



Early Treatment with Hydroxychloroquine Improves Clinical Outcomes and Reduces Hospital Admissions in COVID-19 Associated Pneumonia

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This work was carried out in collaboration among all authors. Author AD'A conceptualization, investigation, formal analysis, writing. Author MC conceptualization, investigation data curation and manuscript revision. Author CL investigation, data curation. Author MU investigation, data curation, manuscript revision. Author FB investigation, statistical analysis. Author GC investigation. Author DDM investigation. Author AF investigation. Author CG investigation. Author FM investigation, formal analysis, manuscript revision. Author MM investigation. Author SO investigation. Author NS investigation. Author MT investigation. Author PDP conceptualization, statistical analysis, formal analysis, writing the original draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Hydroxychloroquine (HCQ) has been used against SARS-CoV-2, but large non randomized studies failed to show any clinical benefit. However, in these studies the drug was started in the hospital setting, a significant time after the onset of symptoms.

Aim of the Study: to verify if HCQ, given early after the onset of symptoms and in the outpatient setting, can reduce hospital admissions and improve clinical outcomes of COVID-19

Methods: We performed a retrospective study on 824 patients with COVID-19 pneumonia in the Bergamo province (Lombardy, Italy). Patients were divided in 2 cohorts: the first included 354 outpatients attended by Primary Care Physicians (PCP), the second included 470 patients admitted to an Emergency Department (ED) during the same period. We compared rate of hospital admission and clinical outcomes in patients treated with HCQ within 6 days from the onset of symptoms, with all other patients. Clinical outcomes were evaluated at a 1-month time-point.

Results: Patients who received early treatment with HCQ showed a lower rate of hospital admissions ($p<0.0001$), mechanical ventilation ($p<0.0022$) and need for oxygen supplementation at home ($p=0.002$) in the first cohort and improved survival in the second ($p=0.03$). Early treatment with HCQ was the only independent variable influencing all predefined outcomes on multivariate analysis.

Conclusions: Treatment with HCQ, initiated within 6 days from the onset of COVID-19 may improve both clinical outcome and rate of hospital admissions.

Keywords: SARS-CoV-2; COVID-19; hydroxychloroquine.

ABBREVIATIONS

PCP : (Primary Care Physicians)
ICU : (Intensive Care Unit), ED (Emergency Department)
NIV : (Non Invasive Ventilation)
CRP : (C-Reactive Protein)
LMWH : (Low Molecular Weight Heparin)

1. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in December 2019 in the province of Hubei (China), and spread rapidly across the world causing a high death toll and disruption of social life. Up to now nearly 111 million cases have been diagnosed in the world, with a global death rate of 2.4 million people [1]. Although the majority of patients have mild or moderate disease, up to 5-10% develop a severe, potentially life threatening course, so that effective antiviral drugs are urgently needed. Several drugs, including remdesivir, favipiravir, beta-interferon, ribavirin, lopinavir-ritonavir and chloroquine or hydroxychloroquine, have been tested in clinical trials on the basis of promising in vitro results and preliminary clinical studies [2-5]. However, none of them, with the exception of remdesivir, has yet been incorporated into clinical practice. Remdesivir itself, which has been endorsed by the FDA on the basis of favorable results in 2 studies [6,7], can only be administered by intravenous route, which makes

its utilization unfeasible in the outpatient setting. Furthermore, in another randomized Chinese study, this drug failed to show any clinical benefit or reduce mortality in patients with COVID-19 pneumonia [8].

Chloroquine and hydroxychloroquine (HCQ) have received great attention after in vitro studies reporting interference with SARS-CoV-2 receptor binding and reduction of ensuing infection [9]. Subsequent non randomized clinical studies showed that these drugs were associated with reduced progression of the disease, decreased duration of symptoms [10] and accelerated viral clearance from nasopharyngeal swabs [11]. HCQ is a safer and more potent drug than Chloroquine in terms of in vitro SARS-CoV-2 suppression [9], acting via an increased endosomal pH and reducing SARS-CoV-2 endocytic host cell entry into the lung epithelium.. It was also suggested that the addition of azythromicin could potentiate the beneficial effect observed with HCQ [11-13]. However, large observational studies failed to confirm these encouraging preliminary data and in addition raised important safety concerns about the risk of QTc prolongation and dangerous cardiac arrhythmias [14-17]. Despite the impressive number of patients included in these observational studies, the potential activity of chloroquine and its analogues in COVID-19 is still an open issue [17-21]. Remarkably, all patients enrolled in studies highlighting the inefficacy of HCQ had received the first dose of

the drugs in the hospital setting, a significant amount of time after the onset of symptoms. Giving HCQ and antiviral drugs in the early phase, before the virus has triggered lung damage, would be crucial since the efficacy of these drugs is greater when promptly started [22].

SARS-CoV2 infection is a biphasic illness. In the first phase, the virus infects the respiratory cells by binding to angiotensin-converting enzyme 2 (ACE-2) receptors and causes a flu-like syndrome, which in many patients is mild or asymptomatic. In a minority of patients, however, the virus triggers a second phase, characterized by a dysregulated cytokine storm which leads to extensive interstitial pneumonia, acute respiratory distress (ARDS) and death. The use of antivirals in this second phase would be less effective because the virus has already triggered the cytokine storm. Therefore we decided to undertake a retrospective study on patients treated with hydroxychloroquine shortly after the onset of symptoms and before hospital admission to establish if the drug was effective in reducing hospitalization and improving clinical outcomes.

2. METHODS

2.1 Epidemiological Context

From February 2020 through April 2020 the COVID-19 pandemic has ravaged across the Lombardy Region of Northern Italy, causing 87.000 documented cases of infection and 14.500 deaths. The death toll was probably higher because the health system was overwhelmed and many patients died at home without being tested.

At the beginning of the pandemic the Italian Society of Infectious Disease (SIMIT) issued guidelines recommending early treatment with HCQ to all COVID-19 patients with documented pneumonia or with mild symptoms, older than 70 years of age and with comorbidities [23]. Outpatient treatment by Primary Care Physicians (PCP) was encouraged by local health authorities in order to relieve the overburdened hospital facilities. Due to the diffusion of the disease, the lack of protective personal equipment and the urgency of the situation, PCP were allowed to order imaging studies and prescribe drugs by remote. HCQ treatment was initiated no later than 6 days from the onset of symptoms in the majority of cases. The recommended dosage by the local Health

Authority and followed by all PCP was 400 mg b.i.d. orally on the first day followed by 200 mg b.i.d. for 10 days.

2.2 Patients and Study Design

The design of the study is represented in Fig. 1. We retrospectively studied two different populations from the province of Bergamo, one of the areas of Lombardy most heavily affected [24]. The first population consisted of a cohort of patients with COVID-19 pneumonia included in a database of 35 PCP from two districts of the North-Eastern part of the province. The data base was elaborated by a pulmonologist (MC) who coordinated the PCP group. The study was conducted according to STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology) for cohort studies, according to the Declaration of Helsinki principles and was approved by the Internal Board for the evaluation of Scientific Studies of Istituti Ospedalieri Bergamaschi- Gruppo San Donato University and Research Hospitals, which in our hospital fulfils the role of a Research Ethical Committee.

Study participants were recruited from the adult general population (≥ 18 years old), among the residents of the Bergamo province and followed-up from March 2, 2020 until April 20, 2020, The electronic medical records of the recruited outpatients were accessed by the respective providers and data were manually abstracted. Patient's privacy was protected by assigning an anonymous identification code, and the electronic data were stored in a locked, password-protected computer. The diagnosis of COVID-19 pneumonia was established in all cases by Chest X-ray or CT scan in addition to respiratory symptoms. All consecutive, incident cases of COVID-19 pneumonia were collected during the study period. A nasopharyngeal swab polymerase test result was not strictly required for the diagnosis, according to The Regional Health Authority policy during the first phase of the pandemic. Patients with at least 2 of the 4 following symptoms: fever $\geq 37.5^{\circ}\text{C}$, cough, chest pain, and dyspnea and radiographically documented pneumonia, were included in the study. Demographic, clinical data, type of treatment and outcomes were collected by PCP through telephone calls or by means of hospital discharge letters.

Patients were divided into two groups: a) group A included all patients treated with HCQ by PCP

within 6 days from onset of symptoms, b) group B included all untreated patients and those who had received HCQ later than 6 days from the onset of symptoms. The choice of starting the drug was left to PCP and depended on clinical judgment, presence of comorbidities, social and psychological conditions of the patient, availability of HCQ in the area, difficulty in ECG monitoring and possible interactions with other drugs.

The second cohort consisted of all consecutive patients who presented to the Emergency Department (ED) of Policlinico San Marco, Zingonia (Bergamo), a 310- bed hospital located in the Southern part of the Province and at that time totally transformed into COVID hospital, from March 2, 2020 until April 20, 2020. All patients underwent a CT scan and tested positive for SARS-CoV-2 polymerase chain reaction from a nasopharyngeal swab performed on the same day of access to emergency care. Only patients with documented viral pneumonia were included into the study. This population was further subdivided into: a) group C if they had received treatment with HCQ before presentation to the ED and within 6 days from onset of symptoms b) group D if they had not been treated with HCQ or later then 6 days. Groups A and C were considered as treated patients, while groups B and D as controls. We analyzed the two cohorts to make sure that no patient of the first cohort was admitted to the ED of our hospital and was counted twice.

2.3 Definition of Outcomes and Statistical Methods

Rate of hospitalization, intubation, need for oxygen supplementation at home and overall survival were defined as clinical endpoints. Patients initially discharged from the emergency department and subsequently admitted to the hospital were counted as admissions. Survival was defined as the percentage of patients alive 1 month after enrollment into the study, while intubation was defined as admission to intensive care unit for mechanical ventilation. The clinical characteristics of treated patients at baseline were compared with controls by means of descriptive statistics using the Chi-square and odds ratio calculation for categorical data. Mann-Whitney U test and student t test were used, where appropriate, for continuous variables. A p value of less than 0.05 was considered as significant. Variables influencing the established endpoints, with a significance level < 0.2, were

also entered into multiple logistic regression analysis to identify independent predictors of each outcome. Statistical analysis was performed with the MedCalc software (Medcalc 2011 Version 11.6.1.0. available at www.medcalc.org).

3. RESULTS

3.1 Baseline Characteristics of the Two Cohorts of Patients

From March 2, 2020 until April 20, 2020 , 354 patients with suspected COVID-19 pneumonia were recruited from the adult general population (≥ 18 years old), of the Bergamo province and attended by PCP at home. All patients underwent Chest CT scan or Chest x ray with typical findings of interstitial pneumonia One-hundred and fifty-five of them (44%) had a positive RT-PCR nasopharyngeal swab for SARS-CoV-2 RNA, in the remaining 199 cases the diagnosis of COVID-19 pneumonia was made by imaging only. One-hundred and seventy-eight of them (50.2%) received HCQ (Group A) and 176 (49.8%) did not (Group B). Both groups were observed during the same period of the pandemic. The median start of follow-up for group A was on March 22,2020 (ranging from March 4 until April 20, 2020), while the median start of follow-up for group B was on March 20, 2020 (ranging from March 3 until April 20, 2020). None of the patients of both groups received HCQ 6 days later from the onset of symptoms. The clinical characteristics of the two groups are reported in Table 1. No significant differences between the two groups were detected at baseline, except for the use of low molecular weight heparin (LMWH), which was more frequent in the treated group. LMWH was given at the prophylactic dose of 4.000-6.000 U/day subcutaneously. The great majority of patients of both groups (99 and 85 % respectively) received concomitant treatment with azithromycin. Adverse events were very mild and mostly included gastrointestinal symptoms. No discontinuation of treatment was required and no cases of torsade de pointe or sudden death were observed.

Four-hundred and seventy patients were admitted to the ED of Policlinico S.Marco Hospital in the same period: 24 (5.1%) had received HCQ before access to the hospital (group C) and 446 (44.9%) had not (group D). The mean duration of HCQ treatment before

presentation to the ED was 4 days (range 1-10). Seventeen patients (70%) of group C continued HCQ during hospitalization, the remaining 7 patients stopped the drug because they had completed the scheduled 10 day course of treatment. Of the 446 patients who had not received HCQ before presentation to the ED, 393 (88%) were subsequently started on HCQ during hospitalization. The baseline characteristics of groups C and D are also represented in Table 1. The two groups were similar with the exception of concomitant treatment with azithromycin, which was more frequent in the group C (37%) than in group D (11%).

3.2 Outcomes

In the cohort of outpatients 34 patients of group A (20%) and 77 patients of group B (43 %) were admitted to hospitals: RR 0.43, 95% C.I. 0.31-0.61; OR 0.32, 95% C.I. 0.20-0.52 (Fig. 2), while thirteen patients of group A (7.3%) vs 36 of group B (20%) were admitted to the ICU and mechanically ventilated: RR 0.35, 95% C.I. 0.19-0.65; OR 0.34, 95% C.I. 0.17-0.68 (Fig. 4 Additional Material). The need for oxygen supplementation for patients attended at home was significantly lower in treated patients than in controls: 59 patient necessitated oxygen

supplementation at home (33%) vs 87 (49%) of controls: RR 0.51, 95% C.I. 0.51-0.86; OR 0.50, 95% C.I. 0.33-0.77 (Fig. 5 Additional Material). The mortality rate in this outpatient population was low with no significant difference between the two groups: two patients of group A died (1.2 %) vs 7 of group B (4 %), RR 0.28, 95% C.I. 0.05-1.34, OR 0.27, 95% C.I. 0.05-1.33 (Fig. 3).

On the contrary the cohort of patients presenting to the ED who had received early out of hospital treatment with HCQ, showed increased survival compared to the other patients: 3 patients of group C died (12.5%) compared to 157 (35%) of group D (Fig. 3). No significant differences were seen in rate of hospital admissions and ICU admissions between the two groups (Fig. 2 and Fig. 4 Additional Material), while data about previous oxygen supplementation at home were not obtainable in this population.

Total lymphocyte count and D-dimer were available at baseline and 7 days after hospital admission in the cohort of patients admitted to the ED of Policlinico S.Marco Hospital. No significant difference was found between the two groups, although a relevant downward trend of CRP levels was observed in HCQ treated patients (Fig. 6 Additional Material).

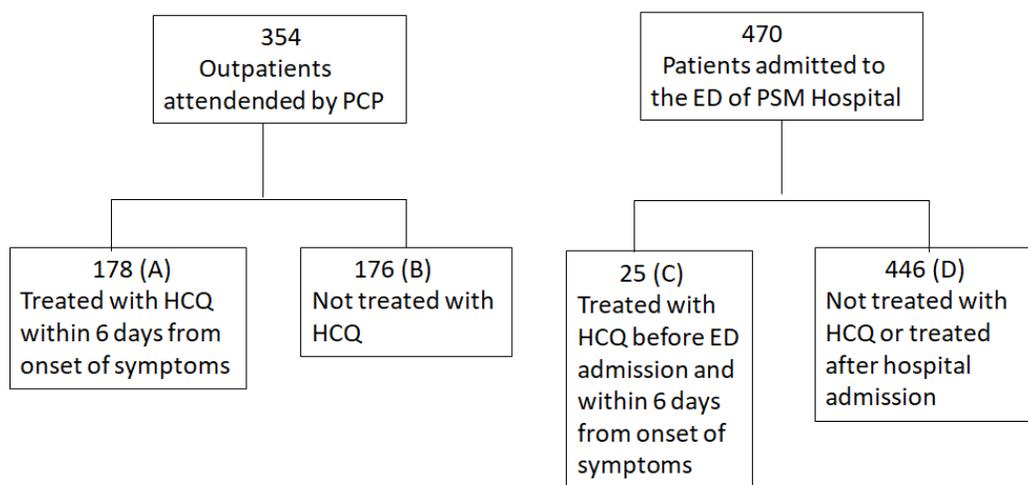


Fig. 1. Flow chart of the study

Table 1. Basal characteristics of the patients. Panel A shows the cohort of patients attended by PCP of Valle Seriana (Bergamo), panel B shows the cohort of patients who presented to the emergency department of Policlinico S.Marco Hospital Zingonia and were subsequently discharged or admitted to the hospital. Patients who received hydroxychloroquine as outpatients are compared with the other patients (Chi square test and Mann-Whitney Rank Sum Test)

Panel A

| | Group A : HCQ (178) | Group B : controls (176) | p |
|---|----------------------------|---------------------------------|----------------------|
| Male sex (%) | 101 (56) | 113 (64) | 0.184 (Chi-sq) |
| Age in years (median \pm 95% C.I.) | 66.8 (64-68) | 64 (62-66) | 0.414 (Mann-Whitney) |
| Body Mass index (median \pm 95% C.I.) | 26 (25-27) | 28 (27-28) | 0.186 (Mann-Whitney) |
| Current or past smokers (%) | 54 (30) | 42 (23) | 0.21 (Chi-sq) |
| Type II Diabetes (%) | 40 (22) | 45 (25) | 0.57 (Chi-sq) |
| Heart disease (%) | 50 (28) | 38 (21) | 0.19 (Chi-sq) |
| COPD (%) | 19 (11) | 19 (11%) | 0.81 (Chi-sq) |
| Hypertension | 78 (44) | 83 (47) | 0.60 (Chi-sq) |
| Concomitant treatment with azithromycin (%) | 177 (99) | 167 (95%) | 0.80 (Chi-sq) |
| Concomitant prophylactic LMWH | 104 (58) | 35 (20) | <0.001 (Chi-sq) |

Panel B

| | Group C: HCQ (24) | Groups D: controls (446) | p |
|---|--------------------------|---------------------------------|----------------------|
| Male sex (%) | 14 (58) | 286 (64) | 0.72 (Chi-sq) |
| Age in years (median \pm 95% C.I.) | 66.6 (60-72) | 68.3 (67-69) | 0.836 (Mann-Whitney) |
| Obesity (BMI >30 Kg/sqm) (%) | 1 (4.1) | 19 (4.2) | 0.61 (Chi-sq) |
| Type II Diabetes (%) | 2 (8.3) | 86 (19) | 0.28 (Chi-sq) |
| Heart disease (%) | 1 (4.1) | 72 (16) | 0.19 (Chi-sq) |
| COPD (%) | 1 (4.1) | 43 (9.6) | 0.59 (Chi-sq) |
| Hypertension | 7 (30) | 197 (44) | 0.26 (Chi-sq) |
| Concomitant treatment with azithromycin (%) | 9 (37) | 48 (11) | 0.003 (Chi-sq) |
| Treatment with HCQ during hospitalization | 17 (70) | 393 (88) | 0.03 (Chi-sq) |
| >50% lung involvement on Chest CT | 2 (8.2) | 55 (12.3) | 0.76 (Chi-sq) |

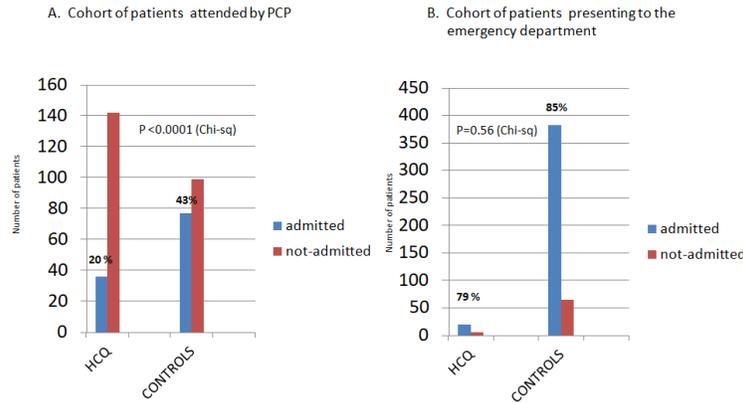


Fig.2 Hospital admissions of COVID-19 patients attended by PCP (panel A) or presenting to the emergency Department of Policlinico S.Marco Hospital (Panel B). The number of patients admitted to hospital in cohort A was significantly lower in patients treated with hydroxychloroquine (Chi Sq: $p < 0.0001$, OR 0.32, 95% C.I. 0.20-0.52). No significant difference was found in cohort B (Chi Sq 0.56, OR 0.63, 95% C.I.: 0.22-1.76).

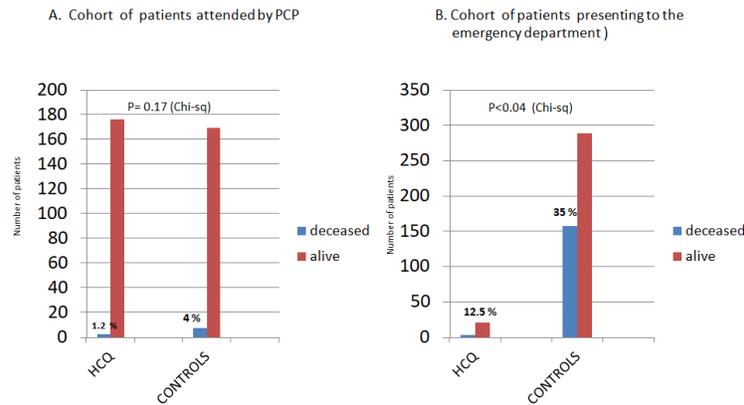


Fig.3 Thirty-day mortality of COVID-19 patients attended by PCP (panel A) or admitted to Policlinico S.Marco Hospital (panel B). Mortality was significantly lower only in the cohort of patients admitted to the Emergency Room of Policlinico S.Marco Hospital and who had received hydroxychloroquine as outpatients (Chi Sq: $p = 0.03$, OR 0.26, 95% C.I. 0.07-0.89), but not in the cohort of outpatients attended by PCP (Chi Sq: $p = 0.17$, OR 0.27, 95% C.I. 0.05-1.33).

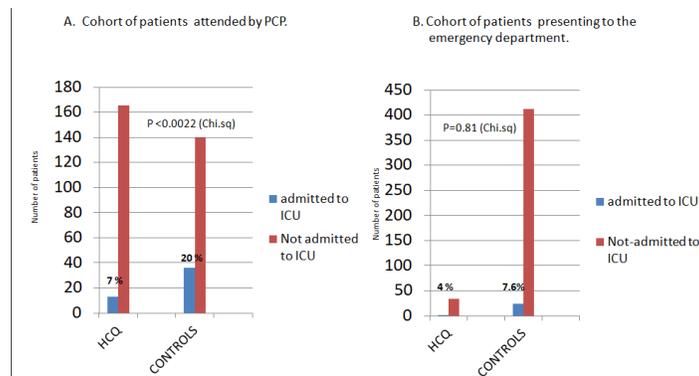


Fig.4 Patients admitted to Hospital Intensive Care Units and undergoing mechanical ventilation. Patients attended by PCP are shown in panel (A), while patients who presented to the emergency Department of Policlinico S.Marco Hospital are shown in panel (B). The number of patients admitted to ICU and undergoing mechanical ventilation in cohort A was significantly lower if the patients had received hydroxychloroquine as outpatients (Chi Sq: $p < 0.0022$, OR 0.34, 95% C.I. 0.17-0.68). No significant difference was found in cohort B (Chi Sq: 0.81, OR 0.52, 95% C.I. 0.06-4.02).

A. Cohort of patients attended by PCP)

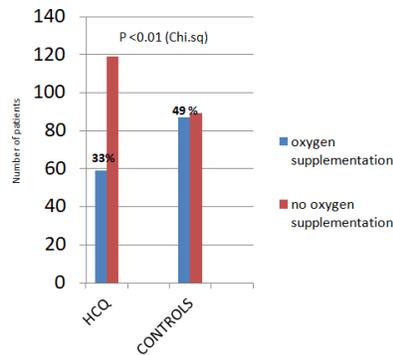


Fig.5 Number of patients who needed oxygen supplementation at home in the cohort of patients attended by PCP. The need for oxygen supplementation was significantly lower in patients who received hydroxychloroquine. (Chi Sq: $p=0.002$, OR 0.50, 95% C.I. 0.33-0.77).

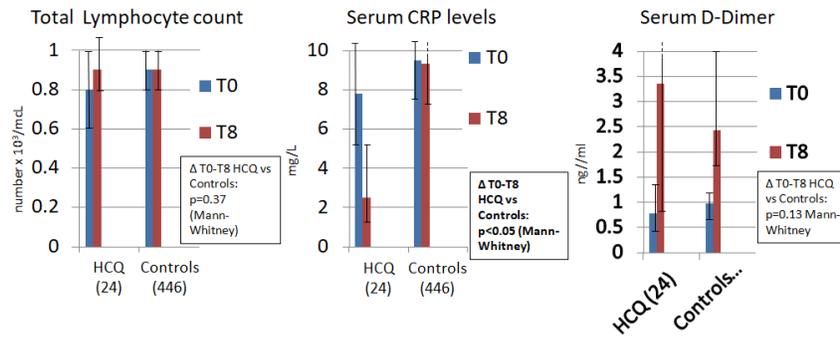


Fig. 6 Total Lymphocyte count, serum CRP (C Reactive Protein) and D-Dimer levels in patients admitted to Policlinico S. Marco Hospital. T0 represents values obtained at baseline and T8 after 7-10 days of hospitalization. Baseline levels of Lymphocytes, CRP and D-Dimer were similar in patients treated with HCQ before hospitalization and controls. After 7-10 days a significant decrease in CRP levels was noted in HCQ treated patients compared with controls ($p<0.05$, MWT: Mann-Whitney Rank-sum test).

We performed univariate and multivariate analysis to assess which variables influenced the established outcomes independently. In order to improve the performance of the multiple regression model we merged the two cohorts and performed both analysis on the entire population of patients. At univariate analysis age older than 65, male sex, presence of heart disease and lack of treatment with LMWH were associated with higher mortality, higher hospital and ICU admissions. At multivariate analysis female sex ($p<0.05$), oxygen supplementation at home ($p<0.0001$) and HCQ treatment ($p<0.0001$) were independently associated with a lower rate of hospital admission. Oxygen supplementation at home ($p<0.0001$) and HCQ ($p<0.05$) were independently associated with lower rate of ICU admission and mechanical ventilation, while

improved survival was only associated with HCQ treatment ($p<0.05$). Concomitant treatment with azithromycin and LMWH were not independently associated with any of the predefined outcomes.

4. DISCUSSION

In our study we investigated the hospitalization risk and the outcomes of community-managed COVID-19 pneumonia in a non-hospital setting, an aspect that has been overlooked so far. Management of the patients by PCP consisted mainly in monitoring oxygen saturation at home with the prescription of antipyretics as needed, antibiotics and HCQ as an antiviral agent. HCQ was given according to a well-established protocol In spite of the official recommendations nearly half of the patients did not receive the

drug, which may be explained by many factors, such as the enrollment of some patients before publication of the guidelines, the reluctance of PCP to use the drug without ECG monitoring, the presence of cardiac comorbidity and the reduced availability of the drug during the pandemic. However, none of these factors generated a selection bias because the baseline clinical characteristics of both groups were similar. We found that treatment with HCQ, initiated early in the course of the disease and in the outpatient setting, decreased hospital and ICU admissions and in addition reduced the need for oxygen therapy at home. Due to the low number of deaths in patients followed by PCP, we could not find a reduction in mortality in these patients. However, a significantly higher survival was noted in more severely affected patients, admitted to the ED and who had received HCQ at home. Treatment was initiated in all cases within 6 days from the onset of symptoms, which is probably the maximum lag time in which HCQ may exert its favorable effects. Therefore early administration of HCQ is paramount to exert its antiviral and immunomodulatory properties. Delayed therapy, in patients that have already progressed into critical illness, is less likely to be of benefit, as shown in a recent meta-analysis of the large different trials [25] in which HCQ was given in the hospital setting and after the patient had slowly deteriorated at home. Our results underline the need to counter COVID-19 at the community level, as hospitalized patients, not diagnosed in the community, often present to the ED at a late stage and may rapidly deteriorate with fulminant illness [26,27].

An early antithrombotic prophylaxis with low molecular weight heparin might add additional value to HCQ treatment. However, it should be noted that, at multivariate analysis, only HCQ and not heparin had a positive independent impact on the analyzed endpoints, therefore no firm conclusion can be made on a putative synergistic effect of heparin. Likewise, we cannot draw any conclusion on the efficacy of azithromycin, since the drug was given to nearly all patients.

Similarly to other large retrospective studies we found an excellent safety profile of HCQ even if co-administered with azithromycin [17-19,28]. Our data base did not contain the ECG reports and therefore we could not extract data on the QTc interval. However no significant clinical toxicity or drug interruption was reported.

Strengths of our study are the inclusion of a non-hospital based population, a well-defined treatment protocol and the addition of a cohort of patients admitted to the ED. It is noteworthy that the control group of this cohort was represented by hospitalized patients who received HCQ after hospital admission and nevertheless had a high in-hospital mortality, which was significantly higher than that observed in patients who had received HCQ as outpatients in the first 6 days of the disease. Nevertheless, we should highlight some limitations of our study. Firstly its retrospective nature, with its inherent problems, may have produced occult biases affecting the distribution of treated and untreated groups. Secondly the fact that only 44% of our outpatient population had a positive RT-PCR nasopharyngeal swab for SARS-CoV-2 RNA. Our patients were diagnosed with COVID-19 pneumonia according to a typical pattern of CT or X-ray features. Obviously, the standard for diagnosis of SARS-CoV-2 virus is reverse transcription polymerase chain reaction (RT-PCR) test but, in real life and during the first wave of the pandemic, obtaining a swab in non-hospitalized patients in Lombardy was practically impossible. Italy was one of the countries with the highest reported case fatality rate [29]. Therefore from February 28th 2020 onwards testing was limited to severely symptomatic patients, in order to avoid collapse of the authorized COVID-19 laboratories. It should also be stressed that Chest CT shows a high sensitivity for diagnosis of COVID-19 and may be considered as a primary tool for COVID-19 detection in epidemic areas [30-31]. Thirdly, in spite of a lower number of deaths in the treated group of outpatients, we could not detect a significant difference in mortality between the two groups. This was attributable to the mild course of the disease in our cohort of outpatients compared with hospitalized patients, where a higher number of events generated a significant difference.

5. CONCLUSION

Our study, similar to the only other study performed on outpatients [32], found that HCQ, started within 6 days from the onset of symptoms, reduced the rate of hospital admission and other important clinical outcomes, with a good safety profile. It is possible that the addition of low molecular weight heparin as antithrombotic prophylaxis might have played a synergistic role. The correct timing to start anti-COVID drugs should be taken into consideration

in designing future randomized trials and we advocate the involvement of PCP in these trials.

CONSENT AND ETHICAL APPROVAL

The authors declare that they received no funding for this study, that they have no conflict of Interest, that the study was approved by the local ethical board and that an informed consent was obtained by patients admitted to the Emergency Department of Policlinico S.Marco Hospital.

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This article is dedicated to the memory of all the doctors and allied health personnel who have sacrificed their own lives to save those of their patients. We wish to honor their competence, braveness and generosity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Johns Hopkins University of Medicine, Coronavirus Research Center. Available: <https://coronavirus.jhu.edu/map.html>
2. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of covid-19 associated pneumonia in clinical studies. Biosci Trends. 2020;14:72-3. DOI:10.5582/bst.2020.01047
3. Wang M, Cao R, Zhang L. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *In vitro*. Cell Res. 2020;30:269-71. DOI:10.1038/s41422-020-0282-0
4. Yao X, Ye F, Zhang M. *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. DOI:10.1093/cid/ciaa237
5. Hung IF, Lung KC, Tso EY, Liu R, Chung TW, Chu MY, et al. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: An open-label, randomised, phase 2 trial. Lancet. 2020;395:1695-704.
6. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe COVID-19. NE Eng J Med April 10; 2020. DOI: 10.1056/NEJMoa2007016
7. Beigel J, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of COVID-19 – preliminary report New Eng J Med. 2020;383:1-12. DOI: 10.1056/NEJMoa 2007764
8. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: A randomised, double-blind, placebo-controlled, multicentre trial. Lancet; 2020. Available: [https://doi.org/10.1016/S0140-6736\(20\)31022-9](https://doi.org/10.1016/S0140-6736(20)31022-9)
9. 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 Discovery; 2020. DOI: 10.1038/s41421-020-0156-0.15
10. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Bioscience Trends. 2020;14:72. DOI: 10.5582/bst.2020.01047
11. Gautret P, Lagier JG, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. International Journal of Antimicrobial Agents; 2020. DOI: 10.1016/j.ijantimicag.2020.105949
12. 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. Available: <https://doi.org/10.1093/cid/ciaa237>
13. Gautret P, Lagier JG, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: An observational study. Travel Medicine and Infectious Disease; 2020.

- DOI: 10.1016/j.tmaid.2020.101663
14. Rosenberg ES, Dufort EM, Tomoko U, Wilberschied LA, Tesoriero J, Weinberg P, et al. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State JAMA; 2020. DOI:10.1001/jama.2020.8630
 15. 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;383:1-8. DOI: 10.1056/NEJMoa2012410
 16. Mahévas M, Tran VT, 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;369:1844. Available: <http://dx.doi.org/10.1136/bmj.m1844>
 17. Million M, Gautret P, Colson P, Roussel Y, Dubourg G, Chabriere E, et al. Clinical efficacy of chloroquine derivatives in COVID-19 infection: Comparative meta-analysis between the Big data and the real world, New Microbes and New Infections; 2020. Available: <https://doi.org/10.1016/j.nmni.2020.100709>.
 18. Lagier JC, 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 Medicine and Infectious Disease; 2020. DOI: <https://doi.org/10.1016/j.tmaid.2020.101791>
 19. Levasseur A, Mailhe M, Ravoux I, Richez M, Roussel Y, Seng P, et al. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis, Travel Medicine and Infectious Disease; 2020. DOI: <https://doi.org/10.1016/j.tmaid.2020.101791>
 20. Mikami T, Miyashita H, Yamada T, Harrington M, Steinberg D, Dunn A, et al. Factors for mortality in patients with COVID-19 in New York City. J Gen Intern Med Ahead of print; 2020. DOI: 10.1007/s11606-020-05983-z
 21. Arshad A, 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 Inf Dis Ahead of print; 2020. Available: <https://doi.org/10.1016/j.ijid.2020.06.099>
 22. Lipworth B, Chan R, Lipworth S, Kuo C. Weathering the cytokine storm in susceptible patients with severe SARS-CoV-2 infection. J Allergy Clin Immunol Pract. Ahead of Print; 2020. Available: <https://doi.org/10.1016/j.jaip.2020.04.014>
 23. S I M I T. Società Italiana di malattie infettive e tropicali sezione regione lombardia. Linee guida sulla gestione terapeutica e di supporto per pazienti con infezione da coronavirus COVID-19 Available: <https://www.omceoch.it/storage/attachments/Ebka.COVID19%20linee%20guida%20trattamento%2001MAR.pdf.pdf>
 24. Faggioli S, Lorini FL, Remuzzi G. Adaptations and lessons in the province of Bergamo. N Engl J Med. 2020;382:71. DOI: 10.1056/NEJMc2011599
 25. Fiolet T, Guihur A, Rebeaud MA, Mulot M, Smadja NP, Saleh YM. Effect of hydroxychloroquine with or without azithromycin on the mortality of coronavirus disease 2019 (COVID-19) patients: A systematic review and meta-analysis. Clin Microbiol Infect. 2021;27:19-27.
 26. Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for coronavirus disease, 2019 (COVID-19): A review. JAMA; 2020. DOI: 10.1001/jama.2020.6019. Online ahead of print
 27. Synowiec A, Szcepanski A, Barreto-Duran E, Lie LK, Pyrc K. Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2): A systemic infection. Clin Microbiol Rev. 2021;34:1-31. DOI:10.1128/CMR.00133-20
 28. White N, Watson JA, Hoggund RM, Chan XH, Cheah PY, Tarning J. COVID-19 prevention and treatment: A critical analysis of chloroquine and hydroxychloroquine clinical pharmacology. PLoS Med. 2020;17(9):1-24. Available: <https://doi.org/10.1371/journal.pmed.1003252>

29. Onder G, Rezza G, Brusaferro S. Case fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA; 2020.
DOI: 10.1001/jama.2020.4683. Online ahead of print.
PMID: 32203977
30. Tao Ai, Yang Z, Hou H, Zhan C, Chen C, Li W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: A report of 1014 cases radiology. Online ahead of print. Radiology. 2020;200642. DOI: 10.1148/radiol.2020200642.
31. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. Lancet Infect Dis. 2020;20(4): 425-434.
32. Guérin V, Lévy P, Thomas JL, Lardenois T, Lacrosse P, Sarrazin E, et al. Azithromycin and hydroxychloroquine accelerate recovery of outpatients with mild/moderate COVID-19 AJMAH. 2020;18(7):45-55.

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