Original Article

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Efficacy of early hydroxychloroquine treatment in preventing COVID-19 pneumonia aggravation, the experience from Shanghai, China

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SUMMARY The aim of this study is to assess the efficacy of multiple treatments, especially hydroxychloroquine, used in different disease stages of coronavirus disease 2019 (COVID-19). All consecutive patients with COVID-19 admitted to Shanghai Public Health Clinical Center (Shanghai, China) between January 20, 2020, and April 30, 2020, were enrolled, and their clinical data were retrospectively collected. Binary logistic regression was used to screen the factors associated with disease aggravation, and multivariable analyses with the Cox proportional hazards model were used to estimate the effects of prognostic factors on the improvement time and PCR conversion days in throat swabs and stool swabs. A total of 616 patients, including 50 (8.11%) severe and 18 (2.92%) critical patients, were enrolled in our retrospective cohort study. The early use of hydroxychloroquine was a protective factor associated with disease aggravation (95% CI: 0.040-0.575, $p = 0.006$). Clinical improvement by 20 days was significantly different between patients with hydroxychloroquine used early and those with hydroxychloroquine not used ($p = 0.016$, 95% CI: 1.052-1.647). The median time to clinical improvement was 6 days in the hydroxychloroquine used early group, compared with 9 days in the without hydroxychloroquine used group and 8 days in the with hydroxychloroquine not used early group ($p < 0.001$). Hydroxychloroquine used early was associated with earlier PCR conversion in both throat swabs (HR = 1.558, $p = 0.001$) and stool swabs (HR = 1.400, $p = 0.028$). The use of hydroxychloroquine at an early stage is a potential therapeutic strategy for treating patients before irreversible severe respiratory complications occur. The early use of hydroxychloroquine decreased the improvement time and the duration of COVID-19 detection in throat and stool swabs.

Keywords clinical management, treatment, hydroxychloroquine, COVID-19, SARS-CoV-2, coronavirus

1. Introduction

The outbreak of COVID-19 has spread around the world and become a public health emergency of international concern. There have been nearly 60 million infections and over 1.4 million deaths reported as of November 24, 2020, since the first case, which was identified in December 2019. Though the majority of patients have mild disease (*1*), the large number of severe and critical cases pose great challenges to the global healthcare system.

Many studies have analysed the characteristics of severe COVID-19 and identified biomarkers for prognosis prediction (*2-4*). Drugs are urgently needed for both prophylaxis and the treatment of severely ill patients. A number of drugs that have been approved for other diseases are being tested for the treatment of COVID-19 patients, but there is an absence of data from appropriately designed clinical trials showing that these drugs, either alone or in combination, will prove effective (*5*). Particularly, some of the treatments (*i.e.*, drugs for malaria) are controversial and have caused heated discussion (*6*).

Therefore, as the percentage of severe cases has markedly decreased since March in Shanghai, China, multiple treatments, especially hydroxychloroquine, were retrospectively analysed to assess their efficacy in different disease stages of coronavirus disease 2019 (COVID-19).

2. Materials and Methods

2.1. Study design and participants

This retrospective cohort study included patients (≥ 15 years old) from Shanghai Public Health Clinical Center (Shanghai, China). All patients who were diagnosed with COVID-19 according to the Seventh Edition of the Guidance for COVID-19 of China (*7*) were screened, and those who died or were discharged between January 20, 2020 (*i.e.*, when the first patients were admitted), and April 30, 2020, were included in our study.

The study was approved by the Research Ethics Commission of Shanghai Public Health Clinical Center (V1.1-2020-02.08), and the requirement for informed consent was waived by the Ethics Commission.

2.2. Data collection

Epidemiological, demographic, clinical, laboratory, treatment, and outcome data were extracted from electronic medical records using a standardized data collection form, among which a modified version of the WHO/International Severe Acute Respiratory and Emerging Infection Consortium case record form was used for severe acute respiratory infections. All data were checked by two physicians (YYM and YS), and a third researcher (BJH) adjudicated any differences in interpretation between the two primary reviewers.

2.3. Laboratory procedures

Local Centers for Disease Control and Prevention was responsible for SARA-CoV-2 detection in respiratory specimens by real-time RT-PCR methods. Throat swab specimens and stool swab specimens were obtained for SARS-CoV-2 PCR re-examination every two or three days after the clinical remission of symptoms, including fever, cough, and dyspnoea, but only qualitative data were available. The criteria for discharge were absence of fever for at least 3 days, substantial improvement in chest CT, clinical remission of respiratory symptoms, and two throat swab samples and two stool swab samples negative for SARS-CoV-2 RNA obtained at least 24 h apart.

2.4. Treatment

All the patients were treated in strict accordance with the novel coronavirus infection diagnosis and treatment proposal formulated by the National Health Commission and Health Committee of China (*7*). Oral hydroxychloroquine was prescribed at 400 mg once a day for 10-14 days. Intravenous vitamin C was used at 5-15 g per day for at least 3 days. Subcutaneous thymosin alpha 1 (1.6 mg) was used three times per week. The dose of lopinavir-ritonavir was 0.5 g twice a day for 3-5 days, and arbidol was orally administered at 0.2 g three times a day for 5-7 days. Corticosteroid and immunoglobulin therapy were empirically administered as a combined regimen when severe pneumonia was diagnosed, and the common doses were 40 mg once or twice a day for 3 days and 10 g for 3-5 days, respectively. Low molecular weight heparin was prophylactically used when D-dimer gradually increased to prevent thrombotic events.

2.5. Definitions

The illness grade of COVID-19 was defined according to the Chinese management guideline for COVID-19 (version 7.0) (*7*). Mild disease includes patients with mild symptoms but no manifestation of pneumonia on imaging. Moderate disease includes patients with fever, cough, sputum production, and other respiratory tract or non-specific symptoms along with the manifestation of pneumonia on imaging. Severe pneumonia was defined as the presence of respiratory distress with respiratory frequency ≥ 30/min, SaO_2/SpO_2 below 94% on room air or a PaO_2 to FiO_2 ratio of 300 or lower. Critical disease includes respiratory failure and the need for mechanical ventilation, or shock or combination with other organ failure and the need treatment in an ICU. Disease aggravation indicates that (1) mild or moderate disease on admission progressed to severe or critical disease; or (2) severe disease on admission progressed to critical disease. Disease improvement indicates that the body temperature is lower than before, respiratory symptoms are relieved in mild patients, and lung CT or chest X ray show that the lesions appear to be more absorbed and dissipated than before in moderate, severe or critical patients. Treatments with the same drug were assigned to three groups: (1) not used indicates not starting the treatment; (2) not used early indicates not starting the treatment within 5 days of diagnosis or treatment not lasting for at least 3 days; and (3) used early indicates starting the treatment within 5 days of diagnosis and lasting for at least 3 days.

2.6. Statistical analysis

Depending on the distribution of the data, categorical variables are described as frequencies and percentages, and continuous variables are described as median and interquartile range (IQR) values. Binary logistic regression was used to screen the factors associated with disease aggravation in COVID-19 patients. The improvement time of hydroxychloroquine usage was estimated by the Kaplan-Meier method, and any differences in improvement time were evaluated with the log-rank test. Multivariable analyses with the Cox proportional hazards model were used to estimate the effects of prognostic factors on improvement time, days to negative throat swabs and days to negative stool

swabs. Statistical analyses were performed using SPSS 23.0 software (IBM, Armonk, NY, USA). The figures were constructed using GraphPad Prism 8.0.

3. Results

3.1. Clinical characteristics and outcomes of the study population

As of April 30, 2020, a total of 616 patients were enrolled in this study. The median age was 39 years (IQR, 28-56 years), and 342 (55.52%) patients were male (Table 1). A total of 172 patients (27.92%) had one or more coexisting chronic medical conditions. Hypertension (97 [15.75%]) was the most common comorbidity, followed by diabetes (40 [6.49%]). The Charlson Index was also calculated, and the Charlson Index of 99 patients (16.07%) was equal to or greater than 2. There were 138 mild, 470 moderate, 3 severe and 5 critical patients on admission, and the number increased by 50 for severe patients and 18 for critical patients on discharge. The percentage of severe and critical cases decreased from 22.36% before February 5 to 2.43% from March 6 to April 30 (Table 1). Up to the time of submission, a total of 608 (98.7%) patients were discharged, and 8 (1.3%) patients died.

3.2. Treatments of the study population

All of the patients were admitted to negative pressure isolation rooms. All patients were treated in strict accordance with the novel coronavirus infection diagnosis and treatment proposal formulated by the National Health Commission and Health Committee of China (*7*). As shown in Figure 1, hydroxychloroquine, vitamin C and thymosin alpha 1 were empirically used

Table 1. Clinical characteristics and outcomes

Disease grade on discharge	Mild *n* = 118 (%)	Moderate *n* = 430 (%)	Severe *n* = 50 (%)	Critical *n* = 18 (%)
Age				
≥ 65	0 (0.00)	45 (0.93)	20 (40.00)	6 (33.33)
< 65	118 (100.00)	385 (0.23)	30 (60.00)	12 (66.67)
Sex				
Male	68 (49.15)	228 (53.02)	31 (62.00)	15 (83.33)
Female	50 (42.37)	202 (46.98)	19 (38.00)	3 (16.67)
Comorbidity				
Hypertension	3 (2.54)	67 (15.58)	16 (32.00)	11 (61.11)
Diabetes	1 (0.85)	28 (6.51)	7 (14.00)	4 (22.22)
Cardiovascular disease	0 (0.00)	0 (0.00)	0 (0.00)	2 (11.11)
Chronic lung disease	0 (0.00)	15 (3.48)	1 (2.00)	2 (11.11)
Chronic kidney disease	0 (0.00)	4 (0.93)	0 (0.00)	2 (11.11)
Charlson Index				
< 2	115 (97.46)	343 (79.77)	34 (68.00)	5 (27.78)
≥ 2	3 (2.54)	87 (20.23)	16 (32.00)	13 (72.22)
Grade distribution by different time points				
Before Feb. 5 (*n* = 246)	4 (1.63)	187 (76.02)	41 (16.67)	14 (5.69)
Feb. 6 – Mar. 5 (*n* = 82)	4 (4.88)	72 (87.80)	4 (4.88)	2 (2.44)
Mar. 6 – Apr. 30 (*n* = 288)	110 (38.19)	171 (59.38)	5 (1.74)	2 (0.69)

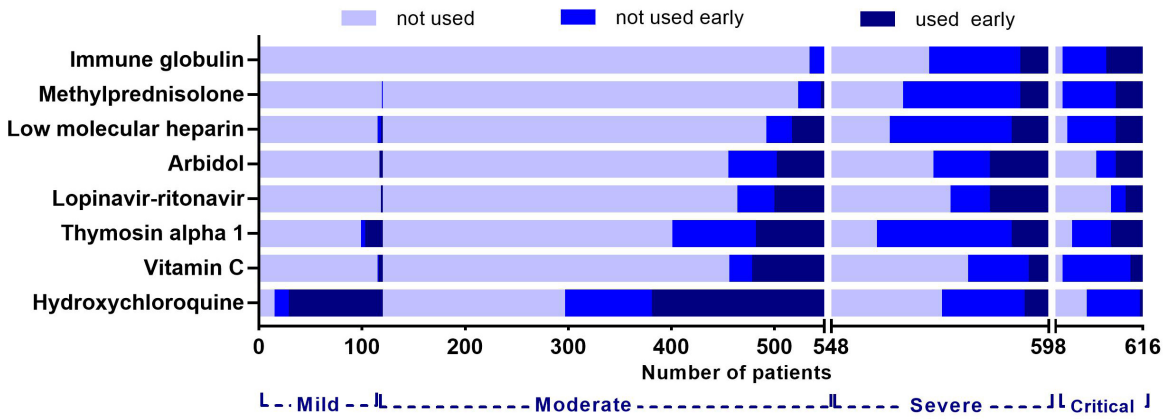

Figure 1. Multiple treatment for patients with different disease aggravation. Treatments that were used early in more than 62 (10%) patients includes hydroxylchloroquine, vitamin C, thymosin alpha 1, lopinavir-ritonavir and arbidol.

according to patient status decided by the attending physicians. Antiviral drugs (*e.g.*, lopinavir-ritonavir and arbidol) were administered to a small proportion of patients. Corticosteroid and methylprednisolone were not normally used unless they were considered necessary (*e.g.*, ARDS) in a panel discussion by experts.

3.3. Factors associated with disease aggravation

A binary logistic regression model was used to identify the factors associated with disease aggravation in patients with COVID-19. Treatments that were used early in 62 (10%) patients were included in this model. As shown in Figure 2, several independent variables were included in this model. Age ≥ 65 years old, thymosin alpha 1, lopinavir-ritonavir and arbidol used in early time were associated with the disease aggravation ($p < 0.05$) and early use of hydroxychloroquine was a protective factor associated with disease aggravation (95% CI: 0.040-0.575, $p = 0.006$).

3.4. Clinical improvement

A Cox proportional hazards model was used to identify the factors associated with improvement within 20 days in patients with COVID-19. As shown in Figure 3, the early use of hydroxychloroquine was the only factor that markedly reduced the improvement time ($p = 0.016$) compared with other treatments used early. Improvement time of different hydroxychloroquine usages were shown in Figure 4.

3.5. Viral clearance

In a Cox proportional hazards regression model, hydroxychloroquine used early was associated with earlier PCR conversion by 20 days in throat swabs (HR = 1.558, $p = 0.001$) and with earlier PCR conversion by 15 days in stool swabs (HR = 1.400, $p = 0.028$) (Figure 5).

4. Discussion

Currently, no specific therapeutic agents or preventive vaccines are available and approved for COVID-19. However, a number of drugs that have been approved for other diseases, some of which have been tried in patients with SARS-CoV and MERS-CoV, are being evaluated for the tresatment of COVID-19. These drugs include remdesivir, baricitinib, chloroquine, hydroxychloroquine, the interleukin-6 (IL-6) receptor monoclonal antibody tocilizumab, and the anti-influenza drugs favipiravir and umifenovir (*8,9*).

Hydroxychloroquine is capable of affecting several cellular pathways and therefore may have several mechanisms of action against SARS-CoV-2 (*10*). In addition, many findings suggest that hydroxychloroquine is effective at impairing SAR-CoV-2 replication *in vitro* (*11,12*). To date, several small studies and subjective reports have published evidence of the effectiveness of hydroxychloroquine for the prevention and treatment of COVID-19 (*13,14*). Furthermore, several official guidelines have already incorporated hydroxychloroquine as the suggested treatment of patients with COVID-19 (*15,16*). However, some articles were published for the ineffectiveness of hydroxychloroquine in the treatment of COVID-19 (*17,18*). Therefore, more clinical studies need to be performed to come to a definite conclusion.

Although adverse events were reported to be higher in hydroxychloroquine recipients than in non-recipients (*17*), another retrospective analysis of 1,061 cases in

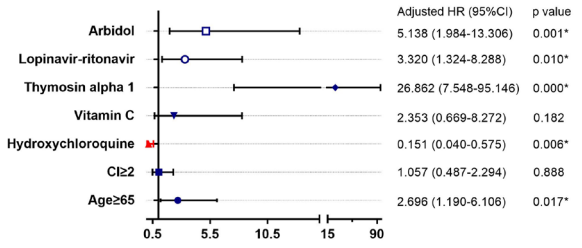

Figure 2. Effect of multiple treatments used early associated with disease aggravation.

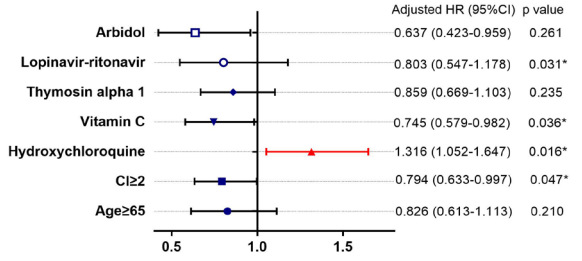

Figure 3. Effect of multiple treatments used early on the improvement time.

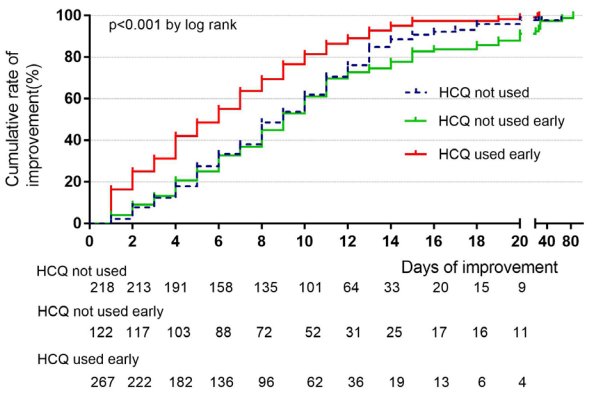

Figure 4. Improvement time of different hydroxychloroquine (HCQ) usages. Kaplan-Meier curves of the time to improvement in days with HCQ used early versus HCQ not used and HCQ not used early in the intention-to-treat population. Clinical improvement by 20 days was significantly different in patients with HCQ used early and with HCQ not used ($p = 0.016$, 95%CI: 1.052-1.647).The median time to clinical improvement was 6 days in the HCQ used early group, compared with 9 days in the without HCQ used group and 8 days in the with HCQ not used early group.

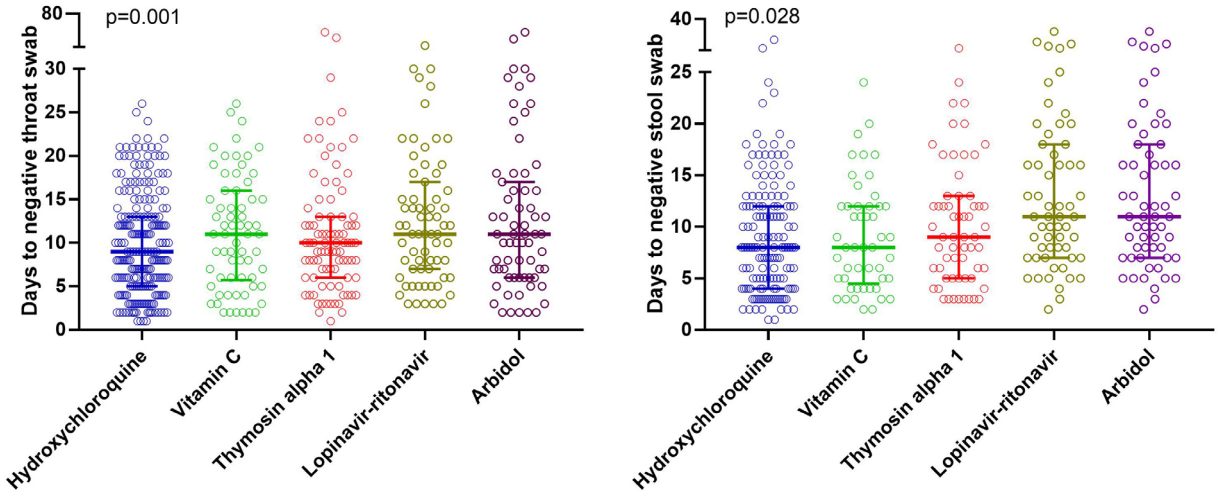

Figure 5. Days to negative results for throat swabs and stool swabs by different treatments used early.

France showed that a total of 2.3% of patients reported mild adverse events (gastrointestinal or skin symptoms, headache, insomnia and transient blurred vision) (*19*). Based on *in vitro* data, doses as high as 800 mg, if not higher, followed by 400 mg for several days, may be required for effective viral clearance in humans (*11*). Chinese official guidelines recommend 500 mg twice a day for 10 days and if severe gastrointestinal reactions occur, 500 mg once a day was administered (*16*). In our research, hydroxychloroquine was orally administered at 400 mg once a day for 10-14 days. As few adverse events were observed and no severe adverse events happened, hydroxychloroquine was recommended as safe with a low incidence of adverse events in patients.

Research has reported that the administration of hydroxychloroquine did not result in a significantly higher probability of negative conversion than the standard of care alone in patients with COVID-19 (*17*). However, in our research, the administration of hydroxychloroquine was specified. The early use of hydroxychloroquine, which is the use of hydroxychloroquine within 5 days of diagnosis, was found to decrease the swab PCR conversion days. Furthermore, the early use of hydroxychloroquine was also found to prevent the progression of the disease aggravation from mild and moderate to severe and critical. The use of hydroxychloroquine at an early stage is a potentially life-saving therapeutic strategy both to treat and cure patients before irreversible severe respiratory complications occur. Though hydroxychloroquine was found to prevent disease aggravation in our study, it was not significantly associated with a decrease in in-hospital mortality (*18*). Therefore, in the late stage of COVID-19 disease, hydroxychloroquine was not recommended as a life-saving treatment.

Though in our study, the early use of thymosin alpha 1 and high-dose intravenous VC was not associated with preventing disease progression and shortened improvement time because of the limitation of grouping and statistics, they were not considered ineffective for COVID-19. Thymosin alpha 1 has been used experimentally, as it has been used in the treatment of viral infections as an immune response modifier for many years, and thymosin alpha 1 supplement has been reported to significantly reduce the mortality of severe COVID-19 patients (*20*). Hemila and colleagues reported that various high-dose intravenous VC infusions (*e.g.*, 200 mg/kg body weight/day, divided into 4 doses) shortened the intensive care unit (ICU) stay by 7.8%, accompanied by a significant reduction in the mortality rate (*21*). Various high-dose intravenous VC infusions (doses varying between 5 g and 15 g per day) have been successfully used in the treatment of moderate to severe COVID-19 patients in China. Given that high-dose VC is safe, well-designed clinical studies are needed for severe and critical cases. Articles suggested that therapies (*i.e.*, ritonavir plus lopinavir) directed at viral replication may prove to be more effective in the early stages of COVID-19 before significant pneumonia symptoms have developed (*5*). However, this conclusion was not drawn in our research. On the one hand, there were few patients who underwent this treatment in our study; on the other hand, the combination of ritonavir plus lopinavir was reported to not provide sufficient benefits over standard care, including the reduction of viral RNA load (*22*). Therefore, future trials in patients with severe illness may help to confirm or exclude the possibility of a treatment benefit.

In conclusion, the use of hydroxychloroquine at an early stage is a potential therapeutic strategy for treating patients before irreversible severe respiratory complications occur. The early use of hydroxychloroquine decreased the improvement time and the duration of COVID-19 detection in throat and stool swabs.

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