v_i + \tau^2$$

Four meta-analyses were performed on:

1. the 31 preselected studies, excluding Kuderer [15], (29 studies for HCQ and 11 for HCQ plus AZI),
2. the 17 studies retained in the meta-analysis [1],
3. our 11 studies we deemed unbiased (Table 1),
4. our 11 unbiased studies plus 2 unfavorable.

2.2 Regarding the toxicity of HCQ combined with AZI

Quantitative analysis was made on the arguments presented in their discussion, notably regarding the toxicity of the association HCQ plus AZI.

3 Results

3.1 Efficacy of HCQ with or without AZI

Among the 31 preselected studies, we disagree concerning the presence or not of critical bias in 18 of them (Table1). Among the 18 studies (including Kuderer) deemed critically unbiased, we found 12 studies with bias which are critical. Conversely, among the 13 studies considered as critically biased, we found 7 studies without bias sufficiently significant to prevent them from entering in the meta-analysis.

In total, we have selected 11 studies that we deemed free of critical bias for HCQ (9 studies)

and HCQ plus AZI (4 studies). Meta-analyses performed on these 11 studies (Table 2) show a mortality reduction of 55% for HCQ and 66% for HCQ plus AZI. For comparison purpose, the combined HRs and 95% CIs calculated for the 31 preselected studies (except Kuderer) and for the 17 studies selected by Fiolet et al. are presented in Table 2.

The effect of heterogeneity in HR and 95% CI calculation, and the differences between the statistical weights obtained with respect to the method used for evaluating variances is illustrated (Table 3) with combined HRs remaining favorable to both treatments.

3.2 Quantitative analysis of the toxicity of HCQ with or without AZI

In the study by Bessière [35], 40 patients in intensive care unit (ICU) have received HCQ or HCQ plus AZI, 20 of whom (50%) have also received another treatment known for causing QTc prolongation on the electrocardiogram. In total, 14 patients presented prolongations of QTc ≥ 60 ms, of whom only 7 (17%) a prolongation of QTc ≥ 500 ms, after 2 to 5 days of treatment. No patient died from cardiac arrest and no ventricular arrhythmia or torsade de pointe was recorded. Bessière admits that his results cannot be generalized outside the ICU setting.

The study by Rosenberg [26] reports the raw rate of cardiac deaths with respect to the number of deceased patients: 35/118 (29.7%) for HCQ plus AZI; 14/38 (36.8%) for HCQ alone; 5/17 (29.4%) for AZI alone and 7/20 (35.0%) for the control group. After adjustment with a logistic regression model, Rosenberg obtains a risk ratio of cardiac arrest of 2.13 [1.12-4.05] for HCQ plus AZI compared with control. This result takes into account all observed cardiac failures in addition to those leading to death. The raw data correspond to a doubling of cardiac failures in the HCQ plus AZI (15.5%) arm with respect to control without treatment (6.8%). However, Rosenberg admits that the patients treated with HCQ or HCQ plus AZI were sicker at the time of their inpatient admission than those in the control arm. This has introduced a selection bias (Table 1) that Fiolet does not take into account in his discussion. The study of Rosenberg presents many limitations: (a) the readmission of patients in other hospitals is not accounted for; (b) mortality is calculated on all hospitalized patients whereas some have been hospitalized only 24 hours and lost to follow-up thereafter; (c) inflammatory markers associated with disease severity were not measured; (d) in some cases, the extremely short delay between inpatient hospital admission and ICU transfer, often concomitantly to treatment initiation with HCQ, does not allow treatment efficacy to be correctly assessed.

Among the 11 preselected studies dealing with the HCQ plus AZI combination, 5 provide quantitative information recorded on the cardiac toxicity. They are without consequence on mortality: (1) Arshad [4]: 783 patients, none with torsade de pointe; (2) Cavalcanti [7]: 217 patients, QTc prolongation observed in 116 patients (QTc > 480 ms within 7 days in 17 patients), no toxic death; (3) Lagier [16]: 3119 patients, QTc prolongation (> 60 ms) observed in 25 patients (0.67%) leading to treatment discontinuation (3 cases with QTc > 500 ms), no torsade de pointe or sudden death; (4) Mahévas [20]: 8 patients (10%) presented an electrocardiographic change with QTc prolongation > 60 ms (1 patient with QTc prolongation > 500 ms); (5) Rosenberg [26]: no significant electrocardiographic difference recorded according to their logistic regression adjusted model.

Table 1: Critical bias analysis

(a yellowish background corresponds to a disagreement with Fiolet)

Studies	Patients (N)	HCQ (N : RR)	HCQ + AZI (N : RR)	Bias[1] Fiolet		Our analysis of the bias	
Non-randomized studies							
Alberici	643	71 OR = 0.44 [0.16-1.24]		Yes	Confounding covariate; selection of patients; classification of intervention	Yes	Bias of classification of intervention : **antiviral therapy based on lopinavir/ritonavir**, univariate analysis only for HCQ effect. Multivariate analysis does not include HCQ.
Arshad	2541	1202 HR = 0.34 [0.25-0.46]	783 HR = 0.29 [0.21-0.40]	Yes	Confounding covariate; selection of patients; classification of intervention	No	Study protocol well established, strategy of treatment based on HCQ, homogeneous cohort of inpatients in a coherent collaborative multi-center setting: The Henry Ford Health System (HFHS) in Southeast Michigan with large six hospital integrated.
Ayerbe	2075	1857 OR = 0.42 [0.32-0.54]		No	Confounding covariate; selection of patients; classification of intervention!	No	Study on heparin (primary endpoint) actually shows the benefit of HCQ. Bias of result selection that underlines the political issue associated with the use of HCQ in Covid-19. The effect of heparin was calculated whereas it was confounding with HCQ or l'HCQ + AZI! Over 2075 included patients, more have received HCQ (1857 pts) than heparin (1734 pts)! All the study authors are Spaniards affiliated to English Universities! The study was published on may 31 after the Recovery trial HCQ arm premature stop was announced.
Barbosa not published	62	31 $Rr_{cal.}$= 2.47 [0.24-24.98] not adjusted		Yes	Confounding covariate; selection of patients; classification of intervention	Yes	**Inconsistent HCQ dosage and too few patients.**

Bousquet	108		27 HR = 0.49 [0.19-1.29]	Yes	Confounding covariate; selection of patients; classification of intervention	No	Study lacking statistical power but a unique strategy of treatment with HCQ + AZI, disease severity well established, conclusion showing a marked efficacy in univariate analysis. Importance of co-medication: 93/108 patients have received an anticoagulant either curative (30%) or preventive.
Cravedi	144	101 $Rr_{cal.}$= 1.53 [0.84-2.80] non adjusted		Yes	Confounding covariate; selection of patients; classification of intervention	Yes	Unadjusted bias: **47/101 patients were from the Bronx** quarter that has a mortality rate of 40 %! Overall mortality rate of the study 46/101 = 32% and for the patients outside Bronx 24%; sample size too small too allow multivariate adjustment; **HCQ** weakly associated with survival in univariate analysis ; confounding factor with co-medication: **40/46 death associated with antibiotics use** (p = 0.023).
Fontana	15	12 $Rr_{cal.}$= 0.50 [0.16-1.55] not adjusted		Yes	Confounding covariate; selection of patients; classification of intervention	Yes	**Too few patients included** (15 pts) and embryonic comparator arm (3 pts).
Gautret	36	20 $Rr_{cal.}$= 3.41 [0.15-77.45] not adjusted		Yes	Confounding covariate; selection of patients; classification of intervention; deviation from intended intervention; missing data; bias of selected results	N.R.	**Study unduly preselected** : the main endpoint was not mortality but the viral load decrease only one patient died on the 3rd day of treatment. The patient was PCR negative at day 3, therefore at a very advanced disease stage. Follow up is only 6 days (patients must be followed over 28 days at least to assess mortality).
Géléris	1376	811 HR=1,04 [0.82-1.32]		No		Yes	No strategy of treatment initiation: for **14% of the pts the treatment started between 48 h after admission and tracheal intubation**, several days later = bias indicated by the non proportionality of the survival

							curves that crosses each other! Combined primary endpoint (intubation or death).
Gupta	2215	1761 $Rr_{cal.}$= 1.06 [0.92-1.23] not adjusted		Yes	Confounding covariate; selection of patients; classification of intervention	Yes	**Study too heterogeneous with 65 hospitals involved**, no treatment strategy, efficacy of **HCQ** is not an endpoint, **HCQ** dosage unknown, treatment duration unknown, inter-institutional variability, competing interest of first author with Big Pharma.
Ip	2512	441 HR=0.99 [0.8-1.22]	1473 HR = 0.98 [0.75-1.28]	No		Yes	The primary endpoint was tocilizumab and the authors conclude to its efficacy with HR = 0.76 [95% CI, 0.57-1.00]. But over the 547 patients who received tocilizumab 486 (89%) have also received **HCQ**! Therefore, **HCQ is a confounding factor of the therapeutic benefit of tocilizumab (bias of selection of the result)**. This interaction is not measured or mentioned by the authors of the study (bias of result selection).
Kuderer	928	89 OR = 1.06 [0.51-2.2]	181 OR = 2.93 [1.79-4.79]	No		Yes	A confounding factor of indication for **HCQ + AZI given to the most severely affected patients** (a bias of indication cannot be excluded according to the study authors because the risk ratio **HCQ** + **QZI HR** = 2·93, [1.79–4.79] is too high. Study is a partial duplicate of the Rivera on the CCC19 cancer registry.
Lagier	702		503 HR = 0.49 [0.25-0.97] p = 0.041	Yes	Confounding covariate; selection of patients; classification of intervention	No	Well established strategy of treatment and clear conditions of **HCQ** + **AZI** administration, including comorbidities and disease severity. HR adjusted for comorbidities (Charlson combined comorbidity index), disease severity (NEWS-2 score) and **HCQ-AZI** > 3 days).

Lecronier	80	38 $Rr_{cal.}$ = 0.58 [0.27-1.24] not adjusted		Yes	Confounding covariate; selection of patients; classification of intervention; deviation from intended	No	Critically ill patients, numerically **HCQ** has less deaths in proportion to the 2 other arms : with 24% versus 41% for SOC and 35% for lopinavir/ritonavir ; the treatment bias declared by the authors (all patients received lopinavir/ritonavir at the beginning of the trial in the 3 arms) is not relevant since finally **HCQ** is at the end the best treatment with respect to the 2 other arms, meaning that the switch to **HCQ** was in any case favorable to the patients.
Luo	102	35 OR = 1.03 [0.26-3.55]		Yes	Confounding covariate; classification of intervention	Yes	**HCQ** dose and posology unknown, no treatment strategy, possible bias of indication: **only the most severely affected patients received HCQ** ; authors have too many competing interests with the pharmaceutical industry.
Magagnoli	807	198 HR = 1.83 [1.16-2.89]	214 HR = 1.31 [0.80-2.15]	No	Selection of patients; classification of intervention; missing data	Yes	The indication bias is the most important according to the authors, **HCQ** + **AZM** has more efficacy if administered before mechanical ventilation. **HCQ** not controlled : 25% of the patients have received > 480 mg d'**HCQ**/day without knowing the maximum dose... The article seems to indicate the possibility of overdosing : "Of note, a randomized, controlled trial of high-dose chloroquine, the parent compound of **HCQ**, was halted prematurely due to cardiac toxicity and higher fatality rates in the high dose chloroquine-treated COVID-19 patients." It is likely that only **only the most severely affected patients received HCQ or HCQ + AZI** as the median hospitalization times indicate : "after propensity score adjustment, the length of hospital stay was 33%

							(95% CI, 6%–67%; p = 0.01) longer in the HC group and 38% (95% CI, 6%–67%; p = 0.004) longer in the HC+AZ group (Table 4) when compared to the no HC group."
Mahévas	181	84 HR = 1,2 [0.4 - 3.3]		No		Yes	Critical bias: **10% of the patients have received HCQ treatment beyond the first 48 hours following admission** + no multivariate analysis (no sufficient data according to the authors them-selves)! None of the 15 patients who received a combination of hydroxychloroquine and azithromycin was transferred to intensive care and none died.
Membrillo MedRxiv	166	123 OR = 0.07 [0.012-0.402]		No	Selection of patients; classification of intervention	No	A non critical bias in the most severely affected patients (**HCQ** not given to the oldest patients). HR adjusted for comorbidities.
Mikami	6493	2813 HR = 0.53 [0.41-0.67]		No	Classification of intervention	No	Clinical conditions and **HCQ** treatment well defined, primary endpoint on **HCQ** efficacy, statistical significance of **HCQ** in multivariate analysis for hospitalized patients (p < 0.001) adjusted for age, gender, human population group, smoking status, asthma, hypertension, diabetis, cancer, hypertension, O2 saturation, lymphocytes level and hydroxychloroquine use.
Paccoud	84	38 HR=0.89 [0.23-3.47]		No		No	Well defined clinical stage of the disease, treatment strategy and dosage of **HCQ**.
Rivera	2186	335 OR = 1.11 [0.71-1.74]	203 OR = 2.15 [1.15-3.06][2]	No		Yes	Adjusted risk ratio aOR = 2.15 for HCQ + any other treatment, is not very credible: possible indication of bias and **study too complicated to provide correct interpretation of the data**; **HCQ** + **AZI** given quasi

							exclusively (10 times more in adjusted OR) to hospitalized patients, compared with ambulatory patients, and more particularly to ICU patients (6 times more); **HCQ** + **AZI** given 5 times more often (adjusted OR) to patients with renal disorder with respect to remdesivir = bias of indication (Gilead recommandation was to avoid to give remdesivir to patients with renal disorder). On the other hand, 14% of the text of the article is spent in listing the competing interest of the authors!
Rogado	45		18 OR = 0.02 [0.01-0.73]	Yes	Confounding covariate; selection of patients; classification of intervention	No	Very few patients included but no critical bias. Significant benefit in multivariate analysis (p=0.03) adjusted for age, histology and cancer stage, cancer treatment type and hypertension.
Rosenberg	1438	271 HR = 1.08 [0.63-1.85]	735 HR = 1.35 [0.76-2.4]	No		Yes	The second author involved with the design of the study, the extraction and interpretation of the data has **competing interest with Gilead**. Strangely, **AZI** administered alone has efficacy. Several bias acknowledged by the study authors. Also **probably a bias of patients selection** as well: over 7914 pts in the initial registry, 70% are randomly eliminated and among the 2362 remaining patients, 887 are eliminated due to incomplete review of their files!.
Sanchez	868	629 OR = 0.47 [0.28-0.79]		No	Confounding covariate; selection of patients; classification of intervention	No	**HCQ** significantly beneficial for the subgroup of patients under chronic hemodialysis: p = 0.005. Factors associated with mortality (age and pneumonia) are identified logistic by regression.
Sbidian	4642	623	227	No		Yes	Study is not yet published, **important bias between the**

MedRxiv		HR = 1.05 [0.77-1.33]	HR = 1.40 [0.98-1.81]				**study arms at baseline**, possible bias of selection and of indication + multivariate analysis that totally inverses the univariate results.
Singh MedRxiv	3372	326 HR = 0.95 [0.74-1.23]	799 HR = 1.19 [0.89-1.60]	No	Classification of intervention	Yes	Important patient selection bias : **only severely affected patients were actually treated** and thus excluded from the control group, collecting the less severe cases as demonstrated by a reduced mortality of 12% (twice lower than currently recorded mortality rates around 25%). Conversely, the **HCQ** and **HCQ** + **AZI** groups collected the most severe cases for which a treatment was attempted. It is probable that **HCQ** and **HCQ** + **AZI** had actually a beneficial effect by reducing mortality to the range of less severely affected patients.
Wang MedRxiv	7592	591 OR = 0.96 [0.69-1.34]	2301 OR=0.94 [0.73-1.21]	Yes	Classification of intervention; missing data	Yes	The primary endpoint was the social determinants of mortality (not treatments efficacy). As a result the conclusions on the effects of **HCQ**, **HCQ** + **AZI** and **AZI** alone are not solid. A mortality rate of 5% is reported for the Bronx whereas it reaches 40% elsewhere. Too many element of appreciation of the validity of the results are missing. Concerning treatments administration, the study does not distinguish the seven different neighborhoods of New York from each others whereas they exhibit mortality rates between 2 and 17%. The study does not distinguish outpatients from hospitalized patients. The univariate analysis presents **totally aberrant risk ratios between 5. et 7. for the treatments** (OR = 7,2 for **AZI** alone). This indicates considerable bias of indication and patients

							selection in addition to bias of the selected results. Multivariate adjustment is performed but one can doubt it has entirely corrected the bias. This study was **not reviewed by peers and is not yet published**.
Yu	550	48 HR = 0.36 [0.18-0.75]		No	Classification of intervention; missing data	No	Homogeneous cohort (all patients are critically ill in ICU) and they have all received the same strategy of treatments and supporting cares. HR is adjusted for age, gender, hypertension, coronary disease, diabetis, SpO2, chronic obstructive lung disease, body temperature.
Randomized studies							
Cavalcanti	504	221 HR = 1.47 [0.48-4.53]	217 HR = 0.64 [0.18-2.21]	No		Yes	Inpatients and outpatients are mixed and the primary endpoint is not the efficacy of **HCQ**. Mortality is too low ≤ 3% in the two treatment arms: 5 pts for **HCQ**, 5 pts for **HCQ** + **AZI** and 6/66 (10%) for the control. Strange enough the adjusted HR is > 1 for **HCQ** and < 1 for **HCQ** + **AZI** with the same initial proportion of deaths. The control mortality is too low 10% compared with the currently recorded rates of 20 to 30 % and the adjusted **HR are unrealistic** with respect to the very few deaths recorded.
Horby MedRxiv	4716	1561 HR = 1.09 [0.96-1.23]		No		Yes	**Overdosing of HCQ:** 2.4 g administered the first 24 hours and 4 g over the first 3 days, comparable to the dose of the Brazilian study of Borba stopped subsequent to 16 (39%) toxic deaths over 41 patients!
Skipper	423	212 $RR_{cal.}$= 1.01 [0.06-16.09] not adjusted		No		Yes	Only 14 hospitalized patients over 423 patients included: 10 in the placebo group and 4 in the **HCQ** group ; only 1 death recorded (groupe placebo!)**. Therefore the calculated HR is totally unrealistic.**

[1]critical (underlined) and serious bias

[2] the hazard ratio used by Fiolet does not correspond to **HCQ** + **AZI** but **HCQ** + any other treatment (azithromycin, remdesivir, tocilizumab, high dose corticosteroids) according to Rivera et., we don't know which proportion received **AZI**.

Yes: the study is critically biased and should not enter the meta-analysis

No: the study is not critically biased and should enter the meta-analysis

N.R. non relevant for mortality reduction evaluation: no bias but dealt with viral load reduction

RR_{cal} : non adjusted risk ratio, calculated theoretically by Fiolet

Table 2: Fiolet et al. revisited meta-analysis

	Hydroxychloroquine	**Hydroxychloroquine + Azithromycin**
Meta-analysis model[1]	**HR IC 95%CI**	**HR IC 95%CI**
Fiolet et al. 31 preselected studies (except Kuderer)		
fixed effect	0.95 [0.89 - 1.01]	1.04 [0.93 - 1.15]
random effects	0.91 [0.77 - 1.05]	1.05 [0.77 - 1.34]
random effects[2]	0.95 [0.82 - 1.08]	1.02 [0.76 - 1.27]
Fiolet et al. 17 finally selected studies for their meta-analysis		
fixed effect	0.96 [0.88 - 1.03]	1.27 [1.12 - 1.41]
random effects	0.93 [0.76 - 1.10]	1.33 [1.06 - 1.60]
random effects[2]	0.94 [0.79 - 1.09]	1.31 [1.08 - 1.55]
Our 11 studies we consider unbiased		
fixed effect	0.45 [0.31 - 0.59]	0.34 [0.06 – 0.61]
random effects	0.45 [0.31 - 0.59]	0.34 [0.06 - 0.61]
random effects[2]	0.46 [0.36 - 0.56]	0.36 [0.17 - 0.54]

[1]p values > 0.001 are indicated

[2]variance calculated with logrank and an expected 26% mortality rate

Table 3: Fiolet et al. revisited meta-analysis

	Hydroxychloroquine			Hydroxychloroquine + Azithromycin		
Selected studies[1]	**Patients[2] (N)**	**HR 95%CI**	**Weight[3] (% / %)**	**Patients[2] (N)**	**HR 95%CI**	**Weight[3] (% / %)**
Arshad	1202	0.34 [0.25 - 0.46]	18.6 / 18.8	783	0.29 [0.21 - 0.40]	56.2 / 46.2
Ayerbe	1857	0.42 [0.32 - 0.54]	25.2 / 12.1			
Bousquet				27	0.49 [0.19 - 1.29]	6.4 / 3.5
Cravedi[4]	101	1.53 [0.84 - 2.80][5]	4.8 / 1.9			
Lagier				503	0.49 [0.25 - 0.97]	12.7 / 24.5
Lecronier	38	0.58 [0.27 - 1.24][5]	3.0 / 0.9			
Magagnoli[4]	198	1.83 [1.16 - 2.89]	8.3 / 8.1	214	1.31 [0.80 - 2.15]	23.9 / 23.9
Membrillo	123	0.07 [0.01 - 0.40]	0.6 / 1.2			
Mikami	2813	0.53 [0.41 - 0.67]	28.6 / 41.8			
Paccoud	38	0.89 [0.23 - 3.47]	0.9 / 1.3			
Rogado				18	0.02 [0.01 - 0.73]	0.9 / 1.9
Sanchez	629	0.47 [0.28 - 0.79]	6.4 / 10.7			
Yu	48	0.36 [0.18 - 0.75]	3.4 / 2.7			
Meta-analysis fixed effect	7047	0.62 [0.48 - 0.75] p < 0.001	100 / 100	1545	0.57 [0.33 - 0.81] p < 0.001	100 / 100
Meta-analysis random effects[6]		0.73 [0.40 - 1.06] p < 0.001	-	-	0.63 [0.14 - 1.13] p = 0.012	-
Meta-analysis random effects[7]		0.70 [0.39 - 1.02] p < 0.001	-	-	0.60 [0.16 - 1.03] p = 0.007	-

[1]our selection of 11 unbiased studies plus 2 unfavorable biased studies

[2] number of patients in the treatment arm

[3]relative weight calculated from the variance : retro-calculated vs. logrank

[4]studies with critical bias

[5]HR not adjusted

[6] model results with retro-calculated variances using the adjusted HR IC 95%

[7]model results with variances calculated with the logrank method under the assumption of an expected 26% mortality rate

4 Discussion

4.1 Comparing the meta-analyses results

Fiolet et al. briefly review other published meta-analyses they deemed of poor quality because of: (a) integrating too few studies, (b) lacking a comparator group, (c) lacking sub-group analysis and sensitivity analysis, and above all, (d) not having studied the sources of heterogeneity in the data published. But the latter point may characterize their study as well.

Surprisingly, despite a statistically significant mortality reduction of 17% (HR = 0.83 [0.65 -1.06]

$p < 0.01$) calculated on the 17 studies they selected, they conclude to the inefficacy of HCQ.

On the same article selection, our method gives the same trends but with higher hazard ratios, HR = 0.93 [0.76 – 1.10] for HCQ and HR = 1.33 [1.06 – 1.60] for HCQ plus AZI, to be compared with their HR = 1.24 [1.04 – 1.54]. This shows that the statistical method employed can influence up to +/- 0.1 the value of the calculated HR and that results should be regarded cautiously when HR values are close to 1. Fiolet et al. have used the method of DerSimonian [36] for their meta-analysis which resulted in statistical weights differing from ours.

Performed on the 11 studies we deem free of critical bias, our meta-analyses shows a very significant efficacy of both treatments with HR = 0.45 [0.31 – 0.59] for HCQ and HR = 0.34 [0.06 – 0.61] for HCQ plus AZI. Overall, our calculations demonstrate that bias analysis is substantially more important than the mathematical technique.

Table 3 shows the effect of heterogeneity on the combined HR and 95% CI. It shows that statistical weights are more important than HR values. Despite HRs markedly > 1., the statistical weight of the unfavorable studies does not exceed 13% (HCQ) and 24% (HCQ plus AZI), so that results are, in this example, still in line with mortality reduction.

4.2 Review of the meta-analysis methodology

4.2.1 The domain of application of the random effects methodology not fully matched

We agree that a random effects model should be applied when combining several studies with heterogeneous results. However, this heterogeneity is not principally due to systematic errors, or statistical bias, but to intrinsic differences between population samples. Main differences are: old or young patients, with or without comorbidities, presenting physiological variations that may influence the effect of treatment when appertaining to different human population groups, or different socio-economical groups. Variation of the clinical practice between institutions may also cause differences in the measured effect for a given treatment. In that case, each study should correspond to a well defined group or type of patients, or a single institution, and the measured average treatment effect (ATE) will be situated around a value corresponding to the real ATE for the group of patients considered. For a different type of patients, the real ATE (mean ATE over all institutions) may be different. Conversely for a different institution, the real ATE (mean ATE over all groups of patients) may differ as well. Subsequently, the random effects model gets closer to the real ATE value, by encompassing all types of patient groups or, alternatively, all institutions. But mixing all types of patient groups with all clinical practices is disastrous for the result of a meta-analysis, when hazard ratios HRs range from 0.4 (treatment completely beneficial) to 2 (treatment totally harmful) for a same treatment.

In the ideal case, where all studies have measured a near statistically significant benefit, a mean treatment effect is produced as well as a reduced confidence interval and a strengthened statistical power.

In the case where the treatment brings a benefit to some types of patients, or within the framework of an institution, but not to other types of patients or not in other institutions, the meta-analysis will get closer to the overall mean value of the ATE. This overall effect of a treatment may be beneficial or null, and sometimes intrinsically harmful due to exacerbated adverse reactions in certain groups of

patients, or harmful due to a deleterious clinical practice. We see that the reasons for a treatment not to be beneficial are diverse and unrelated to its intrinsic curative potential. In any case, the result of a meta-analysis does not mean that an overall null or unfavorable effect abrogates the curative effect measured in certain groups of patients or when the treatment is combined with an adequate clinical practice.

For instance, in the case of the severe acute respiratory syndrome (SARS) due to the infectious Covid-19 disease, the timing of administration of HCQ or HCQ plus AZI as soon as the first day of hospitalization was crucial, as well as appropriate co-medications to fight adverse physiological effects such as coagulation disorders. Observational studies allow clinicians to rapidly report curative tactics developed on patient samples of intermediate sizes (100 to 200 patients). This form of publication allows the medical community to improve its practice for the benefit of patients. For instance, Bousquet [6] conducted on 108 patients a study aimed at measuring the treatment effect of HCQ plus AZI. They concluded to the efficacy of HCQ plus AZI (HR = 0.49) in univariate analysis. Multivariate analysis could not be performed because of the reduced number of patients included in the study; but the severity of the disease was a well established parameter and the strategy of treatment uniquely defined. This study was eliminated from the meta-analysis we review due to a confounding co-variable invoked but not specified. We suppose the authors have probably considered that 93/108 patients having received an anticoagulant was a confounding factor. This understanding was incorrect because administrating an anticoagulant was an adequate co-medication potentiating the treatment effect.

4.2.2 A bias analysis not explicit and quite erroneous

The bias analysis was presented in a 6 item summary table in appendix A of the article with no explicit statements accompanying it.

All preselected studies were either published (21) or deposited (10) without peer review on the site of MedRxiv at the University of Yale. Some present bias that make their evaluation very difficult (Table 1) such as the studies by Horby [13], Sbidian [28], Singh [29] and Wang [31].

Similarly to the study by Wang, the retrospective studies on large samples of patients entering the meta-analysis may mix Covid-19 patients, either hospitalized with patients requiring only ambulatory care. In addition, patient heterogeneity, diversity of clinical and individual clinician practice, severity of the disease, age and comorbidities constitute a broad spectrum of medical conditions.

The 3 randomized studies (Table 1) that the meta-analysis authors consider free of critical and serious bias actually cannot be taken into account for simple reasons.

(a) The first one (Horby [13]) conducted on hospitalized patients conceals an over-dosage of HCQ [38] that has most probably impacted the survival chances of the patients (Table 1). Unfortunately, due to its very small variance, the study has a statistical weight that dominates the meta-analysis.

(b) The second one (Cavalcanti [7]) mixes inpatients and outpatients and the primary endpoint was not the efficacy of HCQ. The published adjusted HR = 1.47 for HCQ is unrealistically unfavorable in front of a raw 3% death rate in the HCQ arm compared with 10% in the control arm. It

is also unrealistic with respect to HCQ + AZI with a HR = 0.64 and a similar 2.5% death rate (Table 1). The internal inconsistency of this randomized study is not discussed. Fiolet et al. categorize it as being very reliable although it demonstrates the benefit of HCQ+ AZI, contrary to their conclusion.

(c) Finally, the third one (Skipper [30]) was remotely conducted on patients staying home with very mild diseases. Over 14 hospitalized patients, only one died in the placebo group. Last but not least, adding to the confusion, only 34% of those patients received appropriate PCR SARS-CoV-2 testing. Surprisingly, they write they have excluded two Chinese studies because no death were reported but they take into account the study of Skipper on the ground it is a randomized study free of bias.

Fiolet et al. did not have access to any patient file, which prevented them from conducting a rigorous meta-analysis. They lacked systematically the necessary information such as disease severity, dosage and number of days treatments were administered.

They claim they have used the ROBIN-I [39] (non randomized studies), and Rob2 [40] (randomized studies) bias evaluation tools as well as the Cochrane on line recommendations [37,41] concerning the conduct of meta-analyses. Although providing useful indications on the nature of classically encountered bias, these tools do not allow the automatic knowledge and detection of all possible bias. They advocate them but do not explicitly explain any of their study selection. Over the 14 studies they eliminated, they invoked the presence of confounding variables in 11 of them without stating them. Strange enough, they included in their calculation the study by Ayerbe they deemed as having a critical bias, and excluded the study by Wang that does not present critical bias according to them.

The study by Rosenberg [26] was categorized as being at low risk of bias (Table 1), although it has several serious limitations (see results section), among which the fact that patients are not consecutive. A drastic random reduction by 70% of the patients took place and, subsequently, over the 2362 remaining patients, 887 were eliminated because the review of their files was incomplete.

Some retained studies (Ip [14] and Geleris [11]) with HR close to 1. did not address HCQ efficacy as primary or secondary endpoints and have overwhelming statistical weight due to their large number of included patients (> 1000), abrogating the potential benefit of HCQ and HCQ plus AZI. We find these studies should have been excluded from the meta analysis (Table 1).

Finally, studies with beneficial effects of HCQ and HCQ plus AZI [4,6,16,17,25] (Table 1) were eliminated although they are characterized by clearly defined treatment strategies, homogeneous patient selection and performed in single institutions or in a connected network of collaborative institutions (Arshad).

4.3 A meta-analysis disconnected from the patient

Covid-19 is a disease with two successive phases: a phase of viral multiplication followed by an inflammatory phase (cytokine storm) where the viral load decreases, while lungs are impaired. Antiviral treatments such as HCQ and/or AZI should be prescribed as early as possible during the first phase, whereas corticosteroids and oxygenation must take place at the very beginning of the second phase. Timing is crucial and may vary according to the groups of patients. Oxygenation may occur by non invasive means or

via tracheal intubation and therapeutic indications may vary according to the medical team. It has evolved according to experience and recommendations, as the clinical aspects of the disease became better known. The timing and dose of the anti-coagulants prescription is an increasing factor of heterogeneity of care, as well as nursing and medical support care.

4.4 Concerning the harmfulness of the association HCQ plus AZI

Any active medication necessarily conceals adverse effects. Physicians are always dealing with them to obtain a therapeutic effect beneficial to the patient. This implies weighting the associated risk. When patients are hospitalized, they easily benefit from clinical monitoring. For instance, routine electrocardiograms allow the early detection of possible cardiac rhythm disorder (e.g. prolongation of QTc interval resulting from a specific treatment toxicity or from unexpected drug interactions); hypokalemia may favor a possible torsade de pointe.

Fiolet et al. cite several studies that would have, according to them, demonstrated the cardiac harmfulness of HCQ plus AZI, but they did not analyze the clinical context and other medicament associations. They don't discuss the inconsistencies inside the studies they selected. For instance, in the study by Magagnoli [19], the cardiac toxicity is suggested, referring to the Rosenberg study, to explain an increased mortality in the HCQ plus AZI arm (HR = 1.31 (p=0.28). But for HCQ alone, mortality increase is even more pronounced (HR = 1.83 (p=0.009), which is inconsistent with the hypothesis of an exacerbated toxicity of the association HCQ plus AZI. The same problem is found in the study of Cavalcanti [7] where HR = 0.64 [0.18-2.21] for HCQ plus AZI whereas HR = 1.47 [0.48-4.53] for HCQ. There are probably some bias of patient selection in these studies: the median hospitalization times reported by Magagnoli indicates that possibly only more severely affected patients have received HCQ or HCQ plus AZI (Table 1).

As ultimate proof of their conclusion, they cite the World Health Organization statistics [42] on adverse reactions recorded in 167,000 patients with auto-immune chronic diseases (lupus, rheumatoid arthritis) receiving long-term HCQ and/or AZI. The measured risk ratio of QTc prolongation, torsade de pointe and ventricular tachycardia is 2.48 [95% CI, 1.28–4.79] for HCQ plus AZI, but event frequencies are very low with 0.3% for HCQ, 0.8% for AZI and 1.5% for their combination. In 263 adverse reactions recorded among 76,215 patients, only 7 patients died (less than 1/10 000) due to torsade de pointe and none following QTc prolongation. This data are for long-term treatments, whereas in the case of Covid-19, the treatment usually lasts 10 days for HCQ and 5 days for AZI, in monitored patients.

In the retrospective article by Harvey Risch [43], we learn that HCQ plus AZI was used in the USA as standard care on more than 300,000 aged patients with multiple comorbidities, 0.047% of whom have developed arrhythmia due to the treatment. Only 9 patients per 100,000 (0.009%) died, which has to be compared with the 10,000 Americans weekly dying of the disease. Lagier et al. [16] have observed QTc > 600 ms in 0.67 % of the patients, without torsade de pointe nor sudden death.

5. Conclusion

Generally speaking, meta-analyses cannot reliably be applied to non randomized heterogeneous studies with hidden multiple bias due to complex confounding factors difficult to identify. This is particularly the case for the studies on Covid-19. Regardless the statistical methodology used, meta-

analyses may unavoidably lead to results with poor or no scientific significance if not rigorously conducted. After thorough discussion of the bias, the results of the meta-analysis remains in favor of the efficacy of HCQ alone or combined with AZI for the treatment of Covid-19. These medications did not demonstrate any significant cardiac toxicity, and were overall well tolerated.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethical Approval

Approval was not required for this work.

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References

1. Fiolet T, Guihur A, Rebeaud ME, et al. Effect of hydroxychloroquine with or without azithromycin on the mortality of coronavirus disease 2019 (COVID-19) patients: a systematic review and meta-analysis. Clinical Microbiology and Infection 27 (2021): 19-27.
2. Alberici F, Delbarba E, Manenti C, et al. A report from the Brescia Renal COVID Task Force on the clinical characteristics and short-term outcome of hemodialysis patients with SARS-CoV-2 infection. Kidney International 98 (2020): 20-26.
3. Ayerbe L, Risco C, Ayis S. The association between treatment with heparin and survival in patients with Covid-19. Journal of Thrombosis and Thrombolysis (2020): 1-4.
4. Arshad S, Kilgore P, Chaudhry ZS, et al. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. International Journal of Infectious Diseases 97 (2020): 396-403.
5. Barbosa J, Kaitis D, Freedman R, et al. Clinical outcomes of hydroxychloroquine in hospitalized patients with COVID-19: a quasi-randomized comparative study. N Engl J Med 1 (2020): 8882.
6. Bousquet G, Falgarone G, Deutsch D, et al. ADL-dependency, D-Dimers, LDH and absence of anticoagulation are independently associated with one-month mortality in older inpatients with Covid-19. Aging (Albany NY) 12 (2020): 11306.
7. Cavalcanti AB, Zampieri FG, Rosa RG, et al. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. New England Journal of Medicine 383 (2020): 2041-2052.
8. Cravedi P, Mothi SS, Azzi Y, et al. COVID-19 and kidney transplantation: results from the TANGO International Transplant Consortium. American Journal of Transplantation 20 (2020): 3140-3148.

9. Fontana F, Alfano G, Mori G, et al. Covid-19 pneumonia in a kidney transplant recipient successfully treated with tocilizumab and hydroxychloroquine. American Journal of Transplantation 20 (2020): 1902-1906.
10. 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. International Journal of Antimicrobial Agents 56 (2020): 105949.
11. Geleris J, Sun Y, Platt J, et al. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. New England Journal of Medicine 382 (2020): 2411-2418.
12. Gupta S, Hayek SS, Wang W, et al. Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. JAMA Internal Medicine 180 (2020): 1436-1446.
13. Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, Wiselka M, Ustianowski A, Elmahi E, Prudon B, Whitehouse A. Effect of Hydroxychloroquine in Hospitalized Patients with COVID-19: Preliminary results from a multi-centre, randomized, controlled trial. MedRxiv (2020).
14. Ip A, Berry DA, Hansen E, et al. Hydroxychloroquine and tocilizumab therapy in COVID-19 patients—An observational study. PloS One 15 (2020): e0237693.
15. Kuderer NM, Choueiri TK, Shah DP, et al. Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. The Lancet 395 (2020): 1907-1918.
16. 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. Travel Medicine and Infectious Disease 36 (2020): 101791.
17. Lecronier M, Beurton A, Burrel S, et al. Comparison of hydroxychloroquine, lopinavir/ritonavir, and standard of care in critically ill patients with SARS-CoV-2 pneumonia: an opportunistic retrospective analysis. Critical Care (London, England) 24 (2020): 418.
18. Luo J, Rizvi H, Preeshagul IR, et al. COVID-19 in patients with lung cancer. Annals of Oncology 31 (2020): 1386-1396.
19. Magagnoli J, Narendran S, Pereira F, et al. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. Med 1 (2020): 114-127.
20. 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. BMJ 369 (2020).
21. Membrillo FJ, Ramírez-Olivencia G, Estébanez M, et al. Early Hydroxychloroquine Is Associated with an Increase of Survival in COVID-19 Patients: An Observational Study. Preprints (2020).
22. Mikami T, Miyashita H, Yamada T, et al. Risk factors for mortality in patients with COVID-19 in New York City. Journal of General Internal Medicine (2020): 1-10.
23. 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. Clinical Infectious Diseases (2020).

24. Rivera DR, Peters S, Panagiotou OA, et al. Utilization of COVID-19 treatments and clinical outcomes among patients with cancer: a COVID-19 and Cancer Consortium (CCC19) cohort study. Cancer Discovery 10 (2020): 1514-1527.
25. Rogado J, Obispo B, Pangua C, et al. Covid-19 transmission, outcome and associated risk factors in cancer patients at the first month of the pandemic in a Spanish hospital in Madrid. Clinical & Translational Oncology (2020): 1-5.
26. 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. JAMA 323 (2020): 2493-2502.
27. Sánchez-Álvarez JE, Fontán MP, Martín CJ, et al. Status of SARS-CoV-2 infection in patients on renal replacement therapy. Report of the COVID-19 Registry of the Spanish Society of Nephrology (SEN). Nefrología (English Edition) 40 (2020): 272-278.
28. 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).
29. Singh S, Khan A, Chowdhry M, et al. 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).
30. Skipper CP, Pastick KA, Engen NW, et al. Hydroxychloroquine in nonhospitalized adults with early COVID-19: a randomized trial. Annals of Internal Medicine 173 (2020): 623-631.
31. Wang AL, Zhong X, Hurd Y. Comorbidity and sociodemographic determinants in COVID-19 mortality in an US urban healthcare system. MedRxiv (2020).
32. Yu B, Li C, Chen P, et al. Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19. Science China Life Sciences 63 (2020): 1515-1521.
33. Borenstein M, Hedges L, Rothstein H. Meta-analysis: Fixed effect vs. random effects. Meta-Analysis. com (2007).
34. Docherty AB, Harrison EM, Green CA, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. BMJ 369 (2020).
35. Bessière F, Roccia H, Delinière A, et al. Assessment of QT intervals in a case series of patients with coronavirus disease 2019 (COVID-19) infection treated with hydroxychloroquine alone or in combination with azithromycin in an intensive care unit. JAMA Cardiology 5 (2020): 1067-1069.
36. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clinical Trials 7 (1986): 177-188.
37. Cochrane Training. Cochrane Handbook for Systematic Reviews of Interventions. Part 3: Special topics. 13.6.2.2 Combining studies (version 6) 2019. https://handbook-5-1.cochrane.org/chapter_13/13_6_2_2_combining_studies.htm (accessed July 28, 2020).
38. Borba MG, Val FF, Sampaio VS, et al. 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: a randomized clinical trial. JAMA Network Open 3 (2020): e208857.

39. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 355 (2016).
40. Sterne JA, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 366 (2019).
41. Cochrane Training. Cochrane Handbook for Systematic Reviews of Interventions. Part 3: Special topics. https://handbook-5-1.cochrane.org/chapter_13/13_5_1_1_sources_of_bias_in_non_randomized_studies.htm
42. Nguyen LS, Dolladille C, Drici MD, et al. Cardiovascular toxicities associated with hydroxychloroquine and azithromycin: an analysis of the World Health Organization Pharmacovigilance Database. Circulation 142 (2020): 303-305.
43. Risch HA. Early outpatient treatment of symptomatic, high-risk coronavirus disease 2019 patients that should be ramped up immediately as key to the pandemic crisis. American Journal of Epidemiology.