Exhibit 335

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https://www.researchgate.net/publication/369821037_Early_Treatment_with_Hydroxychloroquine_and_Azithromycin_A_Real-Life_Monocentric_Retrospective_Cohort_Study_of_30423_COVID-19_Patients

Early Treatment with Hydroxychloroquine and Azithromycin: A 'Real-Life' Monocentric Retrospective Cohort Study of 30,423 COVID-19 Patients

Matthieu MILLION[1,2,3*], Sébastien CORTAREDONA[2,3,4*], Léa DELORME[1,3], Philippe COLSON[1,2,3], Anthony LEVASSEUR[1,2,3], Hervé TISSOT-DUPONT[1,2,3], Karim BENDAMARDJI[1,3], Salima LAHOUEL[1,3], Bernard LA SCOLA[1,2,3], Laurence CAMOIN-JAU[1,2,5], Florence FENOLLAR[2,3,4], Philippe GAUTRET[2,3,4], Philippe PAROLA[2,3,4], Jean-Christophe LAGIER[1,2,3], Stéphanie GENTILE[6,7], Philippe BROUQUI[1,2,3], Didier RAOULT[1,2,3]

*Co-first authors

Corresponding author: Didier RAOULT, Didier.raoult@gmail.com

Alternate corresponding author: Matthieu MILLION, matthieumillion@gmail.com

IHU-Méditerranée Infection, MEPHI, Aix Marseille Université, 19-21 Boulevard Jean Moulin, 13005 Marseille; Phone: (33) 4 13 73 24 01; Fax: (33) 4 13 73 24 02

Affiliations

1. Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France.
2. Aix-Marseille Université, Institut de Recherche pour le Développement (IRD), UMR Microbes Evolution Phylogeny and Infections (MEPHI), Marseille, France.
3. Assistance Publique Hôpitaux de Marseille (APHM), Marseille, France.
4. Aix Marseille Université, Institut de Recherche pour le Développement (IRD), Service de Santé des Armées, AP-HM, UMR Vecteurs Infections Tropicales et Méditerranéennes (VITROME), Marseille, France.
5. Haematology Laboratory, Hôpital de la Timone, APHM, Marseille, France.
6. Equipe de Recherche EA 3279 "Santé Publique, Maladies Chroniques et Qualité de Vie", Faculté de Médecine, Aix Marseille University, 13005 Marseille, France.

7. Service d'Evaluation Médicale, Assistance Publique-Hôpitaux de Marseille, 13005 Marseille, France.

Keywords : SARS-CoV-2, COVID-19, Hydroxychloroquine, Azithromycin, Vaccination, Survival, Mortality, Real-world evidence.

Abstract

Objective To estimate the comparative effectiveness of combination therapy with hydroxychloroquine (HCQ) and azithromycin for coronavirus disease 2019 (COVID-19)-related death based on a large monocentric cohort independent of investigators' putative biases in a real-world setting.

Design Retrospective monocentric cohort study, with comprehensive data collection authenticated by an external bailiff and death reports from a national database (French National Death Registry).

Setting Institut Hospitalo-Universitaire Méditerranée Infection Center in Marseille, France.

Participants All adults older than 18 years with PCR-proven COVID-19 who were treated directly in our centre between 2 March 2020 and 31 December 2021 and did not refuse the use of their data.

Interventions HCQ and azithromycin (HCQ-AZ) as a reference treatment were compared to other regimens containing HCQ, ivermectin and azithromycin alone, combined, or none of these three drugs. The effect of vaccination was also evaluated.

Main outcome measures 6-week all-cause mortality. Multivariable logistic regression estimated treatment effectiveness with adjustments for age, sex, comorbidities, vaccination, period of infection or virus variant, and outpatient or inpatient care.

Results Total 30,423 COVID-19 patients were analysed (86 refused the analysis of their data) including 30,202 with available treatment data, and 535 died (1.77%). All-cause mortality was very low among patients < 50 years (8/15,925 (0.05%)) and among outpatients treated with HCQ-AZ (21 deaths out of 21,135 (0.1%), never exceeding 0.2% regardless of epidemic period). HCQ-AZ treatment was associated with a significantly lower mortality rate than no HCQ-AZ after adjustment for sex, age, period and patient care setting (adjusted OR (aOR) 95% confidence interval (CI) 0.55, 0.45-0.68). The effect was greater among outpatients (71%

death protection rate) than among inpatients (45%). In a subset of 16,063 patients with available comorbidities and vaccinations status, obesity (2.01, 1.23-3.29), chronic respiratory disease (2.93, 1.29-6.64), and immunodeficiency (4.01, 1.69-9.50), on the one hand, and vaccination (0.29, 0.12-0.67) and HCQ-AZ treatment (0.47, 0.29-0.76), on the other hand, were independent factors associated with mortality. HCQ, alone or in any association, was associated with significant protection from death among outpatients (0.41, 0.21-0.79) and inpatients (0.59, 0.47-0.73).

Conclusions HCQ prescribed early or late protects in part from COVID-19-related death. During pandemic health crises, financial stakes are enormous. Authentication of the data by an independent external judicial officer should be required. Public sharing of anonymized databases, ensuring their verifiability, should be mandatory in this context to avoid fake publications.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic was an unprecedented health challenge that led to 677 million cases, 6.9 million deaths and 13 billion vaccine doses administered as of March 2023 (1). The lethality of infection was highly variable according to age, sex, comorbidities, geography, epidemic periods and variants (2). A recent multinational study including 689,572 inpatients found an average case fatality rate of 21% (3). Apart from specific prevention or antiviral treatment, early prehospital management with oxygen saturation monitoring and early oxygen therapy have been shown to reduce mortality (4, 5). As of 2021, vaccination was associated with a decrease in mortality risk, replicated in our centre with a 3-fold decrease in mortality among those aged ≥ 55 years (6). COVID-19 has changed with limited cytokine storm and lung involvement, and mortality has fallen notably since the emergence of the B.1.1.529 Omicron variant (7). From a therapeutic perspective, the newly developed direct antiviral nirmatrelvir has been recommended for early COVID-19 treatment for at-risk patients (8). Repurposed hydroxychloroquine (HCQ) was the most frequently prescribed treatment worldwide during the first months of the pandemic (9) but is not recommended in Europe or the USA (10). However, assessing its efficacy against COVID-19 mortality is critical to clarifying whether drug repurposing is clinically relevant for early treatment in future lethal pandemics.

The story of HCQ for the treatment of COVID-19 began in February 2020 in Wuhan, China, with the testing of seven FDA-approved molecules by Wang *et al*. (11). Chloroquine was included in the panel on careful and unbiased analysis of the literature on severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) (12) and on the accurate understanding of the mechanism of infection (endosomal pathway and glycosylation of the membrane-bound SARS-CoV-2 receptor, angiotensin-converting enzyme 2 (ACE-2) (11). Thus, the same researchers reported that HCQ, a less toxic derivative of chloroquine, was even more effective

(13). Thus, chloroquine and HCQ have been repeatedly found to be some of the most effective potential repurposed drugs by different approaches, such as large-scale SARS-CoV-2 protein interaction map analysis (14, 15) and network medicine frameworks (16), by several teams in the USA and other countries (9). *In vitro* antiviral efficacy at the micromolar range has been confirmed by multiple teams outside (9, 14, 15) and in our centre (17, 18, 19, 20, 21) for HCQ and compounds of the same 4-aminoquinoline family, notably amodiaquine (15, 18). Thus, we previously reported in the clinical setting that off-label HCQ, particularly when associated with azithromycin (AZ), was associated with improved viral clearance (22).

In this context, we therefore decided on a standard of care including HCQ and AZ treatment for COVID-19 patients in our centre starting in March 2020 based on article 37 of the Helsinki Declaration for unproven interventions (23). In the absence of reference treatment, we prescribed off-label, as allowed by the French Public Health Code, this combination of drugs to improve patient outcomes. This decision was based on the *in vitro* antiviral effect already demonstrated by Chinese studies, the binding to the critical sigma receptor target in SARS-CoV-2 infection, the specific immunomodulatory effects of HCQ and AZ, which may prevent the “cytokine storm”, the antithrombotic effects of HCQ useful in the context of COVID-19-associated coagulopathy and pulmonary embolism, the antibiotic effect of AZ against bacterial superinfections, and the reduction in viral shedding, with potential public health effects by reducing the duration of infectiousness (9, 14).

This led us to show that this treatment, when given early, was associated with extremely low mortality (24) and improved survival compared to other regimens (25). We confirmed this in both 10,429 outpatients (26) and 2,111 inpatients (27) treated in our centre in 2020. However, our impartiality, transparency and methodology were questioned. This challenged us to obtain and make public unbiased raw data to provide our impartial methodological criteria (28), and report results in the most transparent way possible. Indeed,

transparency and verifiability of the raw data and their analysis were identified as important issues during the pandemic (29, 30, 31). To this end, we used comprehensive data from administrative sources such as hospital admission files, computerized pharmacy prescription files, and the French National Death Registry of the "Institut National des Statistiques et des Etudes Economiques" (INSEE) (32). The quality control process and sources of data were verified by an independent bailiff.

In the context of optimized data verifiability, the aim of this work was to test whether the combination therapy HCQ-AZ, as a part of our standard of care, was associated with a different mortality compared to other treatments prescribed to all adult COVID-19 patients treated at our centre in 2020-2021. Secondary objectives were to identify whether the effect was different according to age, sex, period, major variants, vaccination status, comorbidities and severity/earliness of treatment (outpatients vs. inpatients).

Methods

Design and methodological criteria

We report a 'real-world' (33) retrospective observational study of a monocentric cohort comparing patients who were exposed or not exposed to antiviral treatment used as a standard of care in our centre (HCQ-AZ). Data from patients cared for in our institute from March 2, 2020, to December 31, 2021, were recorded in the hospital information system. This retrospective study was conducted following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (34) and new criteria identified through a critical review of the literature assessing HCQ for COVID-19 mortality (28). Accordingly, we particularly explicated impartiality (conflicts of interest), transparency (recruiting centre and doctors), and medical expertise (the authors are experts in the field who

directly care for patients, and standard of care and treatment protocols are clearly detailed) in the supplementary data of the present work (28).

Inclusion and exclusion criteria

The data included were those of patients ≥ 18 years of age with PCR-proven COVID-19 regardless of symptoms (asymptomatic or symptomatic) who were treated in our centre, i.e., had a medical examination by one of the doctors in our centre (Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, Marseille, France) either as outpatients or inpatients, i.e., hospitalized on the day of the visit in our outpatient unit following evaluation or directly transferred from another medical ward except the intensive care unit. The reasons for exclusion were erroneous patient identification (identity surveillance and duplicates), lack of available medical data, lack of COVID-19 after checking the medical record (including patients without COVID-19 consulting for a post-COVID-19 syndrome), expression of opposition to the use of their medical data for research purposes (in accordance with the European General Data Protection Regulation), and data from patients hospitalized in our centre after intensive care. Data from COVID-19 outpatients who left against medical advice were excluded. The inclusion period was from 2 March 2020 to 31 December 2021, with a follow-up period of 6 weeks. Consequently, the data extracted from the database were those recorded from 2 March 2020 to 13 February 2022.

Outcomes and exposures

The primary outcome was 6-week all-cause mortality. The objective did not change during this study. The covariates considered were age, sex, epidemic period, virus variants, patient care setting (outpatient/inpatient) and treatment. The epidemic periods were defined and separated by the week with the fewest cases between two epidemic peaks. Information on

vaccination status and comorbidities was available for a subset of patients first entered in our care pathway by our outpatient unit and treated in 2021. Virus variants were characterized and named according to the Pangolin classification as previously reported (35) with the exception of the first epidemic period: The 'W' letter was used here to designate all SARS-CoV-2, Wuhan-derived, that circulated during the first epidemic period in our geographical area (from February to May 2020).

Diagnostic criteria

The diagnostic criteria were PCR-confirmed infection with a cycle threshold (Ct) value < 35 as previously reported (36). Clinical or computed tomography (CT) scan definitions were not sufficient (28).

Treatment groups

The standard of care and full protocol for specific treatment (HCQ, AZ, ivermectin (IVM)) are detailed in the supplementary data and in our previous studies (24, 25, 26, 27). Accordingly, all the treatment protocols included at least one of the 3 (HCQ, AZ and/or IVM) molecules with proven *in vitro* efficacy against SARS-CoV-2 (20). HCQ alone (HCQ only group) was used at the very beginning of the epidemic (March 2020) for the very first patients and then for patients with a contraindication to AZ (mainly allergy and comedication with colchicine). Accordingly, the HCQ-AZ combination was chosen as the standard of care in our centre as soon as the end of March 2020 based on our seminal trial (22). AZ was used alone (AZ only group) for patients for whom HCQ could not be prescribed because of non-reversible contraindications, at the discretion of the doctor or refusal of the patient. From autumn 2020, a combination of IVM and AZ (IVM-AZ) was proposed after the first report of efficacy in the literature (37, 38). In some cases, HCQ was not prescribed at the outset and

was started only after correction of a transient contraindication, such as hypokalaemia, resulting in delayed HCQ (IVM-AZ-delayed HCQ group). Accordingly, we primarily assessed our reference treatment (HCQ-AZ) against other combinations that included HCQ or not (regimens without HCQ: AZ only, IVM-AZ, IVM only, other treatment (no HCQ, no AZ, no IVM); regimens with HCQ: HCQ only, IVM-AZ-delayed HCQ, HCQ-IVM). Some combinations were not in the proposed reference protocols (HCQ-IVM, other treatment), illustrating the freedom for each medical doctor for off-label prescriptions. Measurements, sources of data, and identification of potential sources of bias or confounding factors are extensively detailed in the supplementary data.

Database authentication by a certified bailiff

The database was created by merging several databases from medical records (computer and paper) and professional medical software such as the prescription software or the biological results software, as well as the admission software, which tracks the movements of services within a hospital stay and for patients who died within 6 weeks in the French National Death Registry (32) (see Measurements and Sources of Data in the Supplementary Methods). For inpatients, treatment data came from the database of medicines delivered during hospitalization. For outpatients, these were prescription data (no information on the actual use of the drug, on the dose, compliance, or duration). Once the database was built, an expert data manager carried out and traced a thorough quality control. This quality control lasted one year and allowed us to reanalyse more than 4,500 patient files by doctors in medicine (JCL, PP, HTD, MM). The construction of the database and quality control of the data were recorded by a mandated bailiff who verified and attested to the presence of all the traceability elements guaranteeing the quality of the data in the database. The anonymized database is available online in public open access (see Data Sharing information).

Statistical analysis

As the aim of this work was to test whether HCQ-AZ, as a part of our standard of care, was associated with a different mortality compared to other treatments, we first compared patients treated with or without the reference HCQ-AZ combination. In a secondary analysis, we compared the reference treatment HCQ-AZ at the outset to every other regimen, differentiating regimens without HCQ (AZ only, IVM-AZ, IVM only, other treatment) and regimens with HCQ (HCQ only, IVM-AZ-delayed HCQ, HCQ-IVM). Finally, the role of each antiviral drug (HCQ, AZ or IVM) was analysed regardless of the prescription of any of the two other antiviral drugs. In this last approach, each drug was included as a binary covariate (yes/no) in the models.

We performed stratified univariate and multivariable analyses according to sex, age classes (<50, 50-39, 70-89 and >89 years), periods (or variants) and patient management. Considering that the French National Death Registry (32) is completely exhaustive, we considered that there were no missing data for the outcome. There were no missing data for age, sex or period of admission. A total of 221 patients had missing treatment data. Since the proportion of patients with missing treatment data was very low (0.7%), they were excluded from the univariate and multivariable analyses of associations between treatment and death. A total of 14,360 (47.2%) patients had missing information on vaccination status and comorbidities, and 8,759 (28.8%) patients had a missing or unknown SARS-CoV-2 variant. Comorbidities, vaccinations and variants were used as covariates in different subgroup analyses. A two-sided p value of less than 0.05 was considered statistically significant. For the secondary analysis, differences between the 8 treatment groups were corrected following the Tukey method for multiple testing. Statistical analyses were carried out using SAS 9.4 statistical software (SAS Institute, Cary, NC).

Ethics

The management of the patients and this retrospective study were performed in accordance with the revised Helsinki Declaration in 2013 (23), the international ethical guidelines for health-related research involving humans (39). This study received the approval of the independent ethical committee (Méditerranée Infection N°: 2021-007 for outpatients and 2021-015 for inpatients). The data presented were collected retrospectively from the hospital's information system (patient files, prescription software, biology software, software tracing departmental movements during a stay as well as the mode of discharge). In accordance with European Regulation n° 2016/679 General Data Protection Regulation (GDPR), the protocols were registered in the hospital's GDPR registry n° 2020-151 and 2020-152, and all patients were informed of the potential reuse of their data via the institution's information procedure, which indicated their right to object via the MyAPHM online portal and/or by post or email addressed to the establishment's Data Protection Officer. Patients who objected to the use of their data were excluded before data collection and extraction from the information system.

Results

Participants

Between March 2, 2020, and December 31, 2021, 31,971 patients were potentially eligible, but 1,175 did not meet the inclusion criteria (Figure 1). In total, 30,796 patients aged ≥ 18 years with PCR-positive COVID-19 treated at our centre were eligible. Among those eligible, 86 (0.3%) patients expressed opposition to the use of their data for research purposes. Finally, 30,423 patients were included and analysed. No patient was considered lost to follow-up because the French National Death Registry was used to assess the death outcome. The

demographic characteristics of the 30,423 included patients are detailed in Table 1 and Supplementary Table 1. The number of cases per week was highly variable and allowed the identification of 7 periods (Figure 2, Supplementary Figure 1). Variants were determined for 21,664 (71.2%) patients, with 4 major variants representing 18,874 (87.1%) patients with available variant information (W, n = 4,079 (18.8%) ; B.1.160, n = 4,445 (20.5%) ; B.1.7.7, n = 5,035 (23.2%); B.1.617.2, 5,315 (24.5%)). The mean age of the patients was 48.8 years, 47.7% of whom were men. All patients were followed for 6 weeks after treatment initiation in our centre. Of the 30,423 patients, 30,202 (99.3%) had treatment information available, of which 26,417 (87.5%) were outpatients (mean age 46.4 years) and 4,538 (15.0%) were inpatients (mean age 64.6 years, see Figure 3). A total of 753 (2.5%) patients were common to these two groups since these patients were initially managed on an outpatient basis before being secondarily hospitalized (Figure 3). The characteristics of the 16,063 (53%) patients with available information on vaccination status and comorbidities are detailed in Supplementary Tables 2 and 3.

Covariables associated with HCQ-AZ treatment

Compared to patients without HCQ-AZ treatment, HCQ-AZ treatment was associated with younger patients (mean, 47.0 vs. 54.6 years), higher frequency of patients included during period 1 (15.7% versus 6.5%), patients with the W variant (21.1% versus 9.9%) and outpatients (91.2% versus 75.1%) (Table 1). Accordingly, age, period, variant and outpatient/inpatient setting were potential confounding factors considered in multivariable models and stratification.

All-cause mortality within 6 weeks

There were 535 all-cause deaths, including 52 with initial outpatient management and 483 with conventional hospitalization (CH) without initial outpatient management. Among these 52 deceased outpatients, 24 (46.2%) were admitted to our centre after initial outpatient care. The peak mortality was observed during the winter of 2020/2021 (Period 4, 165/960 (17.2%) for inpatients). The mean age of the deceased patients was 80.1 ± 10.8 years. Among the included variables, age was the strongest risk factor for death with a nonlinear relationship (Supplementary Figures 2, 3 and 4). Indeed, mortality was very low among those aged < 50 years (18-49 years, 8/15,925 patients, 0.05%), increased between ages 50 and 69 (82/10,786 0.76%) and 70 and 89 years (347/3,413 10.17%) and was the greatest among those aged > 89 years (98/299, 32.78%). Male sex was a risk factor for death (men, 2.2% and women, 1.3%, chi-square test $p < 10^{-4}$). A peak of mortality was observed during period 4 (winter 2020/2021) at 3.0%, and a minimum was observed in period 6 (July to September 2021) at 0.93% (Figure 4 and Supplementary Figure 5). Among the 4 major variants, the B.1.160 (Marseille 4) variant was associated with the highest mortality (3.9% vs. 1.3%, chi-square test p<0.0001).

Association between treatment regimen and mortality

Patients with or without HCQ-AZ treatment

Among the 30,202 patients with treatment information, 191/23,172 (0.82%) patients treated with HCQ-AZ died compared to 344/7,030 (4.89%) among those without HCQ-AZ (Figure 3). Overall, HCQ-AZ therapy was associated with a lower mortality than treatment without HCQ-AZ (odds ratio (OR) 95% confidence interval (CI) 0.16, 0.14-0.19). After adjustment for sex, age, period and patient management (out/inpatient), HCQ-AZ remained associated with a significantly lower mortality rate (adjusted OR (aOR) 0.55, 95% CI 0.45-0.68, Table 2). Overall mortality among outpatients treated with HCQ-AZ was extremely low (21/21,135

(0.1%), without substantial variations across periods, and never exceeded 0.2% per month (Supplementary Figure 5).

Information on vaccination status and comorbidities was available for a subset of 16,063 patients who first entered our care pathway by our outpatient unit and were treated in 2021. A total of 1195 (7.4%) patients were hospitalized, including 728 on the day of the first evaluation, and were considered inpatients (see Methods) and 467 outpatients (Supplementary Tables 2 and 3). Among these 16,063 patients, the association between HCQ-AZ and mortality remained unchanged regardless of whether vaccination and comorbidities were considered (aOR 0.47, 95%CI 0.29-0.75) or not (0.47, 0.29-0.76 , Supplementary Table 4). When the model included comorbidities and vaccination, obesity (2.01, 1.23-3.29), chronic respiratory disease (2.93, 1.29-6.64), and immunodeficiency (4.01, 1.69-9.50), on the one hand, and vaccination (0.29, 0.12-0.67) and HCQ-AZ treatment (0.47, 0.29-0.76), on the other hand, were the only independent factors associated with mortality (Supplementary Table 5). Stratification by care setting showed a similar effect of vaccination among outpatients (aOR = 0.32, p = 0.04) and inpatients (0.40, p = 0.07). Among the 21,550 patients with available variant information (Supplementary Figure 1), the lower mortality associated with HCQ-AZ was confirmed after adjustment for age, sex, patient management (out/inpatient) and variant (aOR 0.55; 95% CI 0.44-0.69).

Among outpatients and inpatients, the association between the treatment variable (HCQ-AZ) and the outcome was not significantly different according to sex, period or variant (two-way interaction terms were not statistically significant). However, the association was significantly different according to patient care setting and age, with a maximal effect size among outpatients aged between 50 and 89 years (Figure 4).

Patients treated with HCQ-AZ compared to every other regimen

In our secondary analyses, comparing unadjusted mortality rates between all 8 treatment groups according to age classes (<50, 50-69, 70-89, >89 years), no significant differences were found among those aged < 50 years and among those aged > 89 years (Supplementary Table 6). Between 50-89 years, HCQ-AZ (1.47%) was always better than every other treatment, and the difference was significant compared to AZ only (8.71%), IVM-AZ (5.52%), IVM-AZ-delayed HCQ (7.22%), and other treatments (3.74%). The difference was not significant compared to HCQ alone (2.09%, p=0.962). In the multivariable model (Table 2, Model B), HCQ-AZ was associated with a significantly lower probability of death than AZ only (aOR 0.51, 95% CI 0.35-0.72), IVM-AZ (0.54 0.31-0.97) and other treatments (0.49 0.26-0.93). The difference was not significant compared to HCQ only (aOR 0.85, 95% CI 0.22-3.25). The 3 groups with HCQ at the outset (HCQ-AZ, HCQ only, HCQ-IVM) were indistinguishable in terms of mortality risk (Figure 5), whereas mortality was consistently halved when the reference treatment HCQ-AZ was compared with groups without HCQ at the outset (aOR 0.51 vs. AZ only, 0.54 vs. IVM-AZ, 0.5 vs. IVM only, 0.49 vs. other treatment and 0.44 vs. IVM-AZ-delayed HCQ, Figure 5). This prompted us to clarify the role of HCQ itself.

Regimens with and without HCQ

Therefore, as we observed that the prognosis of the reference group (HCQ-AZ) was not different from each of the groups with HCQ at the outset (HCQ only, HCQ-IVM) but different from all the groups without HCQ with a similar outcome difference (odds ratio reduced 2-fold in the reference group HCQ-AZ compared to AZ only, IVM-AZ, IVM only, and other treatment), we decided to look at the role of HCQ itself, irrespective of the associated treatment. Thus, we performed a multivariable logistic regression including HCQ, AZ and IVM as 3 binary variables. A total of 23,755 (78.7%) patients had a regimen with

HCQ compared to 6,447 (21.3%) without this drug. A total of 27,750 (91.9%) patients had a regimen with AZ compared to 2452 (8.1%) without this drug. A total of 1878 (6.2%) had a regimen with IVM compared to 28,545 (93.8%) patients without this drug. No difference in survival was found for AZ (aOR 0.97, p = 0.861) or IVM (1.08, p = 0.633). Only HCQ was associated with a lower mortality (0.55, 0.44-0.68, p <.0001, Figure 5, Supplementary Table 7), and this was confirmed both for outpatients (aOR 0.31, 95% CI 0.16-0.59, p = 0.0004, Supplementary Table 8) and inpatients (0.52, 0.42-0.65, p <.001, Supplementary Table 9).

Discussion

The essence of this work was to transparently report on two years of activity at the IHU Méditerranée Infection Centre on the management of COVID-19 patients. We were keen to avoid any scientific or malicious criticism of data entry. The entire data collection process was explained and originated from the hospital information system, and this system is independent from our institute (IHU Méditerranée Infection). All PCR-proven COVID-19 patients ≥ 18 years of age treated at the IHU were analysed and, apart from the few who opted out of the use of their data (0.3%), were fully included. This 'whole real-world population' (33) approach prevents selection bias and guarantees research equitability (39). The only outcome analysed was all-cause mortality, which was recorded in the French National Death Registry (32) and chosen because it is the most severe and clinically relevant outcome, irreversible and is not subject to human subjectivity (28). In this context, no excess mortality was found with HCQ treatment, consistent with cardiovascular safety found in our centre (40). In contrast, we found a threefold lower risk of death when HCQ-AZ was prescribed early. Overall, the reference treatment (HCQ-AZ) proposed in our centre was associated with improved survival independent of age, sex, epidemic period, major variants, vaccination status, comorbidities and severity.

The 'real-world' source (33), the comprehensiveness, the verifiability and the transparency (30, 31) of the raw data are the main strengths of this work. Indeed, the database is in public open-access and available to any investigator who wishes to use it. A totally independent bailiff, a sworn officer at the national level, verified the absence of manipulation of the raw data at the medical and computer levels, including the submission of the anonymised database to 2 international open access research data repositories (DRYAD related to the US National Science foundation, and ScienceDB related to the Chinese Academy of Sciences – See Data Availability statement). These elements should avoid any dispute about the reality of the data and/or their potential bias. Impartiality was optimized as none of the investigators had any conflict of interest in this area, which would be highly unlikely given that the drug is generic and not of interest to any pharmaceutical industry.

Our first hypothesis was that the best treatment was the HCQ-AZ combination because of early results obtained in smaller populations and other criteria, such as the anti-inflammatory, antiviral and antibiotic activity of AZ (20, 22, 24, 25, 26, 27). However, we were surprised to see that in fact, the key point of the therapy was the use of HCQ in the therapeutic regimen, regardless of the association with AZ or IVM or used alone. The results are consistent with the exhaustive analysis carried out on the C19early.org website (10) (Supplementary Figures 6 and 7). In this online meta-analysis without any selection bias (all available studies were included), early HCQ treatment (15 studies) showed a 72% mortality protection rate among 52,740 (19,762 treated with HCQ) COVID-19 patients (10) compared to 71% in the present study (Figure 4). In contrast, late treatment for patients with severe forms who were hospitalized showed a lower but significant 19% mortality protection rate among 252,506 (125,494 treated with HCQ) patients (10) that was lower than the 45% found in the present study (Figure 4).

Our study was based on a reasonable HCQ dosage (200 mg *tid*) that after three days achieves a blood concentration of 1 mg/mL of HCQ, which is the effective dose for preventing intracellular multiplication of the virus (11, 41). The earlier the treatment is prescribed, the greater the duration with an efficient blood concentration (> 1 mg/mL) before complications arise. The importance of early treatment could at least in part explain the discrepant results shown in other studies, in which HCQ was prescribed after complications occurred (42).

Considering the Bradford Hill criteria (43) for a link between early HCQ treatment and improved COVID-19 survival, the critical role of earliness, which fulfils the *temporality* and *biological gradient* criteria, is the most convincing evidence. Indeed, antiviral efficacy is expected before the onset of complications (9), as is the case for nirmatrelvir recommended only before the need for oxygen (8). Other criteria include the *strength of association* (3-fold decrease in the risk of death), *consistency* with studies reported by other teams (Supplementary Figures 6 and 7), *plausibility* (shorter viral clearance (25, 44, 45, 46, 47, 48, 49), endosomal pathway and sigma receptor ligand (14)), *coherence* with the natural history of the disease (9), *in vitro experimental* evidence (9, 14, 15, 20), and *analogy* with recognized efficacy of HCQ to treat intracellular infections involving the endosomal pathway, such as Q fever (50).

The natural experimental design (treatment was determined by variation not under the control of the researcher (51, 52)) of the present study, inherent to its 'real-world' setting (33), presents some limitations. Randomization was not used for ethical reasons (53). Indeed, as clinicians and infectious disease specialists, we considered that equipoise was not achieved (53) because HCQ was expected to improve survival based on early Chinese *in vitro* studies (11, 13), our long experience with HCQ and its safety in infectious diseases (54), our seminal trial on SARS-CoV-2 viral clearance (22), and knowledge of COVID-19 (9, 14, 15) (see the

Introduction section). These ethical issues have been discussed as the 'parachute paradigm' (55, 56). The open-label design may have introduced an indication bias because information on some comorbidities, such as dementia, the inability to take oral medication or bedridden condition and severity among inpatients, was not collected. Indeed, multivariate models used in 'real-world' studies cannot control for unobserved or unmeasured confounding factors. However, a Cochrane meta-analysis reported that there is no evidence for significant effect estimate differences between observational studies and randomized controlled trials (RCTs) (57).

Natural experimental studies are used when randomization is unfeasible, impractical or unethical and to avoid the artificiality bias of randomized studies (52). Advantages over planned experiments include the possibility of studying effects in 'real-world' whole populations (33) and studying rare outcomes with greater reach, impact and equity (52). Indeed, our 'real-world' study (33) included the whole population of adult PCR-proven COVID-19 patients initially treated in our centre who gave permission for the use of their data. Outpatient care, associated with very rare outcomes (low case fatality rate (CFR)), is critical for early treatment before complications occur. Lim *et al*. (4) reported that early outpatient care based only on supportive care decreased the case fatality rate from 2.5% to 0.5% (4). With this low 0.5% CFR, identifying a statistically significant 50% death protection rate in an RCT for an experimental drug would have necessitated > 18,000 patients, which is practically unfeasible. Overall, real-world studies are better than RCT for rare events, and evaluate treatment effect in a broader and more representative patient population, improving generalisability (58).

This study was not multicentric, which may limit its generalizability. In fact, our centre carried out a proactive strategy of massive screening before the arrival of the virus in our centre (59), a laboratory-based quarantine for repatriated individuals from China (60), and

an early care and treatment strategy (61) already associated with a dramatic improvement in prognosis in other centres independent of the use of HCQ (4). The prescription of HCQ was well-informed and careful with respect to contraindications and caution with electrocardiography (ECG) and hypokalaemia, sometimes neglected in other centres (28). It is possible that careless use of HCQ in a nonexpert setting for patients with contraindications and those treated late may produce very different results. This centre effect could be responsible for Simpson's paradox in multicentric studies (28).

Some multicentric studies known as megatrials, some of which are well known, theoretically included a large number of hospitals, but the data were inaccessible (30, 31), and the results were improbable, such as the Mehra *et al*. study, which had to be rapidly retracted (29). It should be noted that among the various studies, the heterogeneity of the centres led, in the large studies and RCTs analysed, to the inclusion of patients whose diagnosis had not been made but whose practitioner presumed, without PCR, that they were patients with COVID-19 (42, 62, 63, 64). However, these studies and the particularly the retracted Mehra study (29) immediately led to changes in strategies at the level of the French Ministry of Health (65, 66), and ultimately declarations at the level of the WHO (67). Some RCTs testing early treatment with HCQ before complications arose were stopped before sufficient statistical power could be achieved (68). We were also able to show that conflicts of interest in this situation played a very important role. Most of the authors who had conflicts of interest with the pharmaceutical industry (28, 69, 70) had a negative evaluation of the effect of HCQ.

Among the limitations of our study, vaccination and comorbidity data were not available for all patients. However, the robustness and stability of the treatment effect was verified regardless of the inclusion of these covariables (Supplementary Table 4). In addition, these data, collected systematically mainly from outpatients in 2021, were available for more than half of the whole cohort with a very large sample size (> 15,000 patients). We already

showed that comorbidities did not explain the observed effect in our previous study among 2111 inpatients using a different data entry methodology (27). Overall, this work did not call into question vaccine protection in subjects over 55 years of age, which we have also reported (6). Overall, the significant role of comorbidities and vaccination confirmed here is another argument for the impartiality and external validity of the present data and findings.

Overall, early outpatient and inpatient management using a therapy including HCQ in standardized doses provides a partial solution to the management of patients infected by SARS-CoV-2, essentially among people over 50 years of age. Indeed, as previously reported (6, 71), COVID-19-associated mortality was very low among patients < 50 years of age. Accordingly, any intervention in this population in addition to standard care is likely to have an unfavourable benefit risk ratio (6). Overall, patient management, from screening to diagnosis, including biological assessment and clinical examination, likely explains the low mortality associated with COVID-19 in our centre. Indeed, mortality rate was 0.59% in outpatients without HCQ-AZ similar to 0.65% in an early care German study (4). Among inpatients not treated with HCQ-AZ, mortality was 16.3%, thus lower than the 21.5% mortality rate recently reported in 600 000 inpatients of a multinational study (3).

Another limitation was that treatment data came from the database of medicines delivered during hospitalization (for inpatients) and prescription data (for outpatients). Prescription data do not provide information about the delivery of drugs to patients, and delivery data do not necessarily mean use of the drug. No information on the actual use of the drug, the dose received, compliance or duration of the treatment were available in the database. This potential bias might have resulted in some overestimation of the number of treated patients, especially outpatients.

When a therapeutic trial may lead to a change in prescribing strategies and guidelines, high financial stakes may profoundly bias the analysis of the data. In this context, total

transparency and open accessibility of the data with verification outside the study sponsor should be required (30, 31). Indeed, the largest scientific and medical journals also have conflicts of interest, and their credibility in the future must be guaranteed by the rigor of the methodology to avoid abuses, as seen in trials for rofecoxib (72), oseltamivir (73) and in Lancetgate (29). The main strength of the present work is the certification by external authorities (bailiff) of the outcomes analysed and the total transparency of the data made publicly available for reanalysis.

Acknowledgements

We thank Louisa OUSSENI, Hervé SUBRERO, and Mathilde VERGARA for their technical help. We thank Jean-Marc ROLAIN for a useful discussion on drug repurposing.

Funding

This work was performed by academic doctors working in the IHU Méditerranée Infection. IHU Méditerranée Infection was funded by the French government and benefited from a grant from Agence Nationale de la Recherche: ANR-15-CE36-0004-01 and by ANR "Investissements d'avenir", Méditerranée infection 10-IAHU-03, and was also supported by Région Provence-Alpes-Côte d'Azur.

Conflicts of interest

All authors have completed the Unified Competing Interest form (available on request from the corresponding author). DR declare grants or contracts and royalties or licenses from Hitachi High-Technologies Corporation, Tokyo, Japan. DR is scientific board member of Eurofins company. DR is founder and shareholder of a microbial culture company (Culture Top), two biotechnology companies (Techno-jouvence, and Gene and Green TK), and a rapid diagnosis of infectious diseases company (Pocramé). All authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work. Our group used widely available generic drugs distributed by many pharmaceutical companies.

Details of contributors

Conceptualization : MM, JCL, PB, DR. Methodology: MM, SC, SG, DR. Validation: MM, SC, LD, JCL, PB. Formal analysis: MM, SC, LD, PC, AL. Investigation: MM, PC, HTD, KB, SL, BLS, FF, JCL, PB, PP. Resources : MM, JCL, PB, PP. Data curation: MM, SC, LD, HTD, SG. Writing – original draft: MM. Writing – review & editing: SC, PC, LC-J, PG, JCL, PP, SG, PB, DR. Visualization: MM, SC, LD. Supervision: MM, JCL, SG, PB, DR. Project administration: MM, JCL, SG, PB, DR. Funding acquisition: DR. The guarantors are Matthieu Million (MM) and Didier Raoult (DR).

Transparency declaration

MM and DR (the guarantors) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data and Script Availability Statement

Raw data are publicly available online in two public open access repositories (Science Data Bank, https://doi.org/10.57760/sciencedb.07803 and DRYAD, https://doi.org/10.5061/dryad.ksn02v78v). Conditions of reuse are license Creative Commons Zero (CC0) for both deposits. The SAS code is available upon request from the authors.

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780 **Table 1. Baseline characteristics (n = 30,423)**

	All		HCQ-AZ†			No HCQ-AZ†			Missing data	
	n	%col	n	%col	%row	n	%col	p‡	n	%col
N	30423		23172			7030			221	
Men	14505	47.7	11077	47.8	76.4	3312	47.1	0.310	116	52.5
Age-Mean (std) Q1-Median-Q3	48.8 (17.1)	35-48-60	47.0 (16.1)	34-47-58		54.6 (19.0)	40-55-69		43.9 (15.2)	31-45-54
<50	15925	52.3	12981	56.0	81.5	2805	39.9	<.001	139	62.9
50-69	10786	35.5	8154	35.2	75.6	2560	36.4	0.060	72	32.6
70-89	3413	11.2	1934	8.3	56.7	1470	20.9	<.001	9	4.1
>89	299	1.0	103	0.4	34.4	195	2.8	<.001	1	0.5
Period										
2020/03/03-2020/06/15	4132	13.6	3637	15.7	88.0	459	6.5	<.001	36	16.3
2020/06/16-2020/09/20	3269	10.7	2292	9.9	70.1	880	12.5	<.001	97	43.9
2020/09/21-2020/11/22	4322	14.2	2788	12.0	64.5	1458	20.7	<.001	76	34.4
2020/11/23-2021/03/21	5906	19.4	4536	19.6	76.8	1362	19.4	0.709	8	3.6
2021/03/22-2021/06/27	5621	18.5	4393	19.0	78.2	1225	17.4	0.004	3	1.4
2021/06/28-2021/09/21	4624	15.2	3752	16.2	81.1	871	12.4	<.001	1	0.5
2021/09/22-2021/12/31	2549	8.4	1774	7.7	69.6	775	11.0	<.001	0	0.0
SARS-CoV-2 variants (nmiss=8 759)††	18874		15035			3767			72	
A (Wuhan)	4079	18.8	3598	21.1	88.2	449	9.9	<.001	32	28.1
B.1.160 (Marseille 4)	4445	20.5	3176	18.6	71.5	1231	27.3	<.001	38	33.3
B.1.7.7 (UK)	5035	23.2	3988	23.4	79.2	1045	23.1	0.708	2	1.8
B.1.617.2 (Delta)	5315	24.5	4273	25.1	71.7	1042	23.1	0.006	0	0.0
Outpatients	26638	87.6	21135	91.2	79.3	5282	75.1	<.001	221	100.0
Inpatients	4538	14.9	2530	10.9	55.8	2008	28.6	<.001	0	0.0
Intensive care unit transfer	544	1.8	321	1.4	59.0	223	3.2	<.001	0	0.0
Death‡‡	535	1.8	191	0.8	35.7	344	4.9	<.001	0	0.0

781 †: HCQ: Hydroxychloroquine, AZ: Azithromycin. ‡: Chi-square test (HCQ-AZ vs. no HCQ-AZ). ††: Variants with n<4 000 are not displayed. ‡‡: All-cause deaths within 6 weeks.

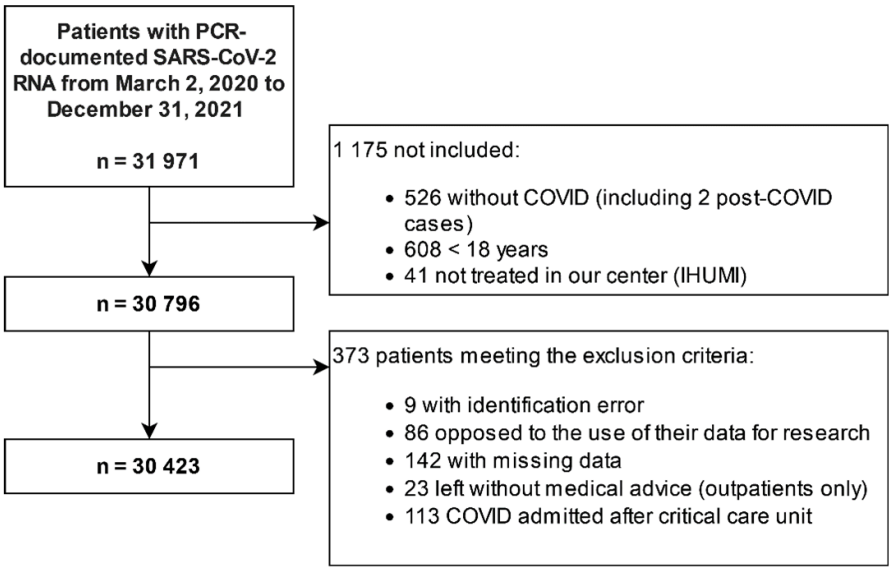
782 **Table 2. Multivariable model of COVID-19 mortality among patients treated in our centre 2020-2021 (n = 30,202†)**

		Model A				Model B				
		OR 95% CI‡	p	aOR, 95% CI††	p		OR, 95% CI‡	p	aOR, 95% CI††	p
Sex (ref. Women)	Men			1.61 1.32-1.96	<.001				1.61 1.32-1.96	<.001
	50-69			6.52 3.21-13.3	<.001				6.47 3.19-13.1	<.001
Age (Ref. <50)	70-89			40.4 20.2-80.7	<.001				39.4 19.7-78.6	<.001
	>89			89.9 43.0-188	<.001				86.4 41.4-180	<.001
Period (Ref. 2020/03/03-2–020/06/15)	2020/06/16-2–020/09/20			0.94 0.61-1.46	0.787				0.92 0.59-1.43	0.704
	2020/09/21-2–020/11/22			1.21 0.83-1.76	0.313				1.16 0.80-1.69	0.438
	2020/11/23-2–021/03/21			1.96 1.39-2.77	<.001				1.90 1.34-2.68	<.001
	2021/03/22-2–021/06/27			1.06 0.71-1.58	0.787				0.99 0.65-1.50	0.958
	2021/06/28-2–021/09/21			1.13 0.72-1.76	0.599				1.06 0.67-1.69	0.789
	2021/09/22-2–021/12/31			1.27 0.83-1.95	0.262				1.22 0.78-1.91	0.395
Outpatients (ref. No)	Yes			0.05 0.04-0.07	<.001				0.05 0.04-0.07	<.001
Treatment (ref. HCQ-AZ‡‡ (n=23 172))	HCQ-AZ vs. No HCQ-AZ‡‡ (n=7 030)	0.16 0.14-0.19	<.001	0.55 0.45-0.68	<.001	HCQ-AZ vs. AZ-only‡‡ (n=3 144)	0.10 0.07-0.13	<.001	0.51 0.35-0.72	<.001
						HCQ-AZ vs. IVM-AZ‡‡ (n=1 434)	0.17 0.11-0.27	<.001	0.54 0.31-0.97	0.029
						HCQ-AZ vs. HCQ-only‡‡ (n=566)	0.67 0.20-2.26	0.974	0.85 0.22-3.25	1.000
						HCQ-AZ vs. IVM-AZ-delayed HCQ‡‡ (n=329)	0.15 0.07-0.33	<.001	0.44 0.17-1.15	0.157
						HCQ-AZ vs. IVM-only‡‡ (n=98)	0.07 0.03-0.21	<.001	0.50 0.15-1.72	0.692
						HCQ-AZ vs. HCQ-IVM‡‡ (n=17)	0.27 0.00-23.9	0.988	0.93 0.00-178	1.000
						HCQ-AZ vs. Other treatment (n=1 771)	0.37 0.21-0.64	<.001	0.49 0.26-0.93	0.018

783
784 †: A total of 221 patients were excluded because of missing treatment data (see Table 1). ‡: Crude odds ratio with 95% confidence interval, ††: Adjusted odds ratio with 95% confidence interval.
785 ‡ ‡: HCQ: Hydroxychloroquine, AZ: Azithromycin, IVM: Ivermectin. Tukey's correction was used to calculate p values and odds ratios for the treatment group variables (model B).

786 **Figures**

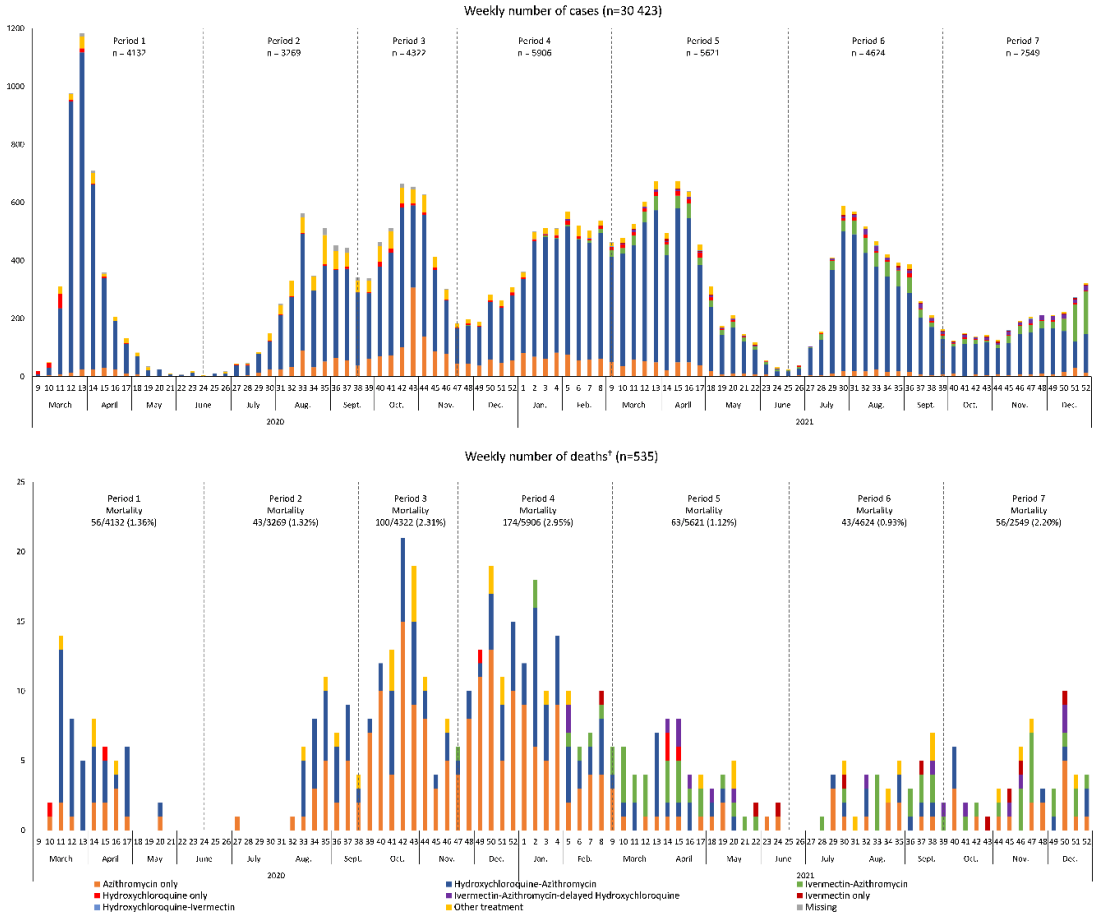
787 **Figure 1. Study flowchart**



788

789 **Figure 2. Number of COVID-19 patients treated in our centre by week, period and**

790 **treatment (n = 30,423)**



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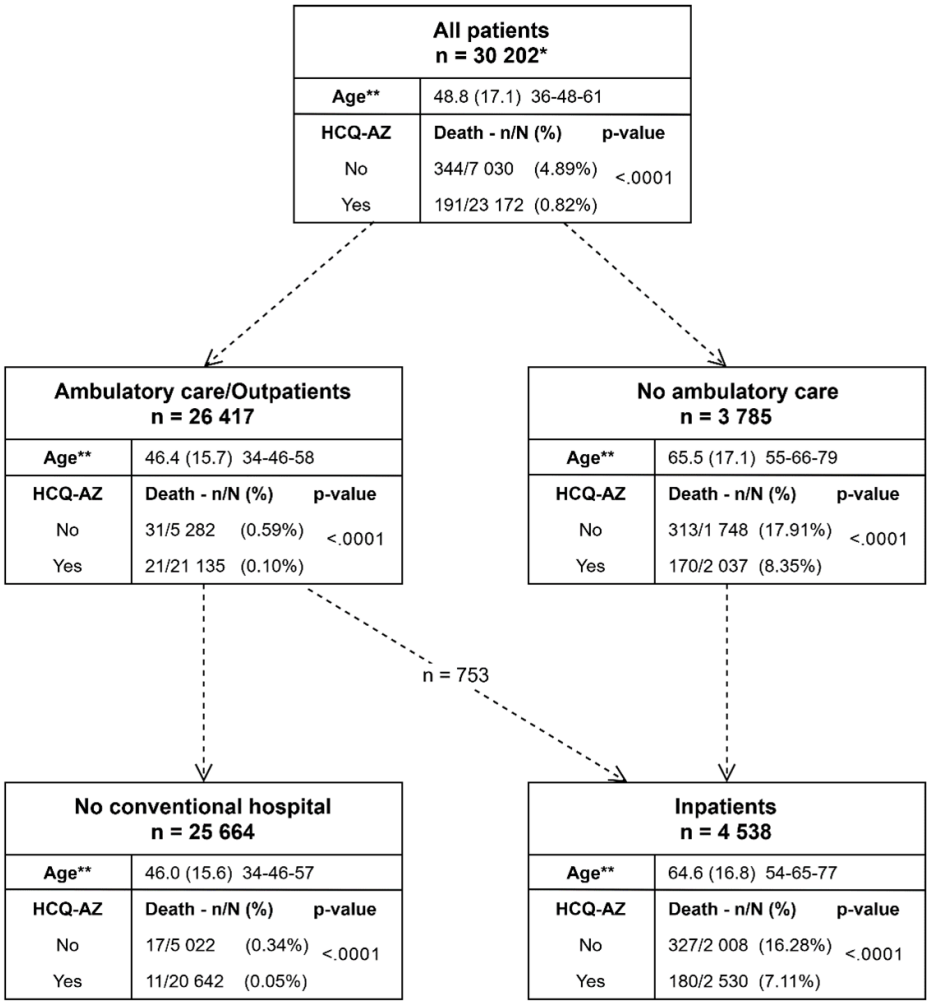
792 †: All-cause deaths within 6 weeks following admission.

793 **Figure 3. Flowchart of health care pathways (n=30,202*)**

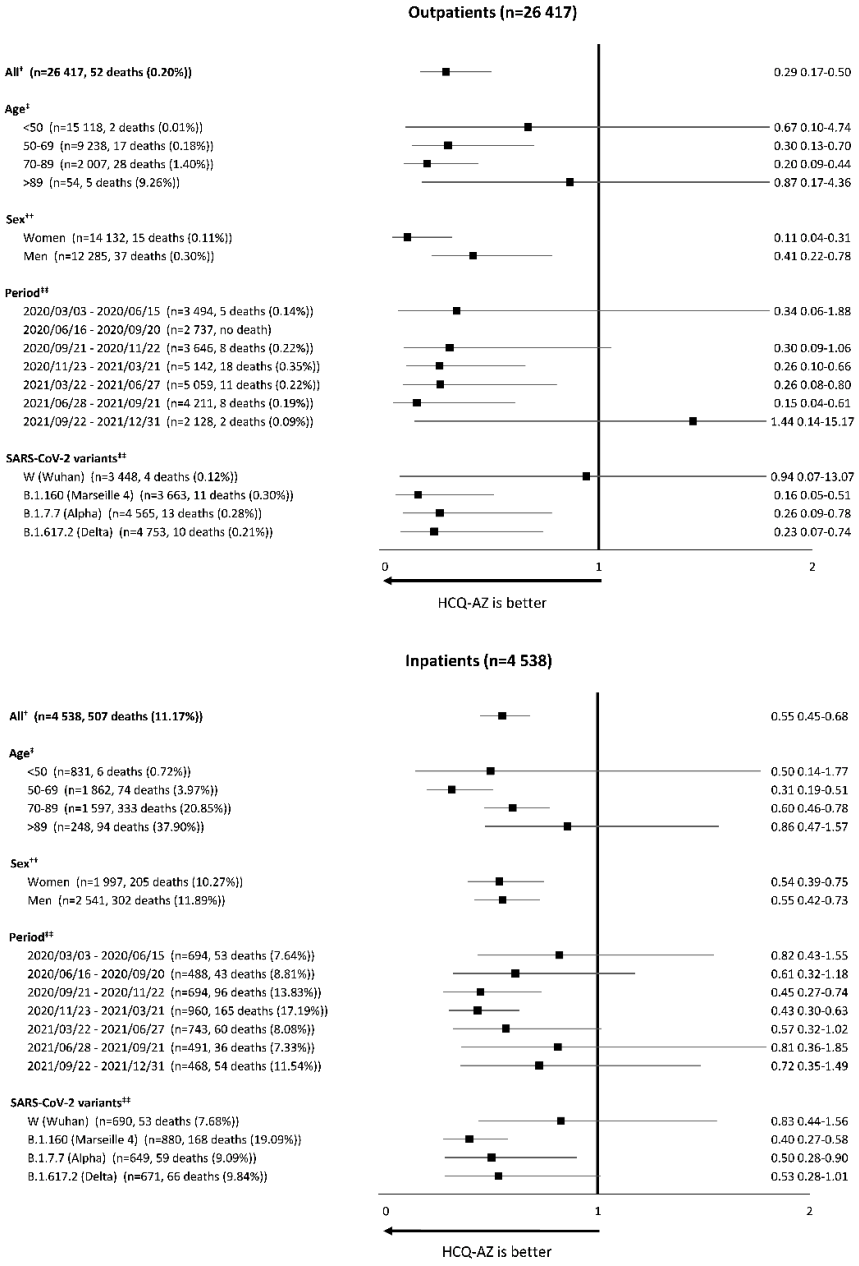


794

795 *221 patients were excluded because of missing treatment data, **Mean (standard deviation)

796 Quartile 1-median-Quartile 3.

797 **Figure 4. Forest plot of the association between HCQ-AZ and 6-week mortality**

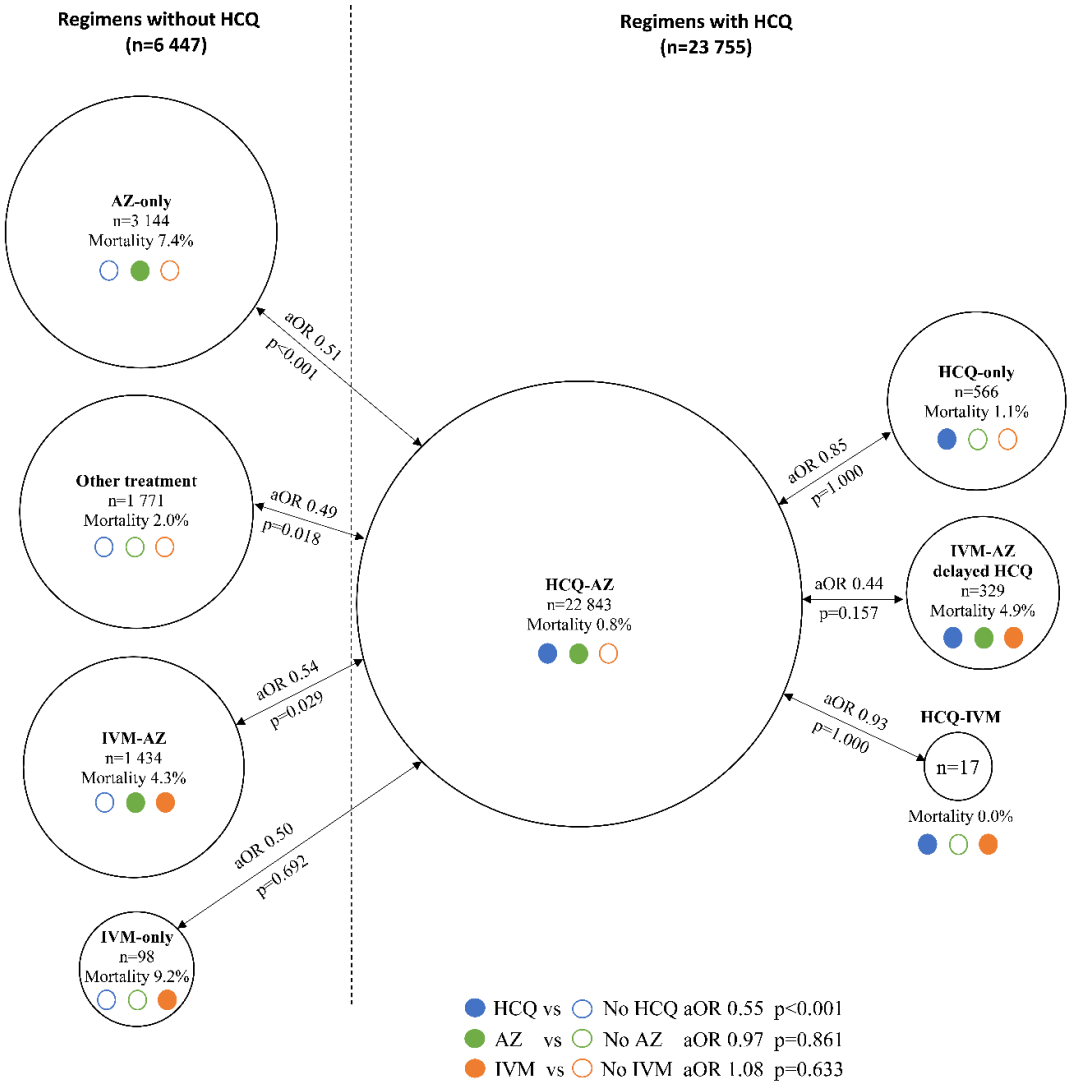


798

799 †: Sex-, age- and period-adjusted odds ratio with 95% CI. ‡: Sex- and period-adjusted odds
800 ratio with 95% CI. ††: Age- and period-adjusted odds ratio with 95% CI. ‡‡: Sex- and age-
801 adjusted odds ratio with 95% CI. A total of 753 patients were both outpatients and inpatients
802 (see Figure 2).

803 **Figure 5. Summary of comparisons between treatment groups and effect on mortality**

804 **associated with each antiviral drug (n = 30,202)**



805

806 HCQ: hydroxychloroquine, AZ: azithromycin, IVM: ivermectin. aOR: adjusted odds ratio.

807 Detailed results with 95% confidence intervals are available in the main text, Tables 1 and 2

808 and Supplementary Table 1.