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Low-dose Hydroxychloroquine Therapy and Mortality in Hospitalized Patients with COVID-19: A Nationwide Observational Study of 8075 Participants

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Highlights

- Hydroxychloroquine (HCQ) 2400 mg during 5 days was used in Belgium for COVID-19
- Impact of HCQ on mortality among 8075 patients with COVID-19 was assessed
- Lower mortality in HCQ-treated patients as compared to supportive care
- Lower mortality is irrespective of symptoms duration

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Low-dose Hydroxychloroquine Therapy and Mortality in Hospitalized Patients with COVID-19: A Nationwide Observational Study of 8075 Participants

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Abstract

Background: Hydroxychloroquine (HCQ) has been largely used and investigated as therapy of COVID-19 across various settings, at total dose usually ranging from 2400 mg to 9600 mg. In Belgium, off-label use of low-dose HCQ (2400 mg in total over five days) was recommended for hospitalized patients with COVID-19.

Methods: We conducted a retrospective analysis of in-hospital mortality in the Belgian national COVID-19 hospital surveillance data. Patients treated either with HCQ alone and supportive care (HCQ group) were compared to patients treated with supportive care only (no-HCQ group) using a competing risks proportional hazards regression with discharge alive as competing risk, adjusted for demographic and clinical features with robust standard errors.

Results: Of 8075 patients with complete discharge data on 24th of May and diagnosed before the 1st of May, 4542 received HCQ in monotherapy and 3533 were in the no-HCQ group. Death was reported in 804/4542 (17.7%) and 957/3533 (27.1%), respectively. In the multivariable analysis, the mortality was lower in the HCQ group compared to the no-HCQ group (adjusted hazard ratio [HR] 0.684, 95% confidence interval [CI] 0.617–0.758). Compared to the no-HCQ group, mortality in the HCQ group was reduced both in patients diagnosed ≤ 5 days ($n=3975$) and > 5 days ($n=3487$) after symptom onset (adjusted HR 0.701, 95% CI 0.617–0.796 and adjusted HR 0.647, 95% CI 0.525–0.797, respectively).

Conclusions: Compared to supportive care only, low-dose HCQ monotherapy was independently associated with lower mortality in hospitalized patients with COVID-19 diagnosed and treated early or later after symptom onset.

Keywords : hydroxychloroquine ; COVID-19 ; SARS-CoV-2 ; mortality ; observational study

1 Introduction

There is currently no robust antiviral or immunomodulatory treatment of SARS-CoV-2 infection, the causative agent of COVID-19. Chloroquine (CQ), an anti-malarial drug, has shown to have *in vitro* anti-viral properties against both SARS-CoV and SARS-CoV-2 by different mechanisms [1–4]. It has also been hypothesized that CQ could have a positive impact on COVID-19 outcome through immunomodulatory properties [5,6]. Hydroxychloroquine (HCQ), a derivative of CQ, has a long clinical track record as treatment of malaria and inflammatory diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), with a favorable safety profile in acute and chronic use [7]. Both CQ and HCQ were selected by the World Health Organization (WHO) for potential repurposing for COVID-19. Early during the amplification phase of the epidemic in Belgium, and pending results of clinical trials, off-label administration of a “low-dose” regimen of HCQ sulphate in monotherapy (400mg twice on day 1, followed by 200mg twice a day from day 2 to 5, i.e. a total dose of 2400 mg) was recommended as an acceptable immediate treatment option for hospitalized COVID-19 patients [8]. This guidance, officially released on March, 13th, was based on the following considerations: (1) HCQ was the only drug with demonstrated *in vitro* effect against SARS-CoV-2 available in Belgium at that time; (2) HCQ exhibited a superior *in vitro* antiviral effect in comparison to CQ, likely explained by the higher intracellular drug accumulated concentrations [9]; (3) limited pharmacokinetic data suggested that the selected dosage should have sufficient antiviral activity [10]; (4) chronic administration of HCQ for rheumatological disorders has not been associated with major safety signals since decades of use; (5) restricting HCQ use to well-selected COVID-19 patients monitored at hospitals appeared as a reasonable risk/benefit compromise considering the well-known dose-dependent cardiotoxicity of the drug; (6) it was advised to Belgian hospitals to administer this off-label regimen whenever possible within clinical studies. Of note, azithromycin (AZM) and systemic use of corticosteroids were not recommended in the guidance [8]. Simultaneously, Sciensano, the Belgian Scientific Institute of Public Health, initiated a national surveillance of COVID-19 hospitalized patients, that included treatments and outcomes among its variables, enabling the clinical surveillance of drug use and outcome.

So far, the impact of HCQ on the outcome of SARS-CoV-2 infection in humans remains undetermined. An increasing number of single- and multi-center retrospective studies using various HCQ dosages are being published with conflicting results [11–19]. Recently, the UK-based RECOVERY and WHO-led SOLIDARITY trials communicated that HCQ at the study dosage of 9200 to 9600 mg over 10 days provided no benefit in hospitalized patients with COVID-19 [20].

In the present study, we retrospectively assessed the association between HCQ monotherapy and in-hospital mortality in a nationwide registry of 8075 COVID-19 patients. Next, the impact of HCQ treatment on mortality was investigated according to the time between symptom onset and COVID-19 diagnosis.

2 Methods

2.1 Data collection

Sciensano's data collection of patients hospitalized with confirmed COVID-19 was initiated on the 14th of March, two weeks after the first symptomatic case was reported in Belgium, and systematic registering was strongly encouraged by health authorities. Two independent online secured questionnaires in LimeSurvey (GmbH, Hamburg, Germany) were made available: one with information after admission and the second after discharge. Information collected at admission included socio-demographic characteristics, clinical presentation, comorbidities, chronic treatment with renin-angiotensin-aldosterone system inhibitors as well as diagnostic workup. Data collected at discharge included COVID-19 treatment details (antiviral and immunomodulatory drugs including date of initiation and termination, mode of administration), clinical and laboratory markers of severity during hospital stay, admission to intensive care unit (ICU), and final outcome at hospital discharge (dead or discharged alive).

2.2 Hydroxychloroquine treatment

On the 13th of March, a task force (ND, SVI and EB, affiliated to the national reference institutions for emerging infections), coordinated by Sciensano, published a guidance for the management of patients hospitalized with COVID-19. Based on the above described rationale, the "low-dose" HCQ regimen

(2400 mg in total over five days) was recommended as a reasonable emergency therapeutic option for hospitalized patients and was centrally provided for free [8]. A set of warnings were provided on its use, including QT corrected (QTc) determination in all admitted patients and close cardiac monitoring in case baseline QTc exceeded 450 msec and in all conditions that could favor arrhythmia (underlying cardiopathy, congenital or acquired QTc prolongation, electrolytic disturbances, or use of other drugs prolonging the QTc duration) [8]. Treatment initiation was advised as soon as diagnosis was made with information to the patient about the off-label use. Final treatment decision was however left at the discretion of the treating physician.

2.3 Inclusion and exclusion criteria

We analyzed all COVID-19 cases for whom both admission and discharge questionnaires were reported up to the 24th of May. The analysis was restricted to those confirmed before the 1st of May by RT-PCR and/or rapid antigen test on respiratory samples, with exclusion of those diagnosed by pulmonary CT Scan only. The SARS-CoV-2 rapid antigen test used in Belgium has a specificity of 99.5% compared to RT-PCR in respiratory samples [21]. Children below 16 years, pregnant and post-partum women as well as patients who were discharged (either alive or dead) within 24 hours after hospital admission or before diagnosis confirmation were excluded. In addition, we removed from this analysis all patients having started any COVID-19-related treatment before symptom onset, including for other clinical indications as well as those having a missing date of diagnosis. To compare patients treated with HCQ alone with those receiving only supportive care, patients treated with another COVID-19-related treatment (macrolides, tocilizumab, lopinavir/ritonavir, remdesivir, atazanavir or anakinra), whether prescribed with or without HCQ, were also excluded.

2.4 Statistical analyses

Participants meeting the inclusion criteria were divided into two groups: (1) COVID-19 patients treated with HCQ monotherapy in addition to supportive care (HCQ group) and (2) those receiving supportive care alone (no-HCQ group). Demographic characteristics, pre-existing conditions, laboratory parameters, clinical features and outcome were described first by discharge status (survivors

versus non-survivors) and secondly by treatment group (HCQ versus no-HCQ). Chi-square tests for categorical variables and Wilcoxon tests for continuous variables were used to assess differences between groups. We considered a p value < 0.05 to be statistically significant.

Missing data among important prognostic baseline covariates were assumed to be missing at random, i.e. independent of the underlying missing values given the observed data. This was handled by tenfold multiple imputation performed in R software through the MICE package version 3.8.0 [22]. A competing risks proportional hazards regression with robust standard errors allowing for clustering within hospitals (R package SURVIVAL version 3.1-12) was then used to analyse in-hospital death competing with alive discharge from hospital. Hazards of this in-hospital death thus dropped to zero post discharge alive. Cause-specific hazards of treatment effect were adjusted for baseline covariates age, sex, comorbidities (cardiovascular disease, arterial hypertension, diabetes, chronic renal, liver and lung diseases, neurological and cognitive disorders, immunosuppressive conditions, malignancies, obesity and smoking status), clinical features (pneumonia diagnosis, acute respiratory distress syndrome, admission to intensive care unit within the 24h following admission, and the time from symptom onset to diagnosis, baseline laboratory parameters of disease severity consisting of lactate dehydrogenase (LDH) ≥ 350 IU/L, C reactive protein (CRP) ≥ 150 mg/L and partial pressure of oxygen (paO₂) < 60 mmHg. As HCQ prescription decreased over time, calendar time of diagnosis was also included in the model.

The propensity of HCQ treatment was estimated from those same baseline covariates (R package IPW version 1.0-11[23]). An inverse propensity weighted standardized cumulative incidence of in-hospital death for each treatment was derived using R package RISCA version 0.8.2[24]. The competing risks analysis was repeated for patients treated within or beyond 5 days of onset of symptoms. Sensitivity analyses were performed (Supplemental Material): they considered additional adjustments in the model, missing data impact and possible immortal time bias associated with delayed treatment receipt. Analyses were performed in SAS Enterprise Guide 7.1 and in R 3.6.3.

2.5 Ethical and privacy considerations

The hospital data collection is being performed by Sciensano, the Belgian Scientific Institute of Public Health, legally entitled for surveillance of infectious diseases in Belgium (Royal Decree of 21/03/2018). This COVID-19 hospital surveillance was authorized by an independent administrative authority protecting privacy and personal data, and approved by the ethical committee of Ghent University Hospital (BC-07507).

3 Results

As recorded on 24th of May, the Sciensano database contained a total of 15544 case records of COVID-19 patients (Figure 1), originating from 109 Belgian hospitals. Among those, both admission and discharge report forms were received for 10920 patients (3311 [20%] discharge forms were missing for patients with admission data and 1313 [8%] admission forms were missing for patients with discharge data). After having excluding patients not meeting inclusion criteria (Figure 1), 8910 cases were included for the descriptive analysis.

About 60% of the hospitalized COVID-19 patients were aged above 65 years (Table 1). In general, participants were severely ill with more than 80% having radiological pneumonia, large proportions presenting with laboratory parameters of severity, including pronounced hypoxemia, and 5% requiring immediate admission in intensive care unit (ICU). Median time from symptom onset to COVID-19 diagnosis was five days. Patients with incomplete discharge data (n=2332) were similar to the study population, in terms of age and sex distribution as well as frequency of pre-existing conditions, except from the proportion of active smokers (Supplemental Table 1). In univariate analysis, compared to survivors, non-survivors were older, more likely to be men and to suffer from pre-existing conditions (Table 1). In addition, non-survivors presented more often with laboratory markers of severity such as high levels of lactate dehydrogenase (LDH>350 IU/L) and C-reactive protein (CRP>150 mg/L), and severe hypoxemia (paO₂<60 mmHg). Time from symptom onset to diagnosis was shorter in non-survivors (median 3 days, versus 6 days in survivors, $p < 0.0001$). Length of hospital stay was similar in both groups.

After further exclusion of patients who received alternative COVID-19 treatments with (n=818, including macrolides, n=761) or without HCQ (n=17), the comparative analysis was restricted to 8075

subjects: 4542 in the HCQ group and 3533 in the no-HCQ group (Fig 1). Of the HCQ-treated patients, 78.2% initiated the treatment within 24h after diagnosis.

As shown in Table 2, COVID-19 patients in the HCQ group were younger and male sex was predominant. Several comorbidities were significantly less frequent in the HCQ group, including cardiovascular diseases, arterial hypertension, chronic renal disease, neurological and cognitive disorders, solid cancer, obesity as well as the proportion of active smokers. On the other hand, at admission, patients in the HCQ group appeared to be sicker as reflected by the higher frequency of radiological pneumonia, acute respiratory distress syndrome, ICU transfer within the 24h after admission and invasive ventilation support as well as the higher frequency of elevated LDH and CRP levels. The case fatality rate of the study population was 21.8% (1761 deaths/8075 patients) but was lower in the HCQ group (804/4542, 17.7%) than in the no-HCQ group (957/3533, 27.1%, $p < 0.001$). Incidental use of steroids was very low in both groups, although slightly higher in the HCQ group (8.1% vs 5.9% respectively). On a side note, mortality in the 761 participants who received HCQ and AZM was 18.9%.

Independent predictors of in-hospital mortality are shown in Figure 2. Age, male sex, cardiovascular disease, diabetes, chronic renal, liver and lung diseases, neurological disorders, immunosuppressive conditions, smoking status, as well as radiological pneumonia, acute respiratory distress syndrome, invasive ventilation support and immediate admission to ICU were all associated with a higher risk of in-hospital death. It was also the case for the measured laboratory parameters of severity at baseline. Treatment with HCQ alone was in contrast independently associated with decreased risk of in-hospital mortality (Adjusted hazard ratio [HR] 0.684, 95% confidence interval [CI] 0.617–0.758) compared to the no-HCQ group. Figure 3 shows the inverse propensity weighted standardized cumulative incidence of in-hospital death for each treatment with multiple imputations. From this model, estimated direct-adjusted mortality at 40 days was 19.1% with HCQ alone, and 26.5% with supportive care only.

Next, we compared the association between HCQ treatment and in-hospital mortality in patients diagnosed and treated within five days after onset of symptoms (early diagnosis, $n = 3975$) to those diagnosed more than 5 days later (late diagnosis, $n = 3487$). Compared to the no-HCQ group,

administration of HCQ appears to be associated to a lower risk of death in both the early diagnosis group (Adjusted HR 0.701, 95% CI 0.617–0.796) and the late diagnosis group (Adjusted HR 0.647, 95% CI 0.525–0.797).

4 Discussion

In this large analysis of patients admitted for COVID-19 in Belgium, HCQ monotherapy administered at a dosage of 2400 mg over five days was independently associated with a lower in-hospital mortality rate compared to patients treated with supportive care only, even after adjustment for age, major comorbidities and disease severity at admission. Importantly, mortality was reduced regardless of the timing from symptom onset to diagnosis and HCQ treatment initiation.

Our study has several limitations and strengths. It is an observational study of data collected using standardized report forms during the most critical phase of the epidemic in Belgium. The cohort was established within an ongoing surveillance that aims at monitoring the epidemic and identifying risk factors for severe COVID-19 and unfavourable outcome. The evaluation of HCQ efficacy in this population was therefore not the primary objective of the data collection itself. Also, the actual HCQ dosage was not systematically checked, but qualitative surveys pointed out that the “low-dose” recommendation was very well adhered to, since the risk of dose-dependent cardiotoxicity and the necessary precautions of use in patients at risk were particularly stressed in the treatment guidance [8]. Not surprisingly, HCQ has been less administered in several groups of patients with pre-existing conditions or co-medications which corresponded to contra-indications of its use (cardiac and renal diseases). The implementation of this surveillance during the initial phase of the epidemic when hospitals were under pressure and its non-mandatory nature resulted in missing admission or discharge report forms for a sizeable proportion of patients. The absence of difference in the baseline characteristics and in outcome for subgroups with missing data and the study population is somehow reassuring, although some hidden sources of bias cannot be fully excluded. Strengths of this study include the very large sample size obtained in a timely manner, the strict comparison between groups exposed to HCQ in monotherapy and to supportive care only (with no other COVID-19 treatments as confounders), the multi-centric design covering the vast majority of Belgian hospitals and the real-life

representativeness of the data. Indeed, this hospital-based surveillance captured complete admission and discharge information for 64% (10920/17052) of all aggregated COVID-19 patients admitted across the country until the 24th of May [25]. Finally, rigorous sensitivity analysis taking into account censored data and immortal bias all confirmed the positive impact of HCQ on in-hospital mortality.

This nationwide observational study provides a robust description of the COVID-19 patients admitted in Belgian hospitals. The demographic and clinical characteristics were similar to hospital cohorts reported in other countries, with a large proportion of patients with well-established risk factors for COVID-19 complications. The observed in-hospital mortality rate (about 21.8%) was in line with that of previous observational studies and ongoing trials [26–28]. Risk factors of death were comparable to previous clinical experience. Notably, we found that biological markers previously related to disease severity and mortality in univariate analysis (increased CRP and LDH) [29] were independently associated with mortality in our study. Other well-established predictors of mortality such as lymphopenia and increased levels of D-dimer were not recorded in our dataset. High D-dimer levels have been meanwhile associated with increased risk of thrombotic events, a major cause of death in hospitalized COVID-19 patients [30,31].

Interestingly, we observed that patients who died in the hospital, had on average a shorter duration of symptoms before admission, suggesting that abrupt clinical deterioration is associated with worst prognosis. More research is required to clarify the phenotype of this subgroup of patients, to assess whether patients who develop signs of severity very soon after the onset of symptoms require more intensive care earlier, and to investigate whether very early predictors of subsequent severity and targeted (pre-hospital) interventions could prevent admission due to acute complications.

In the context of the COVID-19 pandemic, HCQ therapy has been in the centre of debates, between hype and bashing, within and beyond the scientific community. Uncertainty about treatment efficacy relies mainly on the observational nature of the published studies so far and the major risks of bias and confounders. Many small single-centre retrospective studies did not find any impact of HCQ treatment on outcome in hospitalized COVID-19 patients [11,13–16], but were not powered to explore associations with mortality endpoint through robust multivariate analysis. Recently, larger

observational studies found that the use of HCQ alone or in combination with AZM was independently associated with lower in-hospital mortality [17–19], in line with our results. Some therapeutic differences have however to be highlighted, that impede full comparison of these studies with our data. In Lagier’s study [17], HCQ dosage was 6000 mg in total over 10 days, and the vast majority of participants were given AZM concomitantly. In Mikami’s study [18], the HCQ dosage was not reported, but most hospitalized patients were also exposed to a combination of HCQ and AZM. Finally, Arshad et al. used the same HCQ dosage as in our study, and reported specifically on the subgroup treated with HCQ, but a large proportion of participants were also given steroids, which might have been beneficial in severe COVID-19 cases [19,32]. Of note, in our dataset, corticosteroids uptake was low in both groups. In Belgium, AZM was not recommended due to the lack of robust information on viral efficacy at the time of writing the guidance. It has however been administered in combination with HCQ in 761 patients in our real-life database (but excluded from this analysis). The combination AZM-HCQ treatment was associated to a decrease in mortality similar to that of HCQ alone (data not shown).

Although observational studies, even of large scale, do not provide final conclusions on treatment efficacy, their results are important to consider to guide clinical trials. Well-designed prospective studies combined with large randomized control trials should provide definitive evidence about the clinical impact of HCQ in severe hospitalized and in mild ambulatory COVID-19 patients. Meanwhile, in a preprint publication at the time of writing, the results of the RECOVERY trial did not show any clinical benefit (death or discharge) in the high dose HCQ arm (9200 mg in total over 10 days) compared to usual care in hospitalized COVID-19 patients (median number of days from symptom onset to randomization: 9 days) [20]. Of note, the dose administered during the first 24h (2400 mg) is equivalent to the dose administered during 5 days in Belgium. The pharmacokinetic rationale for the high dosage remains poorly described. Moreover, 10% of the patients included in the HCQ arm had negative SARS-CoV-2 test and mortality was high in both groups (25%), indicating advanced disease.

While both CQ and HCQ have *in vitro* antiviral activity against SARS-CoV-2 [3,4,9], concerns about a real antiviral activity *in vivo* have emerged early in the pandemic based on previous experience in

other viral infections [33]. Antiviral efficacy of HCQ in humans has been poorly studied so far with adequate methods. Questions have also been raised whether safe HCQ dosages are sufficient to reach antiviral activity in target pulmonary cells [34]. Translating *in vitro* data into *in vivo* drug concentration in tissue appears particularly challenging for HCQ, as plasma concentrations do not appear to be a reliable surrogate [35]. Preprint studies in animal models (non-humans primates and Syrian hamsters) also suggest that HCQ has no antiviral efficacy [36,37]. Clinical efficacy might however be mediated through immunomodulatory mechanisms [7], preventing the progression toward severe disease with over-inflammatory responses by dampening the cytokine storm [38]. HCQ has indeed been shown to decrease the production of pro-inflammatory cytokines, both *ex vivo* and in lung explant model [5,39,40]. In the same line, use of low-dose dexamethasone (one of the RECOVERY arm) was recently reported to significantly decrease mortality in COVID-19 patients requiring oxygen [28]. Also, HCQ has been suggested to have some anticoagulant properties that may be beneficial in preventing thrombotic events in complement to low-molecular weight heparin [41].

The potential detrimental effect of HCQ, mainly due to dose-dependent cardiotoxicity, has become a major clinical concern, especially following the publication of an article that reported an association between HCQ therapy and increased in-hospital mortality, but that was retracted shortly after [42]. A randomized controlled trial evaluating high (12000 mg over 10 days) and low CQ dosage was halted prematurely due to serious toxicity in the high dose group [43]. However, many studies have meanwhile reported on the safety of a short-term/low dosage course of HCQ monotherapy [16,44]. Our study provides further support to the claim that this regimen is not associated with increased short term risk of cardiotoxicity and mortality in the hospital setting and in well-selected COVID-19 patients. Accordingly, as of 17 June 2020, the Federal Agency for Medicines and Health Products had registered in total 8 reports of adverse reactions suspected to be associated with HCQ use for the treatment of COVID-19 in Belgium, among which 3 cases of cardiac toxicity (all having received concomitant medication) and no reported deaths [45].

In conclusion, in this large nationwide observational study of patients hospitalized with COVID-19, HCQ monotherapy administered at a dosage of 2400 mg over five days was independently associated

with significant decrease in mortality compared to patients not treated with HCQ. This impact was observed in both early and late treatment group, suggesting that this benefit might be mediated by immunomodulatory properties, a hypothesis worth addressing as evidence of an antiviral activity of HCQ on SARS-CoV-2 appears increasingly inconsistent. Considering the availability and cheapness of HCQ, it seems worth further investigating the clinical effect of optimized dosage of HCQ and designing add-on studies in ongoing trials to monitor, beyond the viral shedding and infectiousness, a relevant set of inflammatory markers during the course of SARS-CoV-2 infection.

Declarations

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References

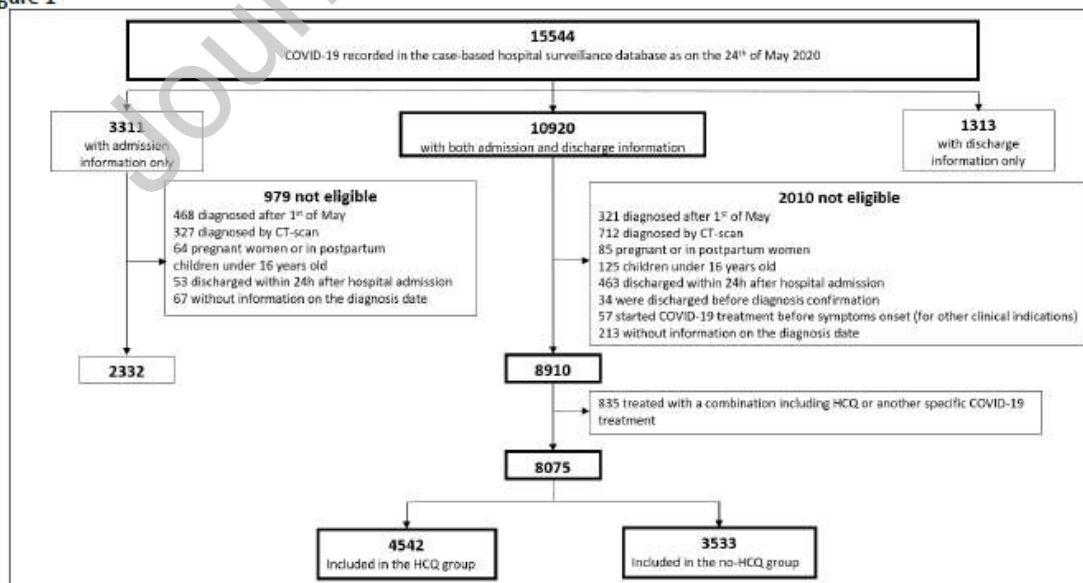
- [1] Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun* 2004;323:264–8. <https://doi.org/10.1016/j.bbrc.2004.08.085>.
- [2] Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology* 2005;2:69. <https://doi.org/10.1186/1743-422X-2-69>.
- [3] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020. <https://doi.org/10.1038/s41422-020-0282-0>.
- [4] Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov* 2020;6. <https://doi.org/10.1038/s41421-020-0156-0>.
- [5] Grassin-Delye S, Salvator H, Brollo M, Catherinot E, Sage E, Couderc L-J, et al. Chloroquine inhibits the release of inflammatory cytokines by human lung explants. *Clin Infect Dis* 2020.
- [6] Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: current state of the science. *Immunity* 2020. <https://doi.org/10.1016/j.immuni.2020.05.002>.
- [7] Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat Rev Rheumatol* 2020;16:155–66. <https://doi.org/10.1038/s41584-020-0372-x>.
- [8] Sciensano. Interim Clinical Guidance for Adults with suspected or confirmed COVID-19 in Belgium n.d.
- [9] Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa237>.
- [10] Perinel S, Launay M, Botelho-Nevers É, Diconne É, Louf-Durier A, Lachand R, et al. Towards Optimization of Hydroxychloroquine Dosing in Intensive Care Unit COVID-19 Patients. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa394>.
- [11] Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* 2020. <https://doi.org/10.1056/NEJMoa2012410>.
- [12] Gautret P, Lagier J, Parola P, Hoang V, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* In Press.
- [13] Mahévas M, Tran V-T, Roumier M, Chabrol A, Paule R, Guillaud C, et al. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data. *BMJ* 2020:m1844. <https://doi.org/10.1136/bmj.m1844>.
- [14] Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J, et al. Association of Treatment With Hydroxychloroquine or Azithromycin With In-Hospital Mortality in Patients With COVID-19 in New York State. *JAMA* 2020. <https://doi.org/10.1001/jama.2020.8630>.
- [15] Paccoud O, Tubach F, Baptiste A, Bleibtreu A, Hajage D, Monsel G, et al. Compassionate use of hydroxychloroquine in clinical practice for patients with mild to severe Covid-19 in a French university hospital. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa791>.
- [16] Sbidian E, Josse J, Lemaitre G, Mayer I, Bernaux M, Gramfort A, 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* 2020:2020.06.16.20132597. <https://doi.org/10.1101/2020.06.16.20132597>.
- [17] Lagier J-C, Million M, Gautret P, Colson P, Cortaredona S, Giraud-Gatineau A, et al. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis. *Travel Med Infect Dis* 2020:101791. <https://doi.org/10.1016/j.tmaid.2020.101791>.

- [18] Mikami T, Miyashita H, Yamada T, Harrington M, Steinberg D, Dunn A, et al. Risk Factors for Mortality in Patients with COVID-19 in New York City. *J Gen Intern Med* 2020. <https://doi.org/10.1007/s11606-020-05983-z>.
- [19] Arshad S, Kilgore P, Chaudhry ZS, Jacobsen G, Wang DD, Huitsing K, et al. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. *Int J Infect Dis* 2020;97:396–403. <https://doi.org/10.1016/j.ijid.2020.06.099>.
- [20] Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, et al. Effect of Hydroxychloroquine in Hospitalized Patients with COVID-19: Preliminary results from a multi-centre, randomized, controlled trial. *MedRxiv* 2020:2020.07.15.20151852. <https://doi.org/10.1101/2020.07.15.20151852>.
- [21] Mertens P, De Vos N, Martiny D, Jassoy C, Mirazimi A, Cuyppers L, et al. Development and Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context. *Front Med* 2020;7:225. <https://doi.org/10.3389/fmed.2020.00225>.
- [22] Buuren S van, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. *J Stat Softw* 2011;45:1–67.
- [23] Wal WM van der, Geskus RB. ipw : An R Package for Inverse Probability Weighting. *J Stat Softw* 2011;43. <https://doi.org/10.18637/jss.v043.i13>.
- [24] Le Borgne F, Giraudeau B, Querard AH, Giral M, Foucher Y. Comparisons of the performance of different statistical tests for time-to-event analysis with confounding factors: practical illustrations in kidney transplantation. *Stat Med* 2016;35:1103–16. <https://doi.org/10.1002/sim.6777>.
- [25] Sciensano. COVID-19 - Bulletin épidémiologique du 25 mai 2020 2020.
- [26] Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, 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* 2020:m1985. <https://doi.org/10.1136/bmj.m1985>.
- [27] Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020. <https://doi.org/10.1001/jama.2020.6775>.
- [28] Group TRC. Dexamethasone in Hospitalized Patients with Covid-19 — Preliminary Report. *N Engl J Med* 2020. <https://doi.org/10.1056/NEJMoa2021436>.
- [29] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* 2020. [https://doi.org/10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3).
- [30] Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med* 2020;46:1089–98. <https://doi.org/10.1007/s00134-020-06062-x>.
- [31] Lippi G, Favaloro EJ. D-dimer is Associated with Severity of Coronavirus Disease 2019: A Pooled Analysis. *Thromb Haemost* 2020;120:876–8. <https://doi.org/10.1055/s-0040-1709650>.
- [32] Lee TC, MacKenzie LJ, McDonald EG, Tong SYC. An Observational Cohort Study of Hydroxychloroquine and Azithromycin for COVID-19: (Can't Get No) Satisfaction. *Int J Infect Dis* 2020. <https://doi.org/10.1016/j.ijid.2020.06.095>.
- [33] Touret F, de Lamballerie X. Of chloroquine and COVID-19. *Antiviral Res* 2020;177:104762. <https://doi.org/10.1016/j.antiviral.2020.104762>.
- [34] Fan J, Zhang X, Liu J, Yang Y, Zheng N, Liu Q, et al. Connecting hydroxychloroquine in vitro antiviral activity to in vivo concentration for prediction of antiviral effect: a critical step in treating COVID-19 patients. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa623>.
- [35] Martin-Blondel G, Ruiz S, Murriss M, Faguer S, Duhalde V, Eyvrard F, et al. Hydroxychloroquine in COVID-19 patients: what still needs to be known about the kinetics. *Clin Infect Dis* 2020. <https://doi.org/10.1093/cid/ciaa558>.
- [36] Kaptein SJ, Jacobs S, Langendries L, Seldeslachts L, ter Horst S, Liesenborghs L, et al. Antiviral treatment of SARS-CoV-2-infected hamsters reveals a weak effect of favipiravir and a complete lack of effect for hydroxychloroquine. *BioRxiv* 2020:2020.06.19.159053. <https://doi.org/10.1101/2020.06.19.159053>.

- [37] Maisonnasse P, Guedj J, Contreras V, Behillil S, Solas C, Marlin R, et al. Hydroxychloroquine in the treatment and prophylaxis of SARS-CoV-2 infection in non-human primates 2020.
- [38] Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* 2020. <https://doi.org/10.1038/s41577-020-0311-8>.
- [39] Jang C-H, Choi J-H, Byun M-S, Jue D-M. Chloroquine inhibits production of TNF- α , IL-1 β and IL-6 from lipopolysaccharide-stimulated human monocytes/macrophages by different modes. *Rheumatology* 2006;45:703–10. <https://doi.org/10.1093/rheumatology/kei282>.
- [40] Silva J, Mariz H, Rocha Jr L, Oliveira P, Dantas A, Duarte A, et al. Hydroxychloroquine decreases Th17-related cytokines in systemic lupus erythematosus and rheumatoid arthritis patients. *Clinics* 2013;68:766–71. [https://doi.org/10.6061/clinics/2013\(06\)07](https://doi.org/10.6061/clinics/2013(06)07).
- [41] Oscanoa TJ, Romero-Ortuno R, Carvajal A, Savarino A. A pharmacological perspective of Chloroquine in SARS-CoV-2 infection. *Int J Antimicrob Agents* 2020:106078. <https://doi.org/10.1016/j.ijantimicag.2020.106078>.
- [42] Ledford H, Noorden RV. High-profile coronavirus retractions raise concerns about data oversight. *Nature* 2020;582:160–160. <https://doi.org/10.1038/d41586-020-01695-w>.
- [43] Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M, 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 Netw Open* 2020;3:e208857. <https://doi.org/10.1001/jamanetworkopen.2020.8857>.
- [44] Lane JCE, Weaver J, Kostka K, Duarte-Salles T, Abrahao MTF, Alghoul H, et al. Safety of hydroxychloroquine, alone and in combination with azithromycin, in light of rapid wide-spread use for COVID-19: a multinational, network cohort and self-controlled case series study. *MedRxiv* 2020:2020.04.08.20054551. <https://doi.org/10.1101/2020.04.08.20054551>.
- [45] Federal Agency for Medicines and Health Products. Summary Adverse Drug Reactions in Corona virus infection 2020.

Figures captions :

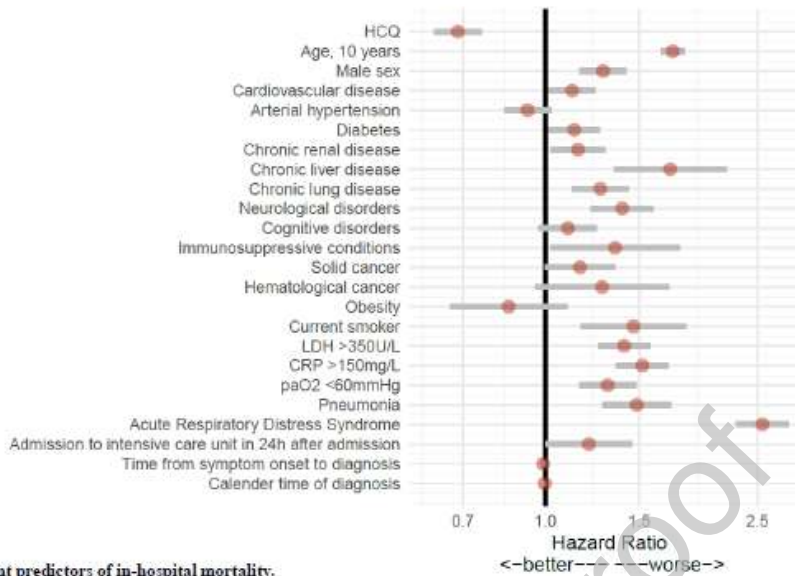
Figure 1



Data flow of COVID-19 patients selection for the observational cohort study

Figure 1 : Data flow of COVID-19 patients selection for the observational cohort study

Figure 2



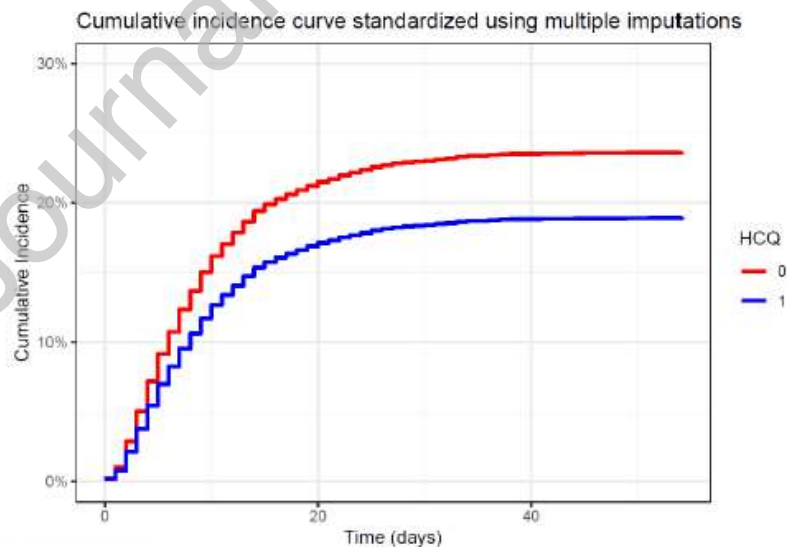
Independent predictors of in-hospital mortality.

Competing risks proportional hazards regression with robust standard errors analysing in-hospital death competing with alive discharge from hospital.

HHCQ : hydroxychloroquine, LDH : lactate deshydrogenase, CRP : C-reactive protein.

Figure 2 : Independent predictors of in-hospital mortality among 8075 patients with COVID-19. Competing risks proportional hazards regression with robust standard errors analysing in-hospital death competing with alive discharge from hospital. HHCQ : hydroxychloroquine, LDH : lactate deshydrogenase, CRP : C-reactive protein.

Figure 3



Cumulative incidence of in-hospital mortality.

Inverse propensity weighted standardized cumulative incidence of in-hospital death according to treatment received.

Hydroxychloroquine (HHCQ) (blue line) vs no-HHCQ (red line).

Figure 3: **Cumulative incidence of in-hospital mortality.** Inverse propensity weighted standardized cumulative incidence of in-hospital death according to treatment received. Hydroxychloroquine (HCQ) (blue line) vs no-HCQ (red line).

Table 1. COVID-19 Patients (n=8910) characteristics by survival or non-survival status during hospitalization

	No./total No. (%)			p value
	Total n=8910	Survivors n=6981	Non-survivors n=1929	
Demographic characteristics				
<i>Age, years</i>				
16-30	149/8906 (1.7)	149/6979 (2.1)	0/1927 (0.0)	
31-44	607/8906 (6.8)	596/6979 (8.5)	11/1927 (0.6)	
45-64	2685/8906 (30.2)	2503/6979 (35.9)	182/1927 (9.4)	<0.0001 [‡]
65-79	2655/8906 (29.8)	2017/6979 (28.9)	638/1927 (33.1)	
>80	2810/8906 (31.6)	1714/6979 (24.6)	1096/1927 (56.9)	
Median (IQR)	71 (57-82)	66 (54-79)	82 (73-87)	
Male sex	4807/8819 (54.5)	3711/6919 (53.6)	1096/1900 (57.7)	0.0017 [‡]
Pre-existing conditions				
Cardiovascular disease	3084/8910 (34.6)	2093/6981 (30.0)	991/1929 (51.4)	<0.0001 [‡]
Arterial hypertension	3622/8910 (40.7)	2641/6981 (37.8)	981/1929 (50.9)	<0.0001 [‡]
Diabetes	1985/8910 (22.3)	1442/6981 (20.7)	543/1929 (28.1)	<0.0001 [‡]
Chronic renal disease	1166/8910 (13.1)	733/6981 (10.5)	433/1929 (22.4)	<0.0001 [‡]
Chronic liver disease	237/8910 (2.7)	160/6981 (2.3)	77/1929 (4.0)	<0.0001 [‡]
Chronic lung disease	1353/8910 (15.2)	976/6981 (14.0)	377/1929 (19.5)	<0.0001 [‡]
Neurological disorders	832/8910 (9.3)	555/6981 (8.0)	277/1929 (14.4)	<0.0001 [‡]

<i>Cognitive disorders</i> ¹	1001/8338 (12.0)	627/6539 (9.6)	374/1799 (20.8)	<0.0001 [±]
<i>Immunosuppressive conditions</i>	248/8910 (2.7)	191/6981 (2.7)	57/1929 (3.0)	0.6049 [±]
<i>Malignancy</i>				
<i>Solid</i>	730/8910 (8.2)	507/6981 (7.3)	223/1929 (11.6)	<0.0001 [±]
<i>Hematological</i>	174 /8910 (2.0)	118/6981 (1.7)	56/1929 (2.9)	0.0007 [±]
<i>Obesity</i> ¹	545/5457 (10.0)	450/4313 (10.4)	95/1144 (8.3)	0.0327 [±]
<i>Current smoker</i>	407/4757 (8.6)	312/3793 (8.2)	95/964 (9.9)	0.1064 [±]
Medications				
<i>Angiotensin converting enzyme inhibitor</i>	1368/8907 (15.3)	1030/6979 (14.8)	338/1928 (17.5)	0.0028 [±]
<i>Angiotensin receptor blocker</i>	806/8907 (9.0)	604/6979 (8.7)	202/1928 (10.5)	0.0135 [±]
COVID-19 treatments				
<i>Supportive care only</i>	3533/8910 (39.6)	2576/6981 (36.9)	957/1929 (49.6)	<0.0001 [±]
<i>HCQ</i>	4542/8910 (51.0)	3738/6981 (53.5)	804/1929 (41.7)	<0.0001 [±]
<i>HCQ + macrolides</i>	761/8910 (8.5)	617/6981 (8.5)	144/1929 (7.5)	0.0561 [±]
<i>Lopinavir/ritonavir</i>	12/8910 (0.1)	7/6981 (0.1)	5/1929 (0.3)	0.2358 [±]
<i>HCQ + lopinavir/ritonavir</i>	18/8910 (0.2)	10/6981 (0.1)	8 /1929 (0.4)	0.0504 [±]
<i>HCQ + tocilizumab</i>	17/8910 (0.2)	12/6981 (0.2)	5/1929 (0.3)	0.4367 [±]
<i>HCQ + tocilizumab + macrolides</i>	7/8910 (0.1)	5/6981 (0.1)	2/1929 (0.1)	0.6565 [±]
<i>HCQ + remdesivir</i>	4/8910 (0.0)	2/6981 (0.0)	2/1929 (0.1)	0.1685 [±]
<i>Others</i>	16/8910 (0.2)	14/6981 (0.2)	2/1929 (0.1)	0.3738 [±]
Laboratory parameters				
<i>Lactate dehydrogenase (IU/L)</i>	343 (258-477)	329 (251-459)	394 (288-548)[<0.0001 [*]
<i>Median (IQR)</i>	[7385]	[5909]	1476]	

	>350	3563/7385 (48.2)	2663/5909 (45.1)	900/1476 (61.0)	<0.0001 [‡]
<i>C-reactive protein (mg/L)</i>					
	Median (IQR)	62 (26-118) [8624]	55.9 (21.8-108.2) [6802]	91.2 (44.4-162) [1822]	<0.0001*
	>150	1487/8624 (17.2)	973/6802 (14.3)	514/1822 (28.2)	<0.0001 [‡]
<i>paO₂ (mmHg)</i>					
	Median (IQR)	66 (57-76) [6013]	67 (70-77) [4713]	61 (52-73) [1300]	<0.0001*
	<60	1834/6013 (30.5)	1221/4713 (25.9)	613/1300 (47.2)	<0.0001 [‡]
Clinical features					
<i>Pneumonia²</i>		7184/8567 (83.9)	5545/6710 (82.6)	1639/1857 (88.2)	<0.0001 [‡]
<i>Acute Respiratory Distress Syndrome</i>		1197/8423 (14.2)	601/6710 (9.0)	596/1713 (34.8)	<0.0001 [‡]
<i>Invasive ventilation support</i>		736/8691 (8.5)	367/6810 (5.4)	369/1881 (19.6)	<0.0001 [‡]
<i>Admission to intensive care unit in 24h after admission</i>		488/8900 (5.5)	298/6974 (4.3)	190/1926 (9.9)	<0.0001 [‡]
<i>Time from symptom onset to diagnosis (days), median (IQR) [No.]</i>		5 (2-9) [8097]	6 (2-9) [6393]	3 (1-7) [1704]	<0.0001*
<i>Length of hospital stay (days), median (IQR) [No.]</i>		9 (5-15) [8894]	9 (5-15) [6970]	9 (5-16) [1924]	0.9320*

All the pre-existing conditions and COVID-19 features were reported as assessed by the clinician.

HCQ: hydroxychloroquine; IQR: Interquartile range; paO₂: partial pressure of O₂

¹Missingness is due to later onset of data collection

²Diagnosis by imaging (Rx and/or CT thorax)

* Wilcoxon test

[‡]Chi-Square test

Table 2. COVID-19 Patients (n=8075) characteristics by treatment group.

	No./total No. (%)		<i>p</i> value
	HCQ n=4542	No-HCQ n=3533	
Demographic characteristics			
<i>Age, years</i>			
16-30	62/4541 (1.4)	72/3530 (2.0)	
31-44	372/4541 (8.2)	175/3530 (5.0)	<0.0001*
45-64	1656/4541 (36.5)	695/3530 (19.7)	
65-79	1395/4541 (30.7)	1015/3530 (28.8)	
>80	1056/4541 (23.3)	1573/3530 (44.6)	
Median (IQR)	66 (54-78)	77 (63-85)	
<i>Male sex</i>	2646/4494 (58.9)	1671/3492 (47.8)	<0.0001 [‡]
Pre-existing conditions			
<i>Cardiovascular disease</i>	1392/4542 (30.7)	1444/3533 (40.9)	<0.0001 [‡]
<i>Arterial hypertension</i>	1757/4542 (38.7)	1513/3533 (42.8)	0.0002 [‡]
<i>Diabetes</i>	998/4542 (22.0)	796/3533 (22.5)	0.5498 [‡]
<i>Chronic renal disease</i>	508/4542 (11.2)	585/3533 (16.6)	<0.0001 [‡]
<i>Chronic liver disease</i>	122/4542 (2.7)	99/3533 (2.8)	0.7511 [‡]
<i>Chronic lung disease</i>	698/4542 (15.4)	517/3533 (14.6)	0.3599 [‡]
<i>Neurological disorders</i>	330/4542 (7.3)	450/3533 (12.7)	<0.0001 [‡]
<i>Cognitive disorders¹</i>	331/4260 (7.8)	582/3266 (17.8)	<0.0001 [‡]
<i>Immunosuppressive conditions</i>	159/4542 (3.5)	78/3533 (2.2)	0.0006 [‡]
<i>Malignancy</i>			
<i>Solid</i>	314/4542 (6.9)	345/3533 (9.8)	<0.0001 [‡]
<i>Hematological</i>	90/4542 (2.0)	70/3533 (2.0)	0.9995 [‡]
<i>Obesity¹</i>	297/2643 (11.2)	186/2284 (8.1)	0.0003 [‡]

<i>Current smoker</i>	183/2390 (7.7)	196/1916 (10.2)	0.0031 [±]
Medications			
<i>Angiotensin converting enzyme inhibitor</i>	669/4541 (14.7)	569/3531 (16.1)	0.0874 [±]
<i>Angiotensin receptor blocker</i>	388/4541 (8.5)	318/3531 (9.0)	0.4665 [±]
Laboratory parameters at admission			
<i>Lactate dehydrogenase (U/L)</i>			
Median (IQR) [No.]	359 (270-497) [3890]	314 (239-442) [2764]	<0.0001 [*]
>350	2036/3890 (52.3)	1146/2764 (41.5)	<0.0001 [±]
<i>C-reactive protein (mg/L)</i>			
Median (IQR) [No.]	68.9 (32.1-125.0) [4461]	50.6 (16.0-105.2)[3340]	<0.0001 [*]
>150	835/4461 (18.7)	471/3340 (14.1)	<0.0001 [±]
<i>paO₂ (mmHg)</i>			
Median (IQR) [No.]	66 (57-75) [3442]	68 (58-80) [1967]	0.0033 [*]
<60	1046/3442 (30.4)	557/1967 (28.3)	0.1084 [±]
Clinical features			
<i>Pneumonia³</i>	4055/4423 (91.7)	2329/3313 (70.3)	<0.0001 [±]
<i>Acute Respiratory Distress Syndrome</i>	720/4306 (16.7)	299/3320 (9.0)	<0.0001 [±]
<i>Invasive ventilation support</i>	503/4407 (11.4)	114/3457 (3.3)	<0.0001 [±]
<i>Admission to intensive care unit in 24h after admission</i>	313/4539 (6.9)	96/3529 (2.7)	<0.0001 [±]
<i>Time from symptom onset to diagnosis (days), median (IQR) [No.]</i>	6 (3-9) [4542]	4 (1-8) [3049]	<0.0001 [*]
<i>Length of hospital stay (days), median (IQR) [No.]</i>	9 (6-15) [3324]	9 (4-17) [3526]	0.2061 [*]
Outcome			
<i>Time from diagnosis to death (days), median (IQR) [No.]</i>	8 (5-13) [4542]	6 (4-12) [3533]	<0.0001 [*]

<i>Death</i>	804/4542 (17.7)	957/3533 (27.1)	<0.0001 [‡]
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All the pre-existing conditions and COVID-19 features were reported as assessed by the clinician.

IQR: Interquartile range; paO₂: partial pressure of O₂

¹Missingness is due to later onset of data collection

²Diagnosis by imaging (Chest X-ray and/or CT-scan)

* Wilcoxon test

[‡]Chi-Square test

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