

## Low dose of hydroxychloroquine reduces fatality of critically ill patients with COVID-19

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Coronavirus disease 2019 (COVID-19) is a pandemic with no specific drugs and high fatality. The most urgent need is to find effective treatments. We sought to determine whether hydroxychloroquine (HCQ) application may reduce the death risk of critically ill COVID-19 patients. In this retrospective study, we included 550 critically ill COVID-19 patients who need mechanical ventilation in Tongji Hospital, Wuhan, from February 1, 2020 to April 4, 2020. All 550 patients received comparable basic treatments including antiviral drugs and antibiotics, and 48 of them were treated with oral HCQ treatment (200 mg twice a day for 7–10 days) in addition to the basic treatments. Primary endpoint is fatality of patients, and inflammatory cytokine levels were compared between HCQ and non-hydroxychloroquine (NHCQ) treatments. We found that fatalities are 18.8% (9/48) in HCQ group, which is significantly lower than 47.4% (238/502) in the NHCQ group ( $P < 0.001$ ). The time of hospital stay before patient death is 15 (10–21) days and 8 (4–14) days for the HCQ and NHCQ groups, respectively ( $P < 0.05$ ). The levels of inflammatory cytokine IL-6 were significantly reduced from 22.2 (8.3–118.9)  $\text{pg mL}^{-1}$  at the beginning of the treatment to 5.2 (3.0–23.4)  $\text{pg mL}^{-1}$  ( $P < 0.05$ ) at the end of the treatment in the HCQ group but there is no change in the NHCQ group. These data demonstrate that addition of HCQ on top of the basic treatments is highly effective in reducing the fatality of critically ill patients of COVID-19 through attenuation of inflammatory cytokine storm. Therefore, HCQ should be prescribed as a part of treatment for critically ill COVID-19 patients, with possible outcome of saving lives.

### hydroxychloroquine, IL-6, mortalities, COVID-19

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## INTRODUCTION

Since the outbreak of infection by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the end of 2019 in Wuhan, China (Guan et al., 2020), the coronavirus disease 2019 (COVID-19) has become a worldwide pandemic, with more than 1,918,432 infections and 123,460

deaths as reported on April 14 of 2020, which have increased rapidly to more than 4,000,000 infections and 277,088 deaths on May 9 of 2020.

To combat this awful epidemic and help frontline physicians to treat patients and save lives, the World Health Organization (WHO), China, and many other countries have issued preliminary guidance on screening and diagnosis of infections in populations as well as managements of infection control. However, because of the lack of effective re-

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medy for COVID-19, the recommended treatment for acutely ill patients are severely limited, with options for compassionate managements that include the use of Chinese herbs (National Health Commission of China, 2020). Although these strategies help many patients, especially in China, severe and especially critically ill patients are with a high risk of death. Recent clinical trials on antiviral drugs such as Arbidol have not shown significant therapeutic benefit and the effect of Remdesivir still needs more evidence (Grein et al., 2020). Recent clinical observation suggests that Tocilizumab against interleukin 6 (IL-6), a major proinflammatory cytokine, to calm down the cytokine storm caused by SARS-CoV-2 infection, could lead to therapeutic effects in a portion of patients (Zhang et al., 2020). More recently, plasma of convalescent patients was used in Wuhan to manage refractory patients successfully, but the scope of this treatment is greatly constrained due to the limited supply of effective plasma.

Chloroquine (CQ) and hydroxychloroquine (HCQ) are two classic drugs (Rainsford et al., 2015), which were originally used to treat malaria. In recent years, repurpose of existing drugs such as chloroquine class of drugs for other diseases has received increasing attention (Rainsford et al., 2015; Plantone and Koudriavtseva, 2018). CQ and HCQ have been used to treat rheumatological and immunological diseases because of their inhibition of immunity and proinflammatory cytokines. CQ and HCQ also have antimicrobial effects and were used to treat infections by bacteria and viruses. Recently, HCQ was reported to be effective in inhibiting SARS-CoV-2 infection in cell-based assays (Bian et al., 2020), and treatment with HCQ was significantly associated with reduction and disappearance of viral load in COVID-19 patients in a small-sized sample (Gautret et al., 2020), which instigated new hope for an effective treatment for COVID-19.

The key to reduce fatality of COVID-19, however, is to cure critically ill patients. This study demonstrates that HCQ, a less toxic derivative of CQ, has dramatically decreased the fatality of critically ill patients of COVID-19 by attenuating the inflammatory cytokine storm.

## RESULTS

### Study patients

From February 1, 2020 to April 4, 2020, a total of 550 critically ill COVID-19 patients were admitted to Tongji Hospital, including 344 males and 206 females. The median age was 68 years old. Forty-eight patients received HCQ treatments (Shanghai Pharmaceutical CO., LTD, 200 mg/tablet) (oral 200 mg twice per day for 7–10 days), and the remaining 502 received basic treatments (non-hydroxychloroquine treatments, NHCQ). There were no difference in baseline

characteristics including age, gender, original comorbidities, as well as severity of disease between these two groups (Table 1).

### Study outcomes

In a total of 550 patients, 247 patients died (the fatality was 44.9%). Nine out of 48 HCQ-treated patients died (18.8%), while 238/502 patients from the NHCQ group died (47.4%,  $P < 0.001$ ). Furthermore, the average hospital stay time was 32 days in the HCQ group and 30 days in the NHCQ-treated patients ( $P = 0.314$ ), but the hospital stay time before death from admission was longer for the HCQ patients than that for the NHCQ patients (15 vs. 8 days,  $P = 0.027$ ), suggesting that the application of HCQ prolonged the survival time of the patients (Table 2, Figure 1A).

For the in-hospital fatality, the HCQ treatment groups did not violate the proportional hazard assumption ( $P = 0.061$ ). Thus, the proportional Cox regression was used to analyze the influence of other factors. The results showed that the use of HCQ was associated with a significantly decreased fatality risk (unadjusted HR: 0.31; 95% CI: 0.16–0.61;  $P = 0.001$ ; adjusted HR: 0.36; 95% CI: 0.18–0.75;  $P = 0.006$ ) (Table 3), which further supports that the critically ill patients can benefit substantially from HCQ treatment.

### Effect of HCQ on inflammatory cytokines

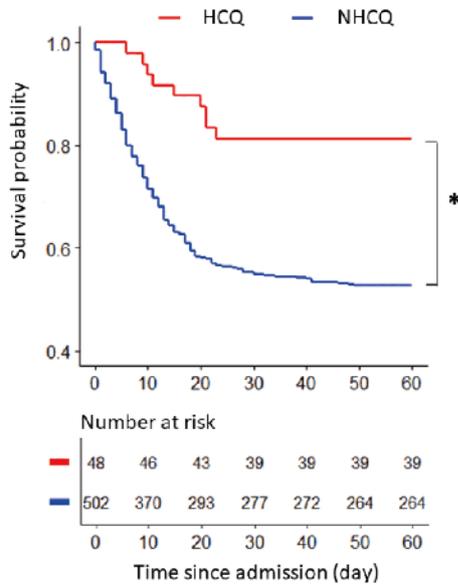
Laboratory tests showed that HCQ significantly reduced IL-6 levels in plasma 22.2 (8.3–118.9)  $\text{pg mL}^{-1}$  vs. 5.2 (3.0–23.4)  $\text{pg mL}^{-1}$  ( $P = 0.002$ ), but there is little change in NHCQ patients (21.3 (8.8–62.8)  $\text{pg mL}^{-1}$  vs. 20.2 (6.1–94.4)  $\text{pg mL}^{-1}$ ,  $P > 0.05$ ). These data suggest that HCQ treatment greatly lowered the inflammatory levels in critically ill COVID-19 patients (Figure 2). Furthermore, we analyzed the change of IL-6 levels during the hospitalization period and found that oral HCQ treatment rapidly resulted in reducing IL-6 level and kept the lower level stable during therapy period. However, after HCQ treatment stopped, the IL-6 level went up to control level (Figure 3).

Furthermore, we analyzed the effects of different IL-6 levels on death risk in response to HCQ treatment and found that when the IL-6 levels were higher than 60  $\text{pg mL}^{-1}$ , patients were more severe. For patients with IL-6 levels higher than 60  $\text{pg mL}^{-1}$ , HCQ treatment decreased IL-6 level from 100.0 (62.6–189.4)  $\text{pg mL}^{-1}$  to 28.4 (10.9–59.3)  $\text{pg mL}^{-1}$  after 5 days' treatment, and reached 9.8 (5.5–24.6)  $\text{pg mL}^{-1}$  after 10 days' treatment. In contrast, in the NHCQ-treated patients, IL-6 levels were elevated from 87.5 (72.9–240.3)  $\text{pg mL}^{-1}$  to 92.4 (34.1–199.3)  $\text{pg mL}^{-1}$  after 5 days, and reached 110.4 (31.6–235.6)  $\text{pg mL}^{-1}$  after 10 days. Moreover, for patients with IL-6 levels higher than 60  $\text{pg mL}^{-1}$ , HCQ treatment reduced the fatality from 71.2% (37/52) to

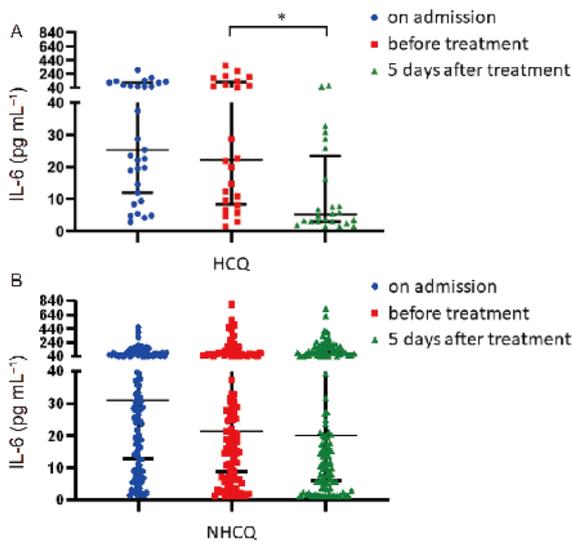
**Table 1** Baseline characteristics of critically ill COVID-19 patients<sup>a)</sup>

	All patients (n=550)	HCQ (n=48)	NHCQ (n=502)	P
Age, years	68 (59–77)	68 (60–75)	68 (59–77)	0.619
Age range, years				
≤60 (%)	139 (25.3)	11 (22.9)	128 (25.5)	0.694
>60 (%)	411 (74.7)	37 (77.1)	374 (71.9)	0.694
Gender, male (%)	344 (62.5)	32 (66.7)	312 (62.2)	0.537
Original comorbidities				
Hypertension (%)	252 (45.8)	23 (47.9)	229 (45.6)	0.760
Coronary heart disease (%)	59 (10.7)	2 (4.2)	57 (11.4)	0.147
COPD (%)	16 (2.9)	0 (0)	16 (3.2)	0.383
Diabetes (%)	94 (17.1)	12 (25.0)	82 (16.3)	0.128
Vital signs				
Body temperature, °C	36.6 (36.3–37.0)	36.7 (36.2–37.3)	36.7 (36.3–37.3)	0.704
Pulse, beats min <sup>-1</sup>	89 (80–102)	91 (80–103)	92 (81–107)	0.594
Respiratory rate, breaths min <sup>-1</sup>	20 (20–22)	25 (20–30)	21 (20–26)	0.052
Systolic blood pressure, mmHg	130 (119–144)	131 (117–149)	133 (118–148)	0.789
Diastolic blood pressure, mmHg	80 (72–89)	79 (70–90)	80 (71–88)	0.608
SpO <sub>2</sub> on admission (%)	97 (95–98)	95 (90–96)	96 (88–98)	0.216
Symptoms, number/total number (%)				
Fever	354/458 (77.3)	29/43 (67.4)	325/415 (78.3)	0.105
Cough	312/458 (68.1)	29/43 (67.4)	283/415 (68.2)	0.920
Sputum production	223/458 (48.7)	26/43 (60.5)	197/415 (47.5)	0.105
Chest tightness	65/458 (14.2)	7/43 (16.3)	58/415 (14.0)	0.680
Shortness of breath	221/458 (48.3)	30/43 (69.8)	191/415 (46.0)	0.003
Nasal congestion	4/458 (0.9)	0/43 (0)	4/415 (1.0)	1
Nausea	18/458 (3.9)	1/43 (2.3)	17/415 (4.1)	1
Diarrhea	100/458 (21.8)	9/43 (20.9)	91/415 (21.9)	0.880
Muscle aches	32/458 (0.7)	1/43 (2.3)	31/415 (7.5)	0.344
Pharynx discomfort	16/458 (3.5)	2/43 (4.7)	14/415 (3.4)	0.655
Fatigue	87/458 (19.0)	8/43 (18.6)	79/415 (19.0)	0.945
Laboratory parameters,				
White-cell count, ×10 <sup>9</sup> L <sup>-1</sup>	7.7 (5.5–11.4)	7.3 (5.3–12.1)	7.7 (5.5–11.4)	0.923
Lymphocyte count, ×10 <sup>9</sup> L <sup>-1</sup>	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.923
Neutrophil count, ×10 <sup>9</sup> L <sup>-1</sup>	6.2 (4.2–10.1)	6.3 (4.2–9.5)	6.2 (4.2–10.2)	0.909
Platelet count, ×10 <sup>9</sup> L <sup>-1</sup>	182.0 (129.8–255.0)	189.0 (137.0–257.0)	182.0 (128.5–255.0)	0.879
Hemoglobin, g L <sup>-1</sup>	125.0 (109.0–139.0)	121.0 (107.5–134.5)	125.0 (110.0–139.0)	0.424
Alanine aminotransferase, U L <sup>-1</sup>	26.0 (16.0–41.0)	30.0 (22.0–52.0)	25.0 (16.0–40.0)	0.009
Aspartate aminotransferase, U L <sup>-1</sup>	35.0 (23.0–53.0)	42.0 (20.0–60.0)	34.0 (24.0–52.0)	0.468
Total bilirubin, μmol L <sup>-1</sup>	10.8 (7.8–15.7)	11.0 (8.2–15.5)	10.7 (7.7–15.8)	0.938
Albumin, g L <sup>-1</sup>	31.4 (28.7–34.5)	31.1 (29.6–33.6)	31.5 (28.7–34.6)	0.527
Lactate dehydrogenase, U L <sup>-1</sup>	395.5 (293.0–536.0)	401.0 (311.5–493.0)	395.0 (291.5–541.0)	0.949
Creatinine, μmol L <sup>-1</sup>	81.0 (62.0–103.0)	86.0 (63.0–110.5)	79.0 (62.0–103.0)	0.440
Blood urea nitrogen, mmol L <sup>-1</sup>	6.4 (4.4–10.4)	5.9 (4.1–9.6)	6.5 (4.5–10.7)	0.392
International normalized ratio	1.1 (1.1–1.3)	1.1 (1.1–1.2)	1.2 (1.1–1.3)	0.034
D-dimer, mg L <sup>-1</sup>	2.3 (1.1–9.6)	2.6 (1.0–10.1)	2.3 (1.1–9.7)	0.895
APTT, s	40.3 (36.6–45.4)	39.0 (36.2–50.7)	40.4 (36.6–44.9)	0.962
C-reactive protein, mg L <sup>-1</sup>	75.7 (36.3–128.9)	84.1 (27.5–117.4)	75.6(37.1–131.5)	0.789
NT-ProBNP, pg mL <sup>-1</sup>	598.5 (223.5–1814.3)	600.5 (191.8–1926.5)	598.5 (224.5–1829.8)	0.803
cTnI, pg mL <sup>-1</sup>	15.9 (6.2–59.5)	12.1 (6.5–47.2)	16.3 (6.2–60.7)	0.582
IL-6, pg mL <sup>-1</sup>	37.4 (14.0–96.1)	25.3 (12.0–111.1)	31.6 (14.0–95.9)	0.593
IL-10, pg mL <sup>-1</sup>	6.5 (5.0–12.7)	6.8 (5.0–9.3)	6.5 (5.0–13.5)	0.706
IL-8, pg mL <sup>-1</sup>	22.4 (12.0–43.8)	23.1 (10.4–30.8)	22.2 (12.1–47.5)	0.471
TNF-α, pg mL <sup>-1</sup>	10.6 (8.2–14.6)	11.0 (9.1–13.7)	10.4 (8.2–14.8)	0.596
IL-1β, pg mL <sup>-1</sup>	5.0 (5.0–5.0)	5.0 (5.0–5.3)	5.0 (5.0–5.0)	0.524
IL-2R, U mL <sup>-1</sup>	1026.0 (679.0–1501.0)	977.5 (749.0–1544.8)	1026.0 (672.0–1490.5)	0.592
Oxygen therapy, number (%)	545 (99.1)	47 (97.9)	498 (99.2)	0.368
Mechanical ventilation, number (%)	349 (63.5)	28 (58.3)	321 (63.9)	0.441

a) Mechanical ventilation contained non-invasive ventilation and invasive ventilation. Data are presented as medians and interquartile range (Q1–Q3). HCQ, hydroxychloroquine treatment; NHCQ, non-hydroxychloroquine treatment; COPD, chronic obstructive pulmonary disease; APTT, activated partial thromboplastin time; NT-proBNP, N-terminal pro-B-type natriuretic peptide; cTnI, cardiac troponin I; IL, interleukin; TNF-α, tumor necrosis factor-α; SpO<sub>2</sub>, percutaneous oxygen saturation.

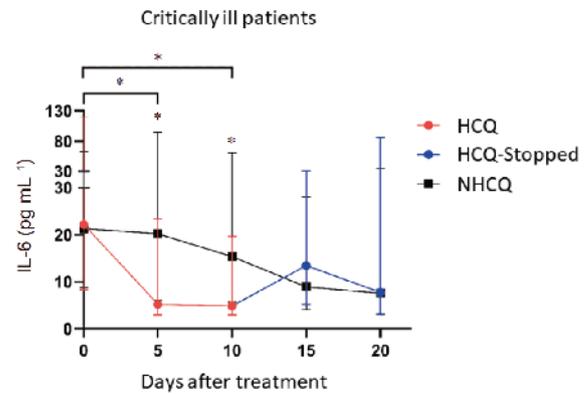


**Figure 1** Kaplan-Meier curve of COVID-19 patients treated with or without HCQ. HCQ treatment significantly reduced the fatality of critically ill COVID-19 patients compared with NHCQ treated patients ( $*P<0.001$ ).



**Figure 2** Effects of HCQ treatment on plasma levels of IL-6. HCQ treatment significantly decreased the levels of IL-6 in about 5 (4–8) days,  $*P<0.05$  (A). The levels of IL-6 were not significantly decreased in NHCQ treatment group at the same observation time (B). Data are presented as medians and interquartile range (Q1–Q3).

44.4% (4/9) in the control ( $P>0.05$ ). For patients with IL-6 levels lower than  $60 \text{ pg mL}^{-1}$  before treated with HCQ, HCQ decreased IL-6 levels from  $12.8 (8.2\text{--}23.5) \text{ pg mL}^{-1}$  to  $3.5$



**Figure 3** Continuous curves of plasma levels of IL-6 showing that the consistency of HCQ treatment period with lowering IL-6 levels,  $*P<0.05$ . Data are presented as medians and interquartile range (Q1–Q3).

$(2.7\text{--}5.6) \text{ pg mL}^{-1}$  after 5 days, and reached  $3.0 (2.1\text{--}14.4) \text{ pg mL}^{-1}$  after 10 days. In the NHCQ patients, IL-6 levels decreased only slightly from  $15.0 (6.4\text{--}26.2) \text{ pg mL}^{-1}$  to  $13.8 (5.7\text{--}43.1) \text{ pg mL}^{-1}$  after 5 days, and reached  $10.8 (5.1\text{--}50.0) \text{ pg mL}^{-1}$  after 10 days. The fatality was 6.3% (1/16) in HCQ patients and 21.3% (27/127) in NHCQ group ( $P>0.05$ ) for these patients with initial IL-6 levels lower than  $60 \text{ pg mL}^{-1}$ . These results indicated that the patients with higher IL-6 levels were severer and had higher fatality than those with lower IL-6 levels, and HCQ had stronger effect on reducing IL-6 levels in these severer patients.

We also analyzed the effect of HCQ treatment starting time after admission on curative response and found that the median time to start HCQ treatment was 10 (3–13) days after admission. Interestingly, the early start of HCQ treatment (within 5 days after admission) resulted in lower fatality than late start of HCQ treatment (5 days after admission, 1/11 (9.1%) vs. 8/37 (21.6%)), which suggests that HCQ treatment should be started earlier after admission, although the result is not statistically significant ( $P>0.05$ ).

## DISCUSSION

This retrospective study found that HCQ treatment was associated significantly with the reduced fatality of critically ill patients with COVID-19 and greatly lowered the levels of IL-6, one of the most inflammatory cytokines. We also found that the time period that led to death in the HCQ-treated

**Table 2** Comparison of clinical outcomes between HCQ-treated and NHCQ-treated patients<sup>a)</sup>

	HCQ	NHCQ	<i>P</i>
Total patients, <i>n</i>	48	502	
Dead patients, <i>n</i> (%)	9 (18.8)	238 (47.4)	<0.001
Hospital stay time before death (d)	15 (10–21)	8 (4–14)	0.027

a) Data are presented as medians and interquartile range (Q1–Q3). HCQ, hydroxychloroquine treatment; NHCQ, non-hydroxychloroquine treatment.

**Table 3** Univariable and multivariable cox proportional hazards model for 60-day fatality after HCQ treatment<sup>a)</sup>

	Crude HR (95% CI), <i>P</i>	Adjusted HR (95% CI), <i>P</i> <sup>*</sup>
HCQ-treated	0.31 (0.16–0.61), 0.001	0.36 (0.18–0.75), 0.006
Age	1.02 (1.01–1.03), <0.001	1.02 (1.01–1.03), 0.004
Gender	1.45 (1.11–1.9), 0.006	1.64 (1.18–2.29), 0.003
Hypertension	0.73 (0.57–0.94), 0.017	0.77 (0.56–1.05), 0.093
Coronary heart disease	1.21 (0.84–1.76), 0.313	1.11 (0.71–1.72), 0.656
COPD	1.12 (0.55–2.26), 0.761	0.77 (0.31–1.93), 0.583
Diabetes	0.64 (0.45–0.93), 0.018	0.57 (0.35–0.92), 0.023
Body temperature	1.34 (1.16–1.56), <0.001	1.3 (1.09–1.54), 0.003
Pulse	1 (1–1), 0.526	–
Respiratory rate	1 (1–1.01), 0.349	–
Systolic blood pressure	1 (0.99–1), 0.401	–
Diastolic blood pressure	1 (0.99–1), 0.104	–
SpO <sub>2</sub> on admission	0.96 (0.95–0.97), <0.001	0.97 (0.96–0.99), <0.001
Fever	0.87 (0.63–1.19), 0.384	–
Cough	0.79 (0.6–1.06), 0.118	–
Shortness of breath	1.05 (0.8–1.39), 0.709	–
Oxygen therapy	0.93 (0.23–3.73), 0.915	0.14 (0.03–0.63), 0.01
Mechanical ventilation	7.4 (4.88–11.2), <0.001	8.07 (4.83–13.47), <0.001

a) \*, The variables with a *P* value less than 0.01 in the univariable Cox models, and those representing the history of diseases were included into the multivariable analysis. HCQ, hydroxychloroquine treatment.

group is longer, suggesting that HCQ prolonged the survival time of critically ill patients even if their lives were eventually lost, which further supports that HCQ has therapeutic effects for critically ill patients with COVID-19. Importantly, we found that the role of HCQ in lowering plasma IL-6 levels was highly consistent with the duration of its administration, and once the drug was stopped, plasma IL-6 levels returned to the control levels.

We have noticed that HCQ has *in vitro* antiviral effects on several viruses, including SARS and SARS-CoV-2 (Al-Bari, 2015; Bian et al., 2020; Gautret et al., 2020; Yao et al., 2020), which may contribute directly to its therapeutic effect on COVID-19 patients. However, the anti-SARS-CoV2 action of HCQ requires a much higher concentration of HCQ than the dose used in this study, and cannot account for the significant efficiency on critically ill patients in our cohort.

COVID-19 patients displayed apparent immunity disorders, showing marked reduction in lymphocyte numbers in peripheral blood and lymphatic tissues, but large amounts of lymphocyte infiltration in lungs as well as in other critical organs such as the heart. Many patients showed symptoms of cytokine storm with markedly elevated levels of the proinflammatory cytokine IL-6, suggesting over-activated immune responses (Bian et al., 2020). Anti-IL-6 antibody, Tocilizumab, has been shown to be an effective treatment option for COVID-19 patients with a risk of cytokine storms (Luo et al., 2020). In this study, we demonstrate that HCQ can mimic the effect of anti-IL-6 antibody by greatly redu-

cing the levels of IL-6 in the critically ill COVID-19 patients. In addition, HCQ can modulate human inflammatory macrophage polarization via downregulating M1 but upregulating M2 macrophages (Shiratori et al., 2018; Sarzi-Puttini et al., 2020), and inhibit proinflammatory cytokines through inhibition of lysosomal-autophagy pathways (He et al., 2011) and formation of double membrane vesicles (He et al., 2011; Plantone and Koudriavtseva, 2018), a process required for genome replication by the SARS coronavirus replication complex (Snijder et al., 2006). Thus, the anti-inflammatory action of HCQ in combination with its inhibitory activity of viral replication may contribute greatly to its therapeutic effects on critically ill COVID-19 patients. In addition, tissue distribution of HCQ is unique and favors therapy of pneumonia because it has much higher concentrations in the lungs (McChesney et al., 1967). Importantly, the CQ class of drugs has been shown to repress inflammation with great synergy with low concentrations of glucocorticoids, one of the most potent classes of anti-inflammatory drugs, pointing to the possibility of combined therapy of HCQ with a low dose of glucocorticoids (He et al., 2011).

Additionally, lower plasma levels of IL-6 after HCQ treatment are highly correlated with the application period of HCQ. IL-6 levels returned quickly to the levels of NHCQ patients after HCQ treatment was stopped. This finding indicates that we need to adjust our treatment regimen by extending the use of HCQ until patients are completely cured.

We also noticed a couple of negative reports about CQ and

HCQ (Geleris et al., 2020; Mahevas et al., 2020; Magagnoli et al., 2020). However, based on the results in these studies, it is very likely that they used high doses of HCQ, which might induce cardiotoxicity and death. They might also have used high doses of HCQ as antiviral agents rather than for anti-inflammation. In contrast, the dose of HCQ used in the present study is much lower than that in previous studies but is the same as used for treating inflammatory diseases, and thus HCQ for COVID-19 patients works more likely as an agent for anti-inflammation and immunomodulation, rather than as a direct antiviral agent. Recently, Geleris et al. (2020) reported their observation study, showing that there was no significant association between hydroxychloroquine use and intubation or death. By carefully re-analyzing this report, however, we noticed that in this study, HCQ-treated patients were more severely ill at baseline (even if after propensity score-matched) than NHCQ-treated patients. In addition, the time of treatment for patients is too short (5 days) to show the efficacy of HCQ. Another concern of this study is that about 60% HCQ-treated patients received azithromycin simultaneously, which increases risk of QT-interval prolongation and sudden death.

In summary, this retrospective study demonstrates that HCQ significantly reduces death risk of critically ill COVID-19 patients without apparent toxicity, and its mechanism of action is likely mediated through its inhibition of inflammatory cytokines on top of its ability in inhibiting viral replication. Therefore, despite the need for randomized-double blind control study, HCQ should be considered as an additional option to treat critically ill COVID-19 patients, which could save lives during the current COVID-19 pandemic. Although this retrospective study was performed on critically ill COVID-19 patients, HCQ could also be an option for patients at an early stage considering its safety records and the long history of its use in treating malaria infections. This study has some limitations. For example, it is a retrospective study and HCQ treatment group only has 48 cases, much less than the number in NHCQ group. Therefore, large-scale random clinical studies are necessary for the treatment of COVID-19 by HCQ.

## METHODS

### Study design

This investigation is a retrospective study involving critically ill patients with COVID-19 in Tongji Hospital, Wuhan, China. These patients were diagnosed according to the WHO interim guideline and the Clinical Guideline for COVID-19 Diagnosis and Treatment published by the National Health Commission of China between February 1, 2020 and April 4, 2020. This study has been approved by the institutional review board of Tongji Hospital (IRBID: TJ-C20200113). The

written informed consent has been waived by the Ethics Committee because of the retrospective and anonymous nature of the data.

### Patients' information

All patients have medical history and imaging characteristics of COVID-19 and have been confirmed with SARS-CoV-2 infection by laboratory tests. In addition, all patients were confirmed by chest computed tomography (CT) and SARS-CoV-2 pathogenic test. The inclusion as critically ill patients had to meet one of the following criteria: (i) patients had respiratory failure and needed mechanical ventilation; (ii) patients had septic shock during hospitalization; (iii) patients with other organ failures that required monitoring and treatment by intensive care unit. In this study, we included all critically ill adult patients (age > 18 years old) with hospitalization during the epidemic period from February 1, 2020 to April 4, 2020.

The baseline treatments were comparable for these two groups, including application of antiviral drugs (Lopinavir and Ritonavir, Entecavir hydrate, or Ribavirin) with 41.7% and 42.0% patients in HCQ and NHCQ, respectively ( $P=0.96$ ); intravenous immunoglobulin in 52.1% patients in HCQ and 48.6% patients in NHCQ, respectively ( $P=0.65$ ); immunoenhancer in 16.7% patients in HCQ and 17.7% patients in NHCQ, respectively ( $P=0.86$ ); antibiotics in 77.1% patients in HCQ and 87.1% patients in NHCQ, respectively ( $P=0.06$ ), but interferon application in 0% patients in HCQ and 10.8% patients in NHCQ ( $P=0.01$ ).

### Data collection of patients

Health care data of COVID-19 patients, including medical history, chest CT, and laboratory tests, in-hospital therapies, and clinical deposits (death or cured discharge), were extracted by data coordinators through the electronic medical records. Laboratory test results included inflammatory cytokines and counts of white blood cells in peripheral blood.

### Statistical analysis

Continuous values were expressed as medians and interquartile range (Q1–Q3) and categorical variables as counts and percentages. The comparisons of continuous values between groups were performed with Wilcoxon rank-sum tests. For paired continuous variables, paired sample Wilcoxon rank test was used. Categorical variables were compared using Chi-square test. Survival curves were described by Kaplan-Meier method and compared with the log-rank test. For the test of primary null hypothesis, the hazard ratios and 95% confidence interval were calculated by proportional Cox regression models if Schoenfeld individual test did not

show a violation of proportional-hazards assumption and extended Cox model where treatment groups were included as a time-varying covariate if they did. All statistical analyses were performed with R packages (version 3.1.4, Vienna, Austria).  $P < 0.05$  was considered to indicate statistical significance, and all comparisons were two-sided.

**Compliance and ethics** *The author(s) declare that they have no conflict of interest. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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