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Early hydroxychloroquine is associated with an increase of survival in COVID-19 patients: an observational study.

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Summary: Hydroxychloroquine has in vitro activity against SARS-CoV-2. We present an observational study. We analysed 164 patients admitted to our hospital with COVID-19 diagnosis. Hydroxychloroquine treatment was associated with an increase in the mean cumulative survival.

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ABSTRACT

Background: there is no treatment proven effective against COVID-19. Several drugs with in vitro potential against SARS-CoV-2 virus have been proposed. Hydroxychloroquine has in vitro anti-viral and immunomodulatory activity, but there is no current clinical evidence of its effectiveness changing the outcome of the disease.

Methods: We enrolled all 18-85 years old inpatients from Central Defense Hospital "Gómez Ulla", Madrid, Spain, who were hospitalised for COVID-19 and had a definitive outcome (dead or discharged). We used a statistical survival analysis to detect treatment differences associated with in-hospital death.

Results: We analysed first 220 medical records. 166 patients met the inclusion criteria. 48,8 % of patients not treated with HCQ died, 22% of those treated with hydroxychloroquine (p=0,002). According to clinical picture at admission, hydroxychloroquine increased the mean cumulative survival in all groups from 1,4 to 1,8 times. This difference was statistically significant in the mild group.

Conclusions: in a cohort of 166 patients from 18 to 85 years hospitalised with COVID-19, hydroxychloroquine treatment with 800mg added loading dose increased survival when patients were admitted in early stages of the disease. There was a non-statistically significant trend towards survival in all groups, which will have to be clarified in subsequent studies.

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FOOTNOTE PAGE

The corresponding author certifies that:

-No funding sources were used in this study

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-This study has not been presented previously

Yours sincerely,

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INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by SARS-CoV-2, a newly discovered coronavirus in China. Until April 29th, 2020, the disease has spread over 213 countries, with almost 3 million infected people and more than 200.000 deaths (1). Globally considered a mild infection in 80% of cases, moderate and severe cases can lead to death. At present, clinical management includes only supportive care, with supplementary oxygen and mechanical ventilatory support when indicated. Many old and novel therapeutic approaches are being evaluated, since there is no specific treatment proven effective (2). Spain is being strongly hit by COVID-19, with more than 200.000 people infected and more than 24.000 deaths.

At the beginning of the pandemic, clinical guideline in Spain was performed by Ministry of Health supported by several study groups and scientific societies. Several drugs were recommended for treatment (3): lopinavir/ritonavir (4,5,6), remdesivir (4,7) (only for severe cases), interferon alfa (8,9) and beta (10) and tocilizumab (11,12). However, the expansion of epidemic, with increasing number of severe cases and deaths, along with stock problems and lack of definitive clinical evidence resulted in different local hospital guidelines. Each guideline was adapted resting on personal experience and limited reports of cases, while others were based on not proven pathophysiological theories. Glucocorticoids (13), azithromycin (14), and other immunomodulators have been used among some others.

Our institution added to clinical protocol the use of hydroxychloroquine (HCQ) after reviewing recommendations of guidelines by the National Health Commission of the People's Republic of China for treatment of COVID-19(15). Chloroquine (CQ), an aminoquinoline that has been used for malaria prevention and treatment, has shown efficacy in vitro against SARS-CoV(16,17) and its use for COVID-19 has been hypothesized. CQ can affect virus infection in many ways, and the antiviral effect depends in part on the extent to which the virus utilizes

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endosomes for entry. Furthermore, HCQ has been found to be more potent than CQ to inhibit SARS-CoV-2 in vitro (18). However, there is no current clinical evidence of use of CQ/HCQ for treatment of COVID-19 from the published literature till date.

We conducted a systematic search of the literature using MEDLINE (covering January 1st, 2000 to April 3rd, 2020) using the following terms: (COVID-19 OR coronavirus OR sars-cov2) AND (hydroxychloroquine OR chloroquine). We excluded duplicated studies, those articles related to coronavirus animal infections, and related to other diseases. The records have been classified by two independent reviewers (kappa=1). We selected 11 original studies (including 2 clinical trials), 11 reviews (1 systematic, 10 non-systematic) and 21 other publications (letters, case reports, news, consensus documents). There were only 2 original articles including patients with COVID-19 infection: 1 randomized clinical trial (19) including 10 patients (3 severe and 7 moderate cases) treated with CQ 500mg orally twice-daily for 10 days, and 12 patients (5 severe and 7 moderate cases) treated with Lopinavir/Ritonavir 400/100mg orally twice-daily for 10 days. The other article is a non-randomized clinical trial (14) including 26 patients receiving HCQ 200 mg, three times per day during ten days (6 of them with azithromycin) and 16 controls.

During the first days of the pandemic, HCQ was not considered in the local protocol. Afterwards, there were shortages in HCQ distribution. Because of that, we had two populations of patients treated and not treated with HCQ although they had similar characteristics. That gave us the opportunity to investigate the differences observed between the two groups. On the other hand, some patients didn't receive HCQ because of potential side effects (arrythmias, drug interactions) or because of patient's denial to give the consent to the out of label use of the drug.

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While awaiting the results from larger clinical trials, confirming with endpoints related to the definitive outcomes of patients, retrospective analysis from regular clinical practice may be useful. The main purpose of this study was to assess the survival in two different therapeutic regimens: with or without HCQ.

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METHODS

-Study design and participants: observational cohort study. We enrolled all adult inpatients from Central Defense Hospital "Gómez Ulla", Madrid, Spain, who were hospitalised for COVID-19 infection and had a definitive outcome (dead or discharged). COVID-19 diagnosis was defined according to Spanish Ministry of Health definitions on 31st March, 2020, including confirmed cases (PCR positive for any SARS-CoV-2 gene in respiratory samples -oropharyngeal swabs or sputum) and probable cases (bilateral interstitial pneumonia with clinical picture compatible with a COVID-19 diagnosis with no laboratory tests or non-concluding SARS-CoV-2 test). We considered discharge, but analysed separately, the discharge to origin residence or to a "hotel-hospital" (temporary low-care medical installations in Madrid hotels adapted for patients in recovery phase, with no oxygen or intravenous requirements, who could be discharged from acute hospital but who had no possibility to correctly complete the quarantine period at home).

The research protocol was approved by the Ethics Committee on Clinical Investigation of the Ministry of Defense of Spain. The requirement for informed consent was waived by the Ethics Committee. After a preliminary exploratory analysis of the outcomes of the first 220 patients, study was stopped, and the investigators considered mandatory a deep analysis and early publishing of the results due to the relevant differences on survival according to the treatment used.

-Data collection: demographic, clinical, laboratory, treatment and outcome characteristics of the patients were extracted from the electronic medical records by two physicians. In case of differences of interpretation, a third investigator checked the medical records and adjudicated any difference. After collecting the data, database was anonymized removing any reference to the patient's ID before the statistical analysis. Primary investigator and statistic analyser had no access to the medical records or patients ID.

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-Laboratory procedures: Methods for laboratory confirmation of SARS-CoV-2 infection have been described elsewhere (20). SARS-CoV-2 PCR test were performed in Clinical Microbiology Laboratory, Central Defense Hospital, Madrid.

Routine blood examinations included whole blood count, coagulation profile, serum biochemical tests (including renal and liver function, lactate dehydrogenase, and electrolytes). Interleukin-6 (IL-6), serum ferritin, myocardial enzymes and procalcitonin were recovered for several but not all patients. Chest radiographs were also done for all inpatients. Frequency of examinations was determined by the treating physician.

-Definitions: severity of clinical picture at admission was defined according to Ministry of Health of Spain medical treatment protocol, March 19th, 2020: mild (no hypoxemia, no respiratory insufficiency), moderate (hypoxemia and / or moderate respiratory insufficiency), severe (severe hypoxemia, severe respiratory distress, poor overall status). Hypertension was defined as previous arterial hypertension requiring any pharmacological treatment. Diabetes Mellitus was defined as previous hyperglycaemia requiring any pharmacological hypoglycaemic treatment. Dyslipidaemia was defined as previous alteration in lipid profile requiring pharmacological treatment. Cardiomyopathy was defined as any previous diagnosis of cardiac chronic disease or acute cardiac event. Respiratory disease was defined as any previous lower respiratory tract chronic disease requiring chronic pharmacological treatment. Cancer was defined as any previously diagnosed malignancy. Dementia was defined as any mental chronic disease altering cognitive capabilities. Lymphopenia was defined as less than 1000 lymphocites/ml. High LDH values were defined as those higher than 400 U/l. High CRP values were defined as those greater than 14 mg/dL. High D-dymer values were defined as those greater than 1000 ng/ml.

- HCQ/CQ selection and dose: Hospital treatment protocol selected hydroxychloroquine over chloroquine considering the best *in vitro* activity profile (EC50=0.72 µM vs. EC50=5.47 µM)

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(18). Despite Yao et al. suggest in their manuscript a loading dose of 400 mg twice daily of hydroxychloroquine (18), we have finally considered a loading dose of 800 mg + 400 mg, followed by a maintenance dose of 400 mg a day based on: 1) the hypothetical benefit of reaching the steady state and the EC90 as soon as possible in the case of this acute viral infection, 2) this is the schedule accepted by the regulatory agency in the attacks of acute malaria treatment, and therefore a safe and well-known doses by the physicians. Nevertheless, a few elder patients didn't receive the extra loading dose of 800mg to avoid said effects and drug interactions.

-Other treatments: patients in both groups were treated with other specific drugs with potential activity against SARS-CoV-2 and/or COVID-19 immune disorders leading to ARDS. These drugs could include antivirals (lopinavir/ritonavir), immunomodulators (interferon beta), and/or anti-inflammatory drugs (steroids and/or tocilizumab)

-Statistical analysis: The quantitative variables were described with the arithmetic mean with its standard deviation and the median with its interquartile range. We used the absolute and relative frequency (%) for qualitative variables. The hypothesis tests used were the Chi2 Pearson or Fisher's exact test, the Student's t test or the Mann Whitney test, the one-way ANOVA, with the Bonferroni test for multiple comparisons, and Kruskal Wallis test. The survival study was performed with the Kaplan Meier test and the comparison of factors with the Mantel Cox Log Rank test. A $p < 0.05$ was considered statistically significant. Logistic regression for multivariant analysis was performed with $p<0,250$ level as a screening criterion for selection of candidate variables. Statistical analysis were done using the software package SPSS Windows (version 25)

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FINDINGS

We studied the first 220 discharge or death medical reports on COVID-19 wards, Central Defense Hospital "Gómez Ulla". We excluded 22 patients who were admitted to a COVID-19 ward but finally didn't have a confirmed clinical or microbiological diagnosis of COVID-19, and 32 patients older than 85 years (in whom the Hospital's COVID-19 treatment protocol decided not to use off-label treatments in the first weeks of the epidemic after evaluating the risk-benefit balance) and 1 patient younger than 18 years (not attended by COVID-19 team). We finally included 166 patients in the study (figure 1). 83 patients had mild clinical picture at admission, 48 moderate and 35 severe. 118 Patients survived (90 were discharged and 29 to "hotel-hospital") and 48 dead.

123 patients were treated with HCQ and 43 patients didn't receive HCQ. 48,8 % of patients not treated with HCQ died and 22% of those treated with HCQ (p=0,002). Age distribution according to the severity at admission was homogeneous between HCQ and non-HCQ treatment groups when the clinical picture was mild (57,6 years HCQ – 58,4 years non-HCQ, p=0,865) or moderate (63,8 years HCQ – 70 years non-HCQ, p=0,269). Patients with severe clinical picture at admission treated with HCQ were younger than those who were not treated (70,4 years HCQ -78,3 years non-HCQ, p=0,036) (table 1).

Comorbidities (table 2) were similar in both groups, except for cardiopathy (p=0,05) and dementia (p=0,022). Differences in treatment according with cardiopathy were expected because of the side effects of CQ/HCQ (prolongation of QT interval). Analytical data at admission had similar values on both groups (table 2).

Mean hospital stay was 6 (5) days in the HCQ group and 5(7) days in the non-HCQ group (no significant difference, p=0,25).

Median (IQR) from symptoms begin to the start of treatment with HCQ: 7(6) days. Mode was 7 days after symptoms onset. The median Md (IQR) of time elapsed from onset of symptoms

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to start of treatment were for the mild group of 7 (7.25) days, for the moderate group of 7 (6.8) days and for the severe of 5 (4) these differences being statistically significant (p = 0.01). Nervertheless, median (IQR) from hospital admission to the start of treatment with HCQ was 1(1) days, with no differences according to clinical picture at admission (p=0,223).

There was a clear increase in the accumulated survival rates at the 3 levels of clinical picture at admission favourable to the group treated with HCQ (table 3). In the mild group: their average cumulative survival is increased 1.8 times in those treated. In the moderate group the cumulative median survival was 1.4 times higher in those treated. In the severe group the cumulative median survival was 1.6 times greater in the those treated.

In the mild group, the mean cumulative survival was 14.4 days (95% CI: 13.7-15.2 days) in those treated with HCQ and 8.2 days (95% CI: 6.5-9.9 days) in the untreated, being this difference of 6.2 days statistically significant (p = 0.032).

In the moderate group, a trend towards longer survival was observed, but without reaching statistical significance. The accumulated survival average was 10.9 days (95% CI: 9.3-12.5 days) in those treated with HCQ and 7.7 days (95% CI: 4.4-10.9 days). In the untreated, this difference of 3.1 days was not statistically significant (p = 0.205).

In the severe group, the mean cumulative survival was 6 days (95% CI: 3.3-8.5 days) in those treated with HCQ and 4 days (95% CI: 1.7-6.1 days) in those not treated. This difference of 2 days was not statistically significant (p = 0.297).

A multi-variant analysis or survival was performed, including comorbidities and analytical values at admission (table 4). The analysis excluded confusion bias on the increased survival in HCQ group: HCQ treatment was an independent predictor of lower mortality (p=0,003, 95% CI 0,012 – 0,402). Other independent predictors of survival were two comorbidities (cardiopathy, p=0,010, 95% CI 0,053-0,672, and dementia, p=0,013, 95% CI 0,002

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to 0,489), and two analytical conditions at admission: lymphopenia (p=0,026, 95% CI for lymphocites higher than 1.000/ml: 1,212-19,686).

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INTERPRETATION

From our known this is the first study showing increase of survival in a significant number of patients with a specific drug treatment for COVID-19. There was a whole increased survival from 1,4 to 1,8 times to survival in those treated with HCQ. The cohort of patients with mild clinic at admission had clearly better outcomes when treated with HCQ.

These results were obtained in patients treated early (first week since symptoms onset, first day after admission). We theorise that the antiviral effect is only effective in early stages of the disease, before the immunomodulated ADRS development (21). This could explain the better outcomes with HCQ in mild patients, treated before the stablishment of ADRS . Similarly, the different outcomes with other drugs with potential activity against SARS-CoV-2 as lopinavir/ritonavir which failed to show effect on mortality reduction with a mean of 14 days since symptoms onset (22). Anyway, our findings in an easily available, low-cost drug with few side effects makes HCQ/CQ a good choice to start clinical trials in hospitalised patients, and to consider an out-of-label most extended use in early stages of the disease (taking care of all the legal and ethical considerations about this use).

Subgroups of patients in moderate and severe condition at admission showed a tendence to survival, but with no statistically significant differences in their outcomes. Probably, due to the physiopathology of COVID-19 (21), once the patient starts the “cytokine storm” phase, antiviral effect it’s less or not useful and immunomodulation of HCQ it’s not enough powerful to cut the progression of ADRS. Anyway, this could be cleared with a higher sample size, which we’ll continue studying for further reports.

There were significant differences in age mean in the HCQ / non-HCQ groups with severe disease at admission. This could be explained due to the higher comorbidities of elder patients with severe condition at admission, what may have led clinicians to decide limitation of therapeutic effort in some of them. Anyway, this should be studied deeper in further studies.

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Dementia patients were less treated with HCQ. This could be explained because of potential drug interactions, that would have made their clinicians decide not to treat with off-label, potentially dangerous drugs, and/or because of the difficulty on administering oral drugs to those patients, some of them with swallowing problems, while not allowed to be accompanied by their relatives due to isolation. This is a data that should be clarified in further studies, because dementia was an independent predictor of mortality in the multi-variant analysis.

Analytical data showed a numeric, non-statistically significant, difference in D-dymer levels between the two groups: 558(716) HCQ group vs 1511(4570,5) non-HCQ group. During the first weeks of the pandemic D-dymer analysis was not performed to all patients at admission, so that 120 (46,9%) patients didn't have this value. This makes difficult to interpreter this data. Anyway, D-dymer levels at admission didn't show as independent predictor of mortality in the multi-variant analysis.

A non-expected data was found when we analyzed the days from symptoms onset to hospital admission. The mean days were lower in those admitted in severe condition. That could mean there are subgroups with a higher risk of quick development of ADRS. Identifying their characteristics would be a key point to understand why COVID-19 course is mild in most patients but severe, devastating and life-threatening in other ones, with still no detected differences explaining the different clinical courses of the disease.

Conclusion: in a cohort of 166 patients from 18 to 85 years hospitalised with COVID-19, hydroxychloroquine treatment with an 800mg added loading dose increased the cumulative mean survival according to the diagnosis of severity at the beginning by 1.4 to 1.8 times. This difference was statistically significant when the clinical picture at admission was mild.

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BIBLIOGRAPHY

1.Coronavirus disease 2019 [Internet]. [cited 2020 Apr 29]. Available from:https://www.who.int/emergencies/diseases/novel-coronavirus-2019

2. Search of: COVID-19 - List Results - ClinicalTrials.gov [Internet]. [cited 2020 Apr 1].Available from: https://clinicaltrials.gov/ct2/results?cond=COVID-19&term=&cntry=&state=&city=&dist=

3. Chen F, Chan KH, Jiang Y, et al. In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. J Clin Virol. 2004;31:69-75. https://doi.org/10.1016/j.jcv.2004.03.003

4. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020;11:222.https://doi.org/10.1038/s41467-019-13940-6

5. Chu CM. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax. 2004;59:252-256.https://doi.org/10.1136/thorax.2003.012658

6. Kuri T, Zhang X, Habjan M, et al. Interferon priming enables cells to partially overturn the SARS coronavirus-induced block in innate immune activation. J Gen Virol. 2009;90:2686-2694. https://doi.org/10.1099/vir.0.013599-0

7. Kim UJ, Won EJ, Kee SJ, Jung SI, Jang HC. Combination therapy with lopinavir/ritonavir, ribavirin and interferon-alpha for Middle East respiratory syndrome. Antivir Ther. 2016;21:455-459.https://doi.org/10.3851/IMP3002

8. Agostini ML, Andres EL, Sims AC, et al. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. mBio. 2018;9(2):e00221-18.https://doi.org/10.1128/mBio.00221-18

9. Turner RB, Felton A, Kosak K, Kelsey DK, Meschievitz CK. Prevention of experimental coronavirus colds with intranasal alpha-2b interferon. J Infect Dis. 1986;154:443-44 7. https://doi.org/10.1093/infdis/154.3.443

10. Morgenstern B, Michaelis M, Baer PC, Doerr HW, Cinatl J Jr. Ribavirin and interferon-beta synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. Biochem Biophys Res Commun. 2005;326:905-908.https://doi.org/10.1016/j.bbrc.2004.11.128

11. Zhang X, Song K, Tong F, Fei M, Guo H, Lu Z, Wang J, Zheng C. First case of COVID-19 in a patient with multiple myeloma successfully treated with tocilizumab. Blood Adv. 2020 Apr 14;4(7):1307-1310. doi: 10.1182/bloodadvances.2020001907.

12. Bersanelli M. Controversies about COVID-19 and anticancer treatment with immune checkpoint inhibitors. Immunotherapy. 2020 Mar 26. doi: 10.2217/imt-2020-0067.

13. Loutfy MR, Blatt LM, Siminovitch KA, et al. Interferon alfacon- 1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA. 2003;290:3222- 3228. https://doi.org/10.1001/jama.290.2 4.3222

14. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020 Mar 20:105949. doi: 10.1016/j.ijantimicag.2020.105949.

15. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends [Internet]. 2020;1–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32074550

16. 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. Virol J[Internet]. 2005 Aug 22 [cited 2020 Apr 2];2:69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16115318

17. Keyaerts E, Vijgen L, Maes P, Neyts J, Ranst M Van. In vitro inhibition of severe acuterespiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun. 2004 Oct 8;323(1):264–8.

18. 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; pii: ciaa237. https://doi.org/10.1093/cid/ciaa237

19. Huang M, Tang T, Pang P, Li M, Ma R, Lu J, Shu J et al. Treating COVID-19 with Chloroquine. J Mol Cell Biol. 2020 Apr 1. pii: mjaa014. doi: 10.1093/jmcb/mjaa014.

20. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020 Mar 11: e203786. Published online 2020 Mar 11.

21. Thevarajan, I., Nguyen, T.H.O., Koutsakos, M. et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nat Med (2020). https://doi.org/10.1038/s41591-020-0819-2

22. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020 Mar 18. doi: 10.1056/NEJMoa2001282.

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***Table 1**. General characteristics of the patients in both treatment groups (with and without HCQ)*

		HCQ Yes n=123	 No n=43	p
Age (years) $\bar{x}(DE)$	Clinical picture at admission	61,5(16,2)	68,7(18,8)	0,012*
	Mild	57,6(15,7)	58,4(17,8)	0,865*
Age (years) $\bar{x}(DE)$	Moderate	63,8(16,5)	70(13,2)	0,269*
	Severe	70,4(13,4)	78,3(7,2)	0,036*
Sex n(%)	Male	76(61,8)	27(62,8)	0,907**
	Female	47(38,2)	16(37,2)	
	Death	27(22)	21(48,8)	
Final outcome n(%)	"Hospital Hotel"	26(21,1)	3(7)	0,002**
	Home	70(56,9)	19(44,2)	

*t Student; **χ2 Pearson

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***Table 2**. Clinical Comorbidities and Analytical Parameters of Patients on Admission in Both Treatment Groups.*

		HCQ Yes n=123 n(%)	No n=43 n(%)	p
Hypertension	Yes	49(69)	22(31)	0,214*
	No	74(77,9)	21(21,1)	
Diabetes	Yes	19(65,5)	10(34,5)	0,246*
	No	104(75,9)	33(24,1)	
Dyslipidaemia	Yes	39(68,4)	18(31,6)	0,190*
	No	84(77,8)	24(22,2)	
Cardiopathy	Yes	23(62,2)	14(37,8)	0,050*
	No	100(78,1)	28(21,9)	
Cancer	Yes	15(65,2)	8(34,8)	0,268*
	No	108(76,1)	34(23,9)	
Dementia	Yes	6(46,2)	7(53,8)	0,022**
	No	117(77)	35(23)	
Pulmonary disease	Yes	15(62,5)	9(37,5)	0,143*
	No	108(76,6)	33(23,4)	
Leukocytes µl		6320(3395)	8004(4530)	0,696***
Lymphocytes µl		1160(545)	860(730)	0,997***
LDH U/l		328(141)	296(216)	0,971***
GOT U/l		36(24)	28,5(37)	0,923***
PCR mg/dl		6,2(10,7)	12,1(16,8)	0,845***
PCT ng/ml		0,13(0,29)	0,59(4,38)	0,730***
Ferritin ng/ml		375(827)	321,5(1158)	0,144***
D dimer ng/ml		558(716)	1511(4570,5)	0,168***

**Chi2 Pearson; **Exact Fisher test; ***Mann Whitney test*

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***Table 3**. Increase of survival with HCQ according to clinical picture at admission (days)*

	Total	HCQ group	Non-HCQ group
Mild	14(IC95%:13-14,8)	14,4(IC95%:13,7-15,2)	8,2(IC95%:6,5-9,9)
Moderate	10,3(IC95%: 8,7-11,9)	10,9(IC95%:9,3-12,5)	7,7(IC95%:4,4-11)
Severe	5,2(IC95%:3,4-7,1)	5,9(IC95%:3,3-8,5)	3,9(IC95%:1,7-6,1)

*Log Rank (mantel Cox)

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***Table 4**. Significant outcomes of the multi-variant analysis of survival*

		B	Standard error	Wald	gl	Sig.	Exp(B)	95% C.I. for EXP(B) Lower	Higher
	Cardiopathy	-1,671	,650	6,609	1	,010	,188	,053	,672
	Dementia	-3,426	1,384	6,132	1	,013	,033	,002	,489
	Lymphopenia	1,586	,711	4,973	1	,026	4,884	1,212	19,686
	High RCP values	-1,413	,645	4,803	1	,028	,243	,069	,861
	HCQ treatment	-2,654	,889	8,914	1	,003	,070	,012	,402

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Figure 1. Patient inclusion flow chart

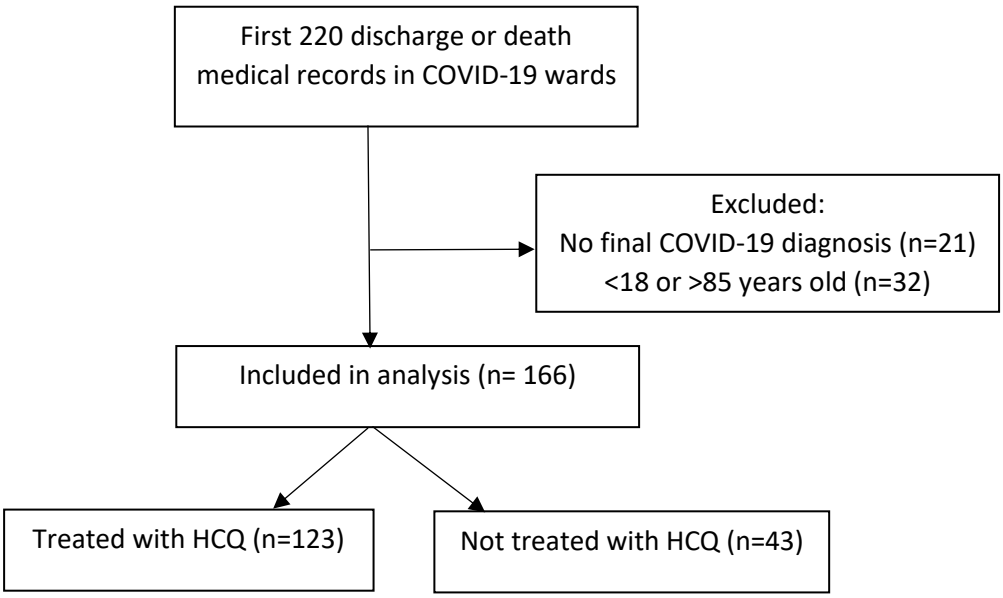

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Figure 2. Age distribution, according to severity at admission

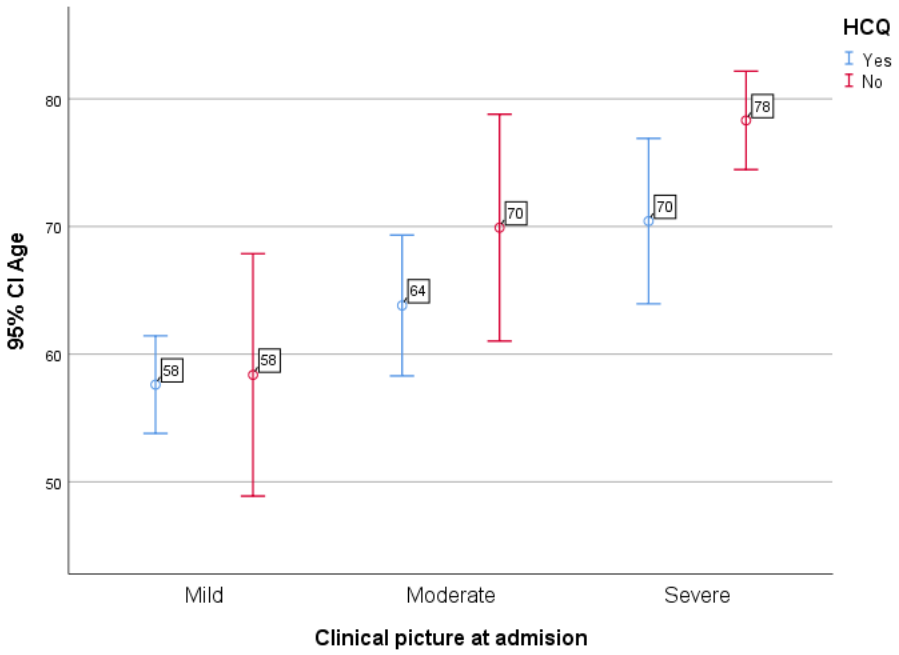

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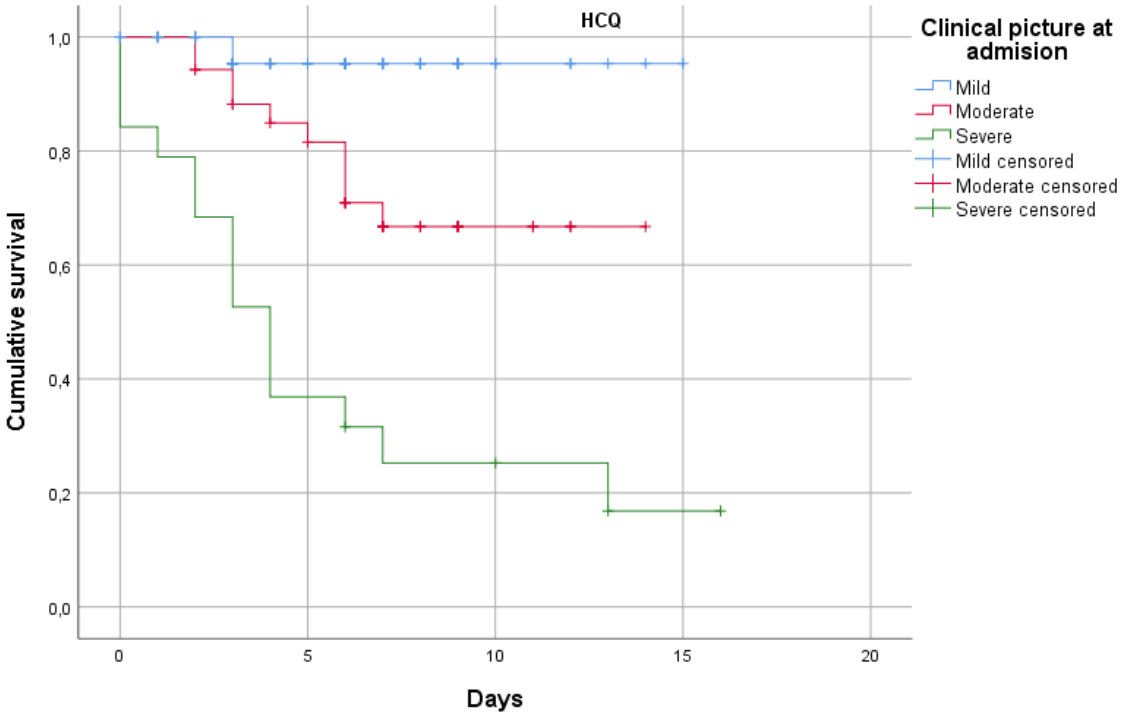
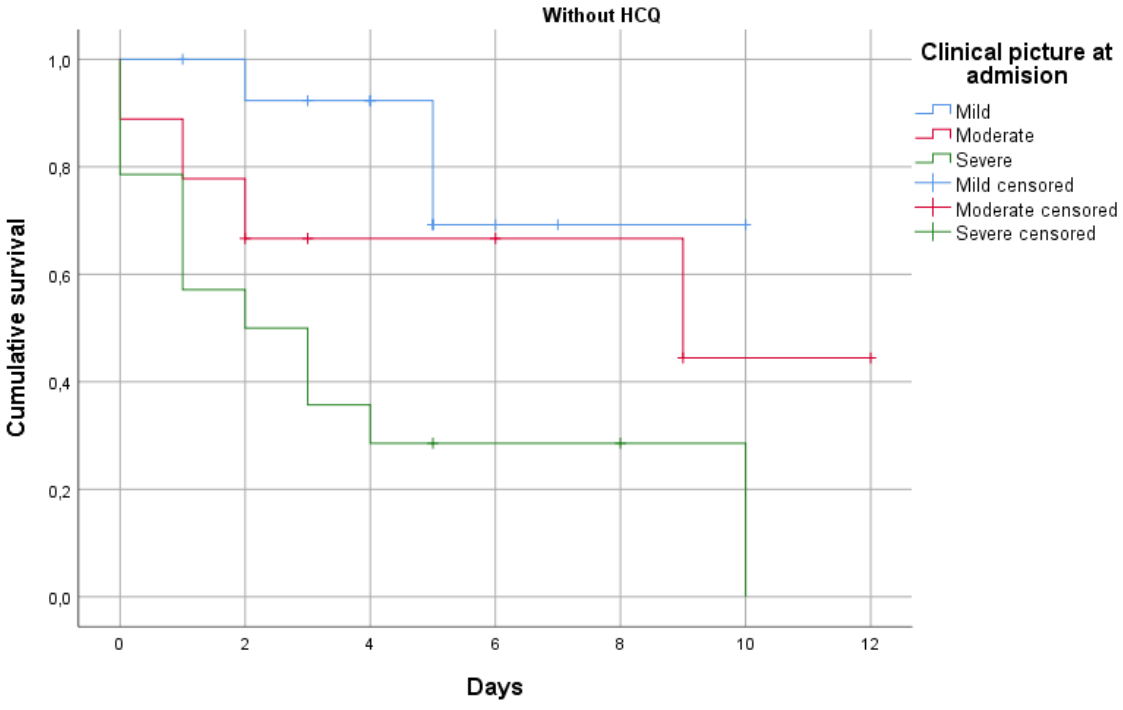
Figure 3. Kaplan-Meier Survival functions with/without HCQ

With HCQ



Without HCQ



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ANNEX 1. MEMBERS OF THE COVID 19 CENTRAL DEFENSE HOSPITAL "GÓMEZ ULLA" TEAM

Allergy Department:

Regina María Paulaskas Vasati, Enrique Gabriel Gómez González.

Anaesthesiology Department:

Pelet Pascual, Elvira. García Aroca, Miguel Ángel. Cantalejo Pérez, Francisco. Gago Sánchez, Alberto José. Rodríguez Martín, Ana. Rodríguez Sánchez, Dolores. Tejeda Fernández, Jose Luis. Santos, Elisabeth. Almagro Vidal, Inés. Álvarez Fdez., Lucía. Arranz Pérez, Rodrigo. Cabrera Serrano, Gemma. Fernández Peña, Alberto. González Del Pozo, Irene. Martín Oropesa, Raquel. Monteserín Matesanz, Cristina. Navarro Echevarría, Patricia. Olivera Moreno, Daniel. Vullo, Paula Agostina.

Cardiology Department:

Salvador Álvarez Antón, David Martí, Concepción Fernández Pascual, María José Morales, Andrea Rueda Linares, Carmen de Juan Bitria, Alexander Félix Marschall, Fredy Andrés Delgado Calva, Maria Belen Biscotti Rodil, Ricardo Concepción Suárez, Dámaris Caballeira Puentes.

CBRN and Infectious Diseases Unit

Lucía Elena Ballester Orcal, Francisco Javier Membrillo de Novales, Yolanda Martínez Martínez, Antonio Fe Marqués.

Clinical Microbiology Department:

María Mateo Maestre, María del Carmen Ybarra de Villavicencio, María Simón Sacristán, Maria Isabel Zamora Cintas, Almudena Rodriguez Aranda, Amelia Montserrat Carmona de Cózar, Maria Encarnación Mérida Arias, Jose Luis Martin Prieto.

Dermatology Department

Leire Sanchez Los Arcos, Cristina Collantes Rodríguez.

Digestive Medicine Department:

Asunción Ramos Meca, Elena Portales, Marian Ángeles García Mayor, Inmaculada Pérez Amarilla, Mar Rodríguez, Enrique de la Fuente, Gema Arranz, María Jesús Callejo, Natalia Zuberoa Rosado Dawid, Sandra María Caro López, Ana Isabel Sáez Sáez, María Domínguez Rodríguez.

Emergency Department:

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Jaime Rossiñol Ruiz, David Coca Benito, M. Luz Cano Izquierdo, M. Lourdes Rojas Bueno, M. Carmen Reche Caballero, Claudio Escobar Bargueño, Silvia Jiménez Zamora, Alfonso López Chollet, Marta Del Nido Alonso, Margarita Del Moral González, Miguel Muro Fernández, Marta Martín Vallejo, Elena Planchuelo Medina, Gabriel González Salazar, Fátima Ibáñez Estéllez, Rolando Sordo Díaz, Noelia Arroyo Pardo, Enrique Portela Filgueira, Ana María Martínez Molina, Beatriz Rato Barrio, Ignazio Taronna Latorre, Jessica D. Peña Vásquez, M. Eugenia Zornoza Pérez, María José Noguera Marín, Capitán Antonio Eloy Seva Delgado, Teniente Darlin M. Guzmán Rosario, Teniente Fabián Manjarrés Henríquez, Teniente Alvaro Rodríguez Rodríguez, Ana Betegón Sanz, Miguel Almazor Iribarren, M. Asunción Sánchez Gil, Juan Carlos Sánchez Sánchez-Gil, Dionisio Alastuey Martínez, Gonzalo Infante Pino, Eduardo De Vicente Cano, Estefanía Ruiz Alcaide, Andrea Matas Escamilla

Endocrinology Service:

Elena Mendoza, Teresa de Grado, Carmen Gil.

General and Digestive Surgery Department:

Oscar Marqueta García, Mariano Javaloyes Rodrigo, Miguel Ángel Sierra Ortega, Tcol Maria Isabel Sanchez-Seco Peña, Ignacio García Marirrodriga, Francisco Sanchez del Valle, Jose Antonio Sáez Montoro, Fernando Fernández Bueno, Yusef Mohamed Al Lal, Cristina López Muñoz, Patricia Tejedor Togores, Guillermo Fernández Díaz, Silvia Maestro Prieto. Luis de Nicolás Navas, Juan José Perez Alegre, Pablo Hernández Sanz.

Intensive Care Department:

Jorge Medina Segovia, Paloma Sanchez Mata, Rosario Fernandez Suero, Felix Maimir Jané, Luis Vicente Saenz Casco, Pilar Borrego Jimenez, Francisco Gijón Gallego, Esperanza Molero Silvero, Cesar Eugenio Gaona Coscia, Javier Sainz Cabrajas.

Infectious Diseases Unit

Germán Ramírez-Olivencia, Miriam Estébanez Muñoz, Begoña de Dios García, María Dolores Herrero Mendoza, Tatiana Mata Forte.

Internal Medicine Department:

María Jesús Sánchez Carrillo, María Navarro Téllez, Belén Esteban Lazareno, Raúl Ruiz Esteban, Javier Rodeles Melero, José María Rodríguez Fernández, María Eugenia Segovia, Elsa Labrada, Ana López Aparicio, Alejandro Estrada, Emma de Pablo, Álvaro Conesa, Ainhoa Gutiérrez, Irene Ruiz, Ana Roel, Xavier Álvarez Granda, Luisa Jimenez Reyes, Laura Checa, Lidia Romero, Paloma Lucena Calvet, Pedro Priego de Montiano, Francisco de Asís Fernández Riestra, Maria Antonia Menendez, Carmen González, Jose Ramón Toral Revuelta, Alba Ibáñez Botella.

Neurology Department:

Manuel Domínguez Salgado, Francisco Valenzuela, María del Rosario Antón Abarca.

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Oncology Department:

Carmen Arlanzón.

Paediatrics Department:

Carlota García, Noelia Valero Flores, Andrés Fernández Flores, María García Baró, Paula Polanco Zea, María José Hernández, Helena Viana Llamas.

Pharmacology Department:

María Henar Gonzalo Salado, Francisco Javier Sanchez Jimenez, Francisco José López Honduvilla, Paloma Sánchez López, Pilar Prats Oliván, María Jesús Méndez Fernandez, Laura Pedraza Nieto, Ana Acuña Vega, Andrea Correa Pérez, Paula Granda Lobato.

Pneumology Department:

Francisco Ramón Villegas Fernández, Andrés Rodero Baño, Gabriel J Caballero Rodriguez, Begoña de Juan Rodrigo, Sergio Campos Tellez, María Jesús Chillón, Alberto González Estebanez, Jose Javier Jareño Esteban, Carolin Wagner Struwing, María Castro Otero, Ana Ochoa Ruiz, Salvador de la Torre Carazo, Ángela Hidalgo Herranz, Marta Perez Gallan, Diogenes José Alfonzo Martinez, Soledad Torres Tienza, Silvia Sans Perez, Cristina Yanlli Bonduki, Juan de Mesa, Carmen Lorenzo.

Preventive Medicine Department:

María Vicenta García Rosado, Ana Isabel López Figueras, Pilar Segura Cebollada, María Teresa Ledo Varela.

Psychiatry Department:

Marta Presa García, Victoria Juarez Calvo, Catalina Iglesias García, Cristina Rodriguez Villarino, Daniel Fernández Faber, Maria Plaza Yuste, Celia María Hernández Caro, Jose David Cozar Ortiz, Coral Esperanza Torrente, Cristina Rodriguez Delgado.

Rehabilitation Department:

Carlos Mora Jordá, Rebeca Maruenda Fernández, Ana Tovar Cifuentes, Guillermo Fernández García Ruiz-Calero, María De los Ángeles Rodríguez Gamero, Cristina Novo Navarro, Maria Victoria Lorenzo Suberviola.

Rheumatology Department:

María Ahijón, Raul Veiga.

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Traumatology Department

Javier Areta Jiménez, Jose Luis Bernacer López, Roberto Trapote Sanmartín, Jose Luis Sopesen Veramendi, Marcos Fernandez Gayol, Jose Adolfo Orellana Gomez-Rico, Francisco González Prieto, Ana Arrollo Perez, Montserrat Martinez Roldan, Diana Crego Vita, Carlos Rodríguez Moro, Arturo Muñoz Ruiz, Rafael García Cañas, Raquel Vallez Romero, Ricardo Vethencourt Koifman, Gonzalo Hernandez Fernandez, Ricardo Baños Turza, Irene Portellano Pascual, Nelson Lasluisa Molina, Monica Huecas Martinez, Alberto Granado Llamas, Azucena Martín Herreros, Alfonso Rodriguez Mejías, Serafín Mihanda Eliquya, Felipe Velasco Vaquero, María Prieto Vazquez.