Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: The RECOVERY Collaborative Group. Effect of hydroxychloroquine in hospitalized patients with Covid-19. N Engl J Med. DOI: 10.1056/NEJMoa2022926

Effect of Hydroxychloroquine in Hospitalized Patients with COVID-19

SUPPLEMENTARY APPENDIX

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Supplementary Methods

Study organization

The RECOVERY trial is an investigator-initiated, individually randomized, open-label, controlled trial to evaluate the efficacy and safety of a range of putative treatments in patients hospitalized with COVID-19. The protocol is available at NEJM.org. The trial was conducted at 176 National Health Service (NHS) hospital organizations in the United Kingdom. The trial was coordinated by a team drawn from the Clinical Trial Service Unit and the National Perinatal Epidemiology Clinical Trials Unit within the Nuffield Department of Population Health at University of Oxford, the trial sponsor. Support for local site activities was provided by the National Institute for Health Research Clinical Research Network.

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Protocol changes

RECOVERY is a randomized trial among patients hospitalized for COVID-19. All eligible patients receive usual standard of care in the participating hospital and are randomly allocated between no additional treatment and one of several active treatment arms. Over time, additional treatment arms have been added (see Table). In version 4.0 of the protocol, a second randomization was introduced for those trial participants with hypoxia (oxygen saturation <92% on air or receiving oxygen) and inflammation (C-reactive protein ≥75 mg/dL), comparing the addition of tocilizumab vs. control on top of the treatment assigned in the first randomization. In version 6.0, a factorial design was introduced to the first randomization such that participants were also randomized to convalescent plasma vs. no additional treatment. As outlined in the protocol, if one or more of the active treatments was not available at the hospital or is believed, by the attending clinician, to be contraindicated (or definitely indicated) for the specific patient, then random allocation was between the remaining treatment arms.

The original and final protocol are included in the supplementary material to this publication, together with summaries of the changes made.

Table. Protocol changes to treatment comparisons

Protocol version	Date	Randomization	Treatment arms
1.0	13-Mar-2020	Main (part A)	No additional treatment Lopinavir-ritonavir Low-dose corticosteroid Nebulised Interferon-ß-1a (never activated)
2.0	23-Mar-2020	Main (part A)	No additional treatment Lopinavir-ritonavir Low-dose corticosteroid Hydroxychloroquine

Protocol version	Date	Randomization	Treatment arms
3.0	07-Apr-2020	Main (part A)	No additional treatment Lopinavir-ritonavir Low-dose corticosteroid Hydroxychloroquine Azithromycin
4.0	14-Apr-2020	Main (part A) Second[a]	No additional treatment Lopinavir-ritonavir Low-dose corticosteroid Hydroxychloroquine Azithromycin No additional treatment Tocilizumab
5.0	24-Apr-2020	-	(no change – extension to children <18 years old)
6.0	14-May-2020	Main (part A) Main (part B factorial) Second[a]	No additional treatment Lopinavir-ritonavir Low-dose corticosteroid[b] Hydroxychloroquine[c] Azithromycin No additional treatment Convalescent plasma No additional treatment Tocilizumab

[a] for patients with (a) oxygen saturation <92% on air or requiring oxygen or children with significant systemic disease with persistent pyrexia; and (b) C-reactive protein ≥75 md/dL)
[b] enrolment of adults ceased 8 June 2020 as more than 2,000 patients had been recruited to the active arm
[c] enrolment ceased 5 June 2020 when the Data Monitoring Committee advised that the Chief Investigators review the unblinded data.

Selection of hydroxychloroquine dose

The hydroxychloroquine dose regimen was based on previous pharmacokinetic modelling of plasma and whole blood hydroxychloroquine concentrations in healthy volunteers, the treatment of malaria and in rheumatological conditions.[1] The choice of dose and predicted safety margins were also informed by pharmacometric studies of chloroquine in the treatment of both severe and uncomplicated malaria and in self-poisoning.[2] In-vitro studies suggest that high concentrations of hydroxychloroquine are required for maximal effects, although inhibitory concentrations derived from static Vero cell cultures are likely to provide, at best, an approximate guide to required in-vivo concentrations.[3] Hydroxychloroquine plasma concentrations in short course regimens are determined primarily by distribution rather than elimination.[1] We reasoned that the target respiratory epithelium was likely to be in a dynamic equilibrium with free plasma concentrations. The objective therefore was to design a regimen that provided free plasma concentrations that were as high as safely possible throughout the treatment period. As a parenteral formulation is not generally available, dosing was designed around currently available hydroxychloroquine sulfate tablets (200mg salt: 155 mg base equivalent). To achieve loading while allowing adequate

distribution, the loading doses (4 tablets) were given at 0 and 6 hours and from 12 hours maintenance doses (2 tablets) were given 12 hourly.

The dosing regimen was based on pharmacometric modelling:[1] All pharmacokinetic models were coded and simulated using the pharmacometric software NONMEM v.7.4.3 (Icon Development Solution, Ellicott City, MD). A small study in healthy volunteers was used for dose simulations[4], reporting a 3-compartment disposition model with a terminal elimination half-life of 50 days. Reported true coefficients and exponents were used to derive mean pharmacokinetic parameters for simulations. Both short course treatments[5] and repeated dosing to steady-state[6] were simulated, to ensure that model-derived concentrations captured the reported drug measurements, resulting in a relative bioavailability parameter of 60% to scale model predictions to reported concentrations. A fixed value of 30% between-patient variability was added exponentially in all parameters in order to capture the approximately 4- to 5-fold variability seen in observed whole blood measurements. Allometric scaling of clearance (exponent of 0.75) and volume (exponent of 1) parameters was implemented in order to simulate different weight groups. A total of 1,000 stochastic simulations were performed and presented as median values and 95% prediction intervals.[1]

Supplementary statistical methods

Sample size

As stated in the protocol, appropriate sample sizes could not be estimated when the trial was being planned at the start of the COVID-19 pandemic. As the trial progressed, the Trial Steering Committee, blinded to the results of the study treatment comparisons, formed the view that if 28-day mortality was 20% then a comparison of at least 2000 patients allocated to active drug and 4000 to usual care alone would yield at least 90% power at two-sided P=0.01 to detect a proportional reduction of one-fifth (a clinically relevant absolute difference of 4 percentage points between the two arms).

Baseline-predicted risk

Baseline−predicted risk of 28-day mortality was estimated through the formula 100 x exp(a)/(1 + exp(a)), where a = −1.23 − 2.85 (if age <50) − 2.03 (if age 50−59) − 1.21 (if age 60−69) − 0.51 (if age 70−79) + 0.42 (if male) − 0.34 (if >7 days since symptom onset) + 0.86 (if on oxygen only at randomization) + 2.18 (if on invasive mechanical ventilation at randomization) − 0.01 (if history of diabetes) + 0.22 (if history of heart disease) + 0.21 (if history of chronic lung disease) + 0.50 (if history of kidney disease). These regression coefficients were derived from a multivariable logistic regression model using data from all trial participants who (at the time of data-lock) had complete 28-day mortality follow-up data. The regression model additionally adjusted for treatment allocation (with usual care designated the reference category) and for all possible two-way interactions between the above baseline characteristics and treatment allocation. These additional terms were ignored when calculating baseline-predicted risk, however, in order to ensure that the estimates corresponded to risk *if assigned usual care*. Patients were then subdivided into three approximately equally-sized groups (across all RECOVERY participants) on the basis of their predicted risk: <30%, ≥30% to <45%, and ≥45%.

Calculation of rate ratio

The RR is derived from the log-rank observed minus expected statistic (O – E) and its variance (V) as the one-step estimate, through the formula exp([O – E] ÷ V), and its 95% CI is given by exp([O – E] ÷ V ± 1.96 ÷ √V).

Ascertainment and classification of study outcomes

Information on baseline characteristics and study outcomes was collected through a combination of electronic case report forms (see below) completed by members of the local research team at each participating hospital and linkage to National Health Service, clinical audit, and other relevant health records. Full details are provided in the RECOVERY Definition and Derivation of Baseline Characteristics and Outcomes Document which was published online (www.recoverytrial.net) on 9 June 2020.

Randomization form

The Randomization form (shown below) was completed by trained study staff. It collected baseline information about the participant (including demographics, COVID-19 history, comorbidities and suitability for the study treatments) and availability of the study treatments. Once completed and electronically signed, the treatment allocation was displayed.

The following modifications were made to the Randomization form during the trial:

Randomization form version	Date of release	Major modifications from previous version
1.0	19-Mar-20	Initial version (protocol V1.0)
2.0	25-Mar-20	For protocol V2.0 • Hydroxycholoroquine added as treatment • Known long QT syndrome added to comorbidities • Severe depression removed from comorbidities
3.0	09-Apr-20	For protocol V3.0 • Azithromycin added as treatment • Suspected SARS-CoV-2 infection included in eligibility criteria
[Second randomization form introduced]	23-Apr-20	For protocol 4.0 • Eligibility criteria for second randomization • Tocilizumab vs control as treatment allocations
4.0	09-May-20	For protocol V5.0 • Age ≥18 years removed from eligibility criteria • Additional questions on child's age and weight added
5.0	21-May-20	For protocol V6.0 • Convalescent plasma added as treatment
6.0	28-May-20	Baseline use of remdesivir

RECOVERY
Randomised Evaluation of COVID-19 Therapy

Test version only (v6.08 - 05/06/20)

Randomisation Program

Call Freefone **0800 138 5451** to contact the RECOVERY team for **URGENT** problems using the Randomisation Program or for medical advice.
All **NON-URGENT queries** should be emailed to **recoverytrial@ndph.ox.ac.uk**

Logged in as: **Barts Health NHS Trust**

Section A: Baseline and Eligibility

Date and time of randomisation: 5 Jun 2020 13:32

Treating clinician

A1. Name of treating clinician

Patient details

A2. Patient surname

Patient forename

A3. NHS number ☐ Tick if not available

A4. What is the patient's date of birth? / /

A5. What is the patient's sex?

Inclusion criteria

A6. Has consent been taken in line with the protocol?
If answer is No patient cannot be enrolled in the study

A7. Does the patient have proven or suspected SARS-CoV-2 infection?
If answer is No patient cannot be enrolled in the study

A8. Does the patient have any medical history that might, in the opinion of the attending clinician, put the patient at significant risk if they were to participate in the trial?

A8B. Is the patient willing to receive convalescent plasma?

A9. COVID-19 symptom onset date: / /

A10. Date of hospitalisation: / /

A11. Does the patient require oxygen?

A12. Does the patient **CURRENTLY** require ventilation or ECMO?
Invasive mechanical ventilation or extra-corporeal membrane oxygenation

Does the patient have any CURRENT comorbidities or other medical problems?

A13.1 Diabetes

A13.2 Heart disease

A13.3 Chronic lung disease

A13.4 Tuberculosis

A13.5 HIV

A13.6 Severe liver disease

A13.7 Severe kidney impairment (eGFR<30 or on dialysis)

A13.8 Known long QT syndrome

A13.9 Current treatment with macrolide antibiotics which are to continue
Macrolide antibiotics include clarithromycin, azithromycin and erythromycin

A13.10 Previous adverse reaction to blood or blood product transfusion

Are the following treatments UNSUITABLE for the patient?
If you answer Yes it means you think this participant should NOT receive this drug.

A14.1 Lopinavir-Ritonavir

A14.3 Azithromycin

A14B.1 Convalescent plasma

Are the following treatments available?

A15.1 Lopinavir-Ritonavir

A15.3 Azithromycin

A15B.1 Convalescent plasma

Current medication

A16 Is the patient currently prescribed remdesivir?

Please sign off this form once complete

Surname:

Forename:

Professional email:

Continue

Cancel

Home

Follow-up form

The Follow-up form (shown on the next page) collected information on study treatment adherence (including both the randomised allocation and use of other study treatments), vital status (including date and provisional cause of death if available), hospitalisation status (including date of discharge), respiratory support received during the hospitalisation, occurrence of any major cardiac arrhythmias and renal replacement therapy received.

The following modifications were made to the Follow-up form during the trial:

Follow-up form version	**Date of release**	**Modifications from previous version**
1.0	30-Mar-20	Initial version
2.0	09-Apr-20	Information on other treatments used during admission: • Azithromycin, IL-6 receptor antagonist Fact and result of SARS-CoV-2 PCR test
3.0	09-Apr-20	Update to functionality; no changes to questions
4.0	23-Apr-20	Duration of treatments added
5.0	12-May-20	Capture of major cardiac arrhythmias added
6.0	28-May-20	Updates to wording of questions. Information on other treatments used during admission: • Remdesivir, convalescent plasma

Hydroxychloroquine for COVID-19

Follow-up

Date of randomisation

Patient's date of birth

yyyy-mm-dd

1. Which of following treatment(s) did the patient definitely receive as part of their hospital admission after randomisation? *

(NB Include RECOVERY study-allocated drug, only if given, PLUS any of the other treatments if given as standard hospital care)

- [ ] No additional treatment
- [ ] Lopinavir-ritonavir
- [ ] Corticosteroid (dexamethasone, prednisolone or hydrocortisone)
- [ ] Hydroxychloroquine
- [ ] Azithromycin or other macrolide (eg, clarithromycin, erythromycin)
- [ ] Tocilizumab or sarilumab
- [ ] Remdesivir

The following questions only appear if the treatments have been allocated at randomisation

Please select number of days the patient received lopinavir-ritonavir

1 2 3 4 5 6 7 8 9 10

Please select number of days the patient received corticosteroid (dexamethasone, prednisolone or hydrocortisone)

1 2 3 4 5 6 7 8 9 10

Please select number of days the patient received hydroxychloroquine

1 2 3 4 5 6 7 8 9 10

Please select number of days the patient received azithromycin

This question and the following question cannot both be zero

0 1 2 3 4 5 6 7 8 9 10

Please select number of days the patient received other macrolides (eg, clarithromycin, erythromycin)

0 1 2 3 4 5 6 7 8 9 10

Please select number of doses of tocilizumab or sarilumab the patient received

1 >1

Please select number of days the patient received remdesivir

1 2 3 4 5 6 7 8 9 10

» Convalescent Plasma

How many convalescent plasma infusions did the patient receive?

This is plasma given as part of trial, not any standard fresh frozen plasma or other blood products that the patient may have been given

0 1 2

Were any infusions stopped early for any reason ie, the patient did not receive the full amount?

Yes No

How many were stopped early?

1 2

» Health Status

2. Was a COVID-19 test done for this patient?

(If multiple tests were done, and the results were positive and negative, please tick Yes – positive result and Yes – negative result)

- [ ] Yes – positive result
- [ ] Yes – negative result
- [ ] Not done

3. What is the patient's vital status? *

- Alive
- Dead

3.1 What is the patient's current hospitalisation status?

Q3.1 is only completed if the patients is alive at Q3

- Inpatient
- Discharged

The patient has been enrolled in the trial for **NaN** days

3.1.1 Date follow-up form completed **Q3.1.1 is only completed if patient is still an inpatient at Q3**

yyyy-mm-dd

3.1.1 What was the date of discharge? **Q3.1.1 is only completed if patient has been discharged at Q3**

yyyy-mm-dd

3.1 What was the date of death? **Q3.1.1 is only completed if patient has died at Q3** *

yyyy-mm-dd

3.2 What was the underlying cause of death? *

This can be obtained from the last entry in part 1 of the death certificate

- ◯ COVID-19
- ◯ Other infection
- ◯ Cardiovascular
- ◯ Other

Please give details

4. Did the patient require any form of assisted ventilation (ie, more than just supplementary oxygen)? *

- ◯ Yes
- ◯ No

Please answer the following questions:

4.1 For how many days did the patient require assisted ventilation? *

4.2 What type of ventilation did the patient receive?

	Yes	No	Unknown
CPAP alone	◯	◯	◯
Non-invasive ventilation (eg, BiPAP)	◯	◯	◯
High-flow nasal oxygen (eg, AIRVO)	◯	◯	◯
Mechanical ventilation (intubation/tracheostomy)	◯	◯	◯

ECMO	◯	◯	◯

Total number of days the patient received invasive mechanical ventilation (intubation/tracheostomy) (from randomisation until discharge/death/28 days after randomisation)

Complete if invasive mechanical ventilation (intubation/tracheostomy) is Yes

5. Has the participant been documented to have a NEW cardiac arrhythmia at any point since the main randomisation?

- ◯ Yes
- ◯ No
- ◯ Unknown

5.1 Please select all of the following which apply

If Q5 is answered Yes, you must select at least one option here

- ☐ Atrial flutter or atrial fibrillation
- ☐ Supraventricular tachycardia
- ☐ Ventricular tachycardia (including torsades de pointes)
- ☐ Ventricular fibrillation
- ☐ Atrioventricular block requiring intervention (eg, cardiac pacing)

6. Did the patient require use of renal dialysis or haemofiltration?

- ◯ Yes
- ◯ No

7. Please enter UKOSS case ID if known *

Enter the full UKOSS case ID ie, COR_123

Complete only if patient was pregnant at randomisation

(select if you do not know the UKOSS case ID)

- ☐ Not known

Cause of death

Cause of death was recorded by the site staff on the Follow-up form. In addition, information about cause of death was obtained from death registration data in England, Wales and Scotland. Where cause of death information was available from both sources, the underlying cause of death from the death registration data was used (in preference to what was recorded on the Follow-up form). In the death registration data, the underlying cause of death is based on the death certificate information completed by the certifying doctor and is recorded using International Classification of Disease 10 codes. These were grouped into relevant categories as described in the Recovery Definition and Derivation of Baseline Characteristics and Outcomes document (see www.recoverytrial.net).

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1. White NJ, Watson JA, Hoglund RM, Chan XHS, Cheah PY, Tarning J. COVID-19 prevention and treatment: a critical analysis of chloroquine and hydroxychloroquine clinical pharmacology. *PLoS Med* 2020; **In Press**.
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3. Yao X, Ye F, Zhang M, 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; ciaa237
4. Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. *Br J Clin Pharmacol* 1989; **27**(6): 771-9.
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6. Carmichael SJ, Charles B, Tett SE. Population pharmacokinetics of hydroxychloroquine in patients with rheumatoid arthritis. *Ther Drug Monit* 2003; **25**(6): 671-81.

Supplementary Tables

Table S1: Baseline characteristics of patients considered unsuitable for randomization to hydroxychloroquine compared with those randomized to hydroxychloroquine versus usual care

	Randomized (n=4716)	Considered unsuitable (n=3199)
Age, years	65.4 (15.3)	67.3 (16.1)
<70	2798 (59.3%)	1712 (53.5%)
≥70 to <80	972 (20.6%)	678 (21.2%)
≥80	946 (20.1%)	809 (25.3%)
Sex		
Male	2934 (62.2%)	2017 (63.1%)
Female	1782 (37.8%)	1182 (36.9%)
Race		
White	3479 (73.8%)	2381 (74.4%)
BAME	857 (18.2%)	508 (15.9%)
Unknown	380 (8.1%)	310 (9.7%)
Number of days since symptom onset	9 (5-13)	8 (4-12)
Number of days since hospitalization	3 (1-5)	2 (1-4)
Respiratory support received		
No oxygen received	1112 (23.6%)	834 (26.1%)
Oxygen only	2811 (59.6%)	2043 (63.9%)
Invasive mechanical ventilation	793 (16.8%)	322 (10.1%)
Previous diseases		
Diabetes	1283 (27.2%)	918 (28.7%)
Heart disease	1211 (25.7%)	1020 (31.9%)
Chronic lung disease	1046 (22.2%)	758 (23.7%)
Tuberculosis	13 (0.3%)	13 (0.4%)
HIV	21 (0.4%)	19 (0.6%)
Severe liver disease	64 (1.4%)	76 (2.4%)
Severe kidney impairment	372 (7.9%)	330 (10.3%)
Any of the above	2689 (57.0%)	1995 (62.4%)

Results are count (%), mean ± standard deviation, or median (inter-quartile range). The 'oxygen only' group includes non-invasive ventilation. Severe liver disease defined as requiring ongoing specialist care. Severe kidney impairment defined as estimated glomerular filtration rate <30 mL/min/1.73m².

Table S2: Treatments given, by randomized allocation

	Treatment allocation	
	Hydroxychloroquine (n=1561)	**Usual care (n=3155)**
Compliance data available	1553	3140
Hydroxychloroquine received	1430 (92.1%)	12 (0.4%)
Other treatments received		
Dexamethasone	142 (9.1%)	288 (9.2%)
Lopinavir-Ritonavir	2 (0.1%)	6 (0.2%)
Azithromycin or other macrolides	289 (18.6%)	638 (20.3%)
Tocilizumab or sarilumab	34 (2.2%)	84 (2.7%)
Remdesivir	1 (0.1%)	2 (0.1%)
Not recorded	3 (0.2%)	0 (0.0%)

Percentages are of those with a completed follow-up form. Remdesivir only became available for use in the UK under the Medicines & Healthcare Products Regulatory Agency Emergency Access to Medicines Scheme on 26 May 2020, 13 days prior to closure of the hydroxychloroquine arm of the study.

Of those allocated hydroxychloroquine who received at least one dose, 69% received it either every day or nearly every day they were in hospital (missing at most 1 dose) while 84% received it on at least half the days they were in hospital.

Table S3: Effect of allocation to hydroxychloroquine on cause-specific 28-day mortality

Cause of death	Treatment allocation		Absolute percent difference (SE)
	Hydroxychloroquine (n=1561)	Usual care (n=3155)	
COVID	374 (24.0%)	743 (23.5%)	0.4 (1.32)
Other infection	8 (0.5%)	5 (0.2%)	0.4 (0.19)
Cardiac	9 (0.6%)	4 (0.1%)	0.4 (0.20)
Stroke	2 (0.1%)	4 (0.1%)	0.0 (0.11)
Other vascular	1 (0.1%)	2 (0.1%)	0.0 (0.08)
Cancer	9 (0.6%)	10 (0.3%)	0.3 (0.22)
Other medical	15 (1.0%)	21 (0.7%)	0.3 (0.29)
External	2 (0.1%)	0 (0.0%)	0.1 (0.09)
Unknown cause	1 (0.1%)	1 (<0.05%)	0.0 (0.07)
Total: 28-day mortality	**421 (27.0%)**	**790 (25.0%)**	**1.9 (1.36)**

RR=Rate Ratio. CI=confidence interval.

Table S4: Effect of allocation to hydroxychloroquine on new major cardiac arrhythmia

	Treatment allocation	
	Hydroxychloroquine (n=1561)	**Usual care (n=3155)**
Number with follow-up form*	735	1421
Atrial flutter or atrial fibrillation	46 (6.3%)	74 (5.2%)
Other supraventricular tachycardia	10 (1.4%)	18 (1.3%)
Subtotal: Supraventricular tachycardia	56 (7.6%)	85 (6.0%)
Ventricular tachycardia	3 (0.4%)	5 (0.4%)
Ventricular fibrillation	2 (0.3%)	0 (0.0%)
Subtotal: Ventricular tachycardia or fibrillation	5 (0.7%)	5 (0.4%)
Atrioventricular block requiring intervention	1 (0.1%)	1 (0.1%)
Total: Any major cardiac arrhythmia	**60 (8.2%)**	**90 (6.3%)**

* Information on new cardiac arrhythmias was only collected on follow-up forms from 12 May 2020 onwards; percentages are of those with such a form completed.

Table S5: Effect of allocation to hydroxychloroquine on need for renal replacement therapy (RRT) among those not on RRT at randomization

	Treatment allocation		
	Hydroxychloroquine (n=1561)	Usual care (n=3155)	RR (95% CI)
Need for renal replacement therapy (among those not on RRT at randomisation)	120/1520 (7.9%)	241/3050 (7.9%)	1.00 (0.81-1.23)

RR=Risk Ratio. CI=confidence interval.