Supplemental Appendix 3 - Online Content

Proactive protection with azithromycin and hydroxychloroquine in hospitalised patients with COVID-19 (ProPAC-COVID): A randomised clinical trial

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

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Section 3. Trial eligibility criteria

The complete inclusion and exclusion criteria from the protocol are given below.

Inclusion criteria

• Patient admitted to Danish emergency departments, respiratory medicine departments or internal medicine departments
• Age ≥ 18 years
• Hospitalised ≤ 48 h
• Positive SARS-CoV-2 test/diagnosis during the hospitalisation (confirmed).
• Men or non-fertile women. Fertile women* must not be pregnant: i.e., negative pregnancy test must be available at inclusion
• Informed consent signed

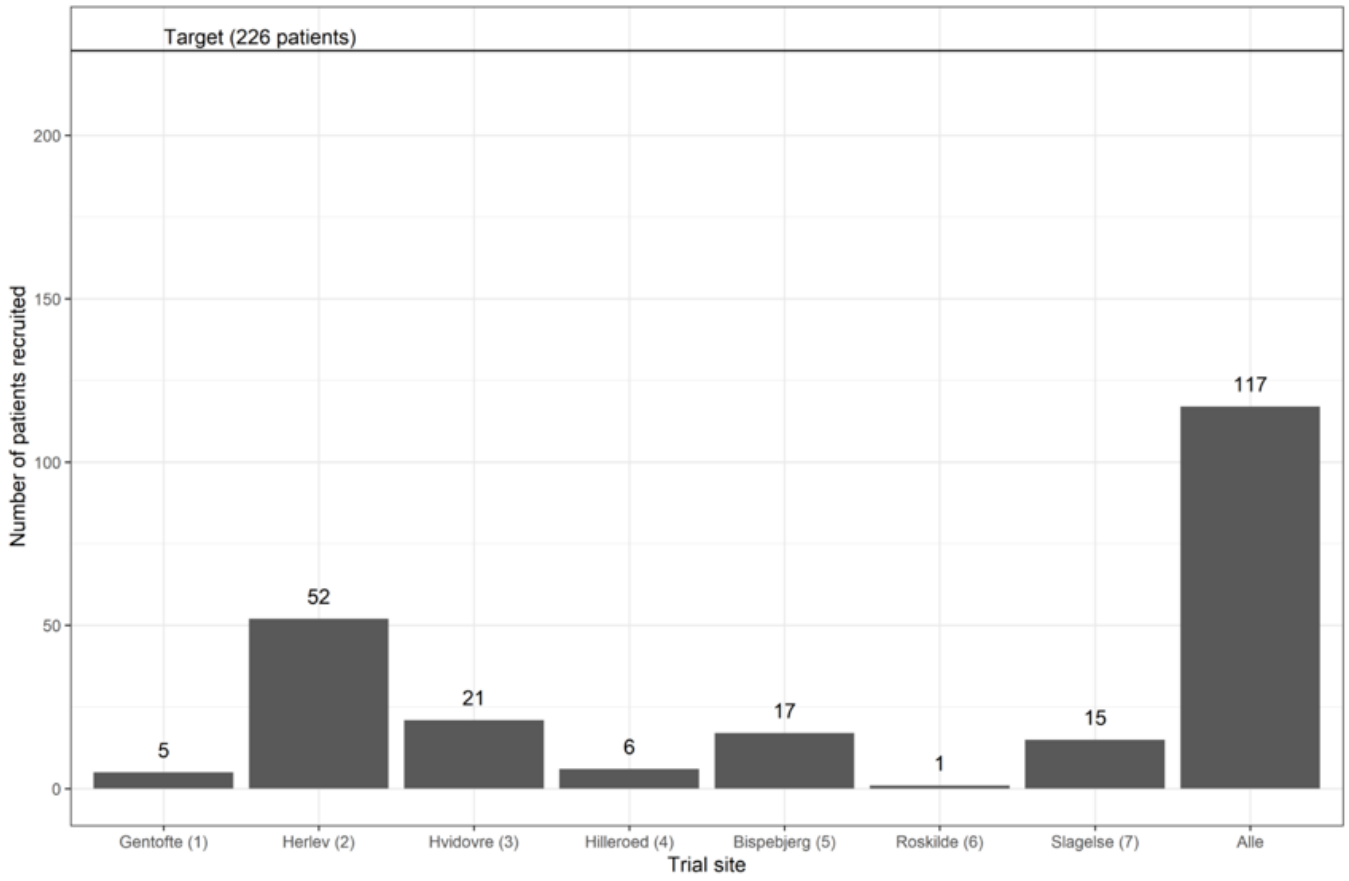
*Defined as after menarche and until postmenopausal (no menstruation for 12 months)

Exclusion criteria
• At the time of recruitment, the patient uses > 5 LO_2/min (equivalent to 40% FiO_2 if measured)
• Known intolerance/allergy to azithromycin or hydroxychloroquine or hypersensitivity to quinine or 4-aminoquinoline derivatives
• Neurogenic hearing loss
• Psoriasis
• Retinopathy
• Maculopathy
• Visual field changes
• Breastfeeding
• Severe liver diseases other than amoebiasis (spontaneous INR > 1.5)
• Severe gastrointestinal, neurological and haematological disorders (investigator-assessed)
• eGFR < 45 mL/min/1.73 m^2
• Clinically significant cardiac conduction disorders/arrhythmias or prolonged QTc interval (QTc (f) of > 480/470 ms).
• Myasthenia gravis
• Treatment with digoxin*
• Glucose-6-phosphate dehydrogenase deficiency
• Porphyria
• Hypoglycaemia (blood glucose at any time since hospitalisation of < 3.0 mmol/L)
• Severe mental illness which significantly impedes cooperation

• Severe linguistic problems that significantly hinder cooperation
• Treatment with ergot alkaloids

*The patient must not be treated with digoxin for the duration of the intervention. For atrial fibrillation/flutter, select according to the Cardiovascular National Treatment Guide (NBV): calcium antagonist, beta blocker, direct current (DC) conversion or amiodarone. In case of urgent need for digoxin treatment (contraindication for the aforementioned equal alternatives), the test drug should be paused, and ECG should be recorded daily.

Figure S1. Total number of patients (*n* = 117) recruited per trial site



Section 4. Interim analyses

An independent data and safety monitoring board (DSMB) oversaw the conduct of the trial and reviewed two interim analyses. Data monitoring guidelines, both interim analysis reports and corresponding DSMB recommendation letters, are attached in Supplemental Appendix 4.

The interim analyses were centred on systematic analyses of
1. Days alive and out of hospital within 14 days after recruitment (primary study outcome)
2. 30-day mortality rate (secondary study outcome)
3. Readmission (all causes) within 30 days (secondary study outcome)

The DSMB recommended stopping enrolment prior to its completion if the intervention was associated with adverse effects that called into question the safety of the intervention.
Moreover, with regards to efficacy, a post-conditional power analysis of the primary outcome was included in the second interim analysis with a pre-specified stopping-criterion for futility set at 0.20.

The date, number of patients reviewed and outcome of each interim analysis are listed in the table below.

Interim Analysis Number (date)	**Enrolled patients with primary outcome data in dataset, no.**	**Enrolled patients with baseline data in dataset, no.**	**DSMB Recommendation**
# 1 (31 May 2020)*	75	65	No safety concerns. Continue trial as planned.
# 2 (28 January 2021)**	117	117	No safety concerns. Stop trial due to very low probability of futility.

* The first interim analysis was conducted in May 2020, as an “acute”, not pre-planned, analysis due to published reports raising concern of severe cardiac side effects attributable to hydroxychloroquine.

**The second interim analysis, in January 2021, was the first pre-planned analysis when half of the patients were recruited.

Table E1. Medication prior to hospital admission
(baseline characteristics in addition to those listed in Table 1)

Medication – no. (%)	All (*n* = 117)	Hydroxychloroquine and azithromycin (*n* = 61)	Placebo (*n* = 56)
Long-acting beta2-agonist	21 (18)	11 (18)	10 (18)
Long-acting muscarinic antagonist	10 (9)	8 (13)	2 (4)
Inhaled corticosteroids	23 (20)	11 (18)	12 (21)
Short-acting beta2-agonist	27 (23)	16 (26)	11 (20)
Short-acting muscarinic antagonist	8 (7)	6 (10)	2 (4)
Roflumilast (PDE4 inhibitor)	0 (0)	0 (0)	0 (0)
Montelukast (Antileukotriene)	1 (1)	1 (2)	0 (0)
Long-term antibiotic treatment	2 (2)	0 (0)	2 (4)
Theophylline	0 (0)	0 (0)	0 (0)
Statins	35 (30)	17 (28)	18 (32)
Long-term oral corticosteroid treatment	6 (5)	3 (5)	3 (5)
Furosemide	11 (9)	7 (11)	4 (7)

Abbreviation: PDE4, phosphodiesterase-4.

Table E2. Chronic conditions
(baseline characteristics in addition to those listed in Table 1)

Chronic condition – no. (%)	**All (*n* = 117)**	**Hydroxychloroquine and azithromycin (*n* = 61)**	**Placebo (*n* = 56)**
Allergy	28 (24)	18 (30)	10 (18)
Diabetes mellitus Type I	1 (1)	0 (0)	1 (2)
Diabetes mellitus Type II	26 (22)	17 (28)	9 (16)
Diabetes mellitus, other type (e.g., mature-onset diabetes of the young)	0 (0)	0 (0)	0 (0)
Diabetes mellitus, unknown type	1 (1)	0 (0)	1 (2)
Atrial fibrillation	21 (18)	15 (25)	6 (11)
Essential hypertension	45 (38)	21 (34)	24 (43)
Osteoporosis	7 (6)	3 (5)	4 (7)
Peripheral vascular disease	3 (3)	1 (2)	2 (4)
Cerebrovascular disease	12 (10)	6 (10)	6 (11)
Haematological diseases	4 (3)	2 (3)	2 (4)
Depression	12 (10)	7 (11)	5 (9)
Past or present lung cancer	1 (1)	0 (0)	1 (2)
Previous cancer (which is not lung cancer)	19 (16)	11 (18)	8 (14)
Former deep vein thrombosis or pulmonary embolism	7 (6)	5 (8)	2 (4)
Liver failure	0 (0)	0 (0)	0 (0)
Rheumatic diseases	14 (12)	6 (10)	8 (14)

Table E3. Medications received during hospital admission (before and after randomisation)

Medication – no. (%)	**All (*n* = 117)**	**Hydroxychloroquine and azithromycin (*n* = 61)**	**Placebo (*n* = 56)**
Any antibiotics	77 (66)	36 (59)	41 (73)
Ciprofloxacin	1 (1)	0 (0)	1 (2)
Piperacillin/tazobactam	54 (46)	24 (39)	30 (54)
Ceftazidime	1 (1)	1 (2)	0 (0)
Meropenem	3 (3)	2 (3)	1 (2)
Colistin	0 (0)	0 (0)	0 (0)
Gentamycin	1 (1)	1 (2)	0 (0)
Amoxicillin	14 (12)	5 (8)	9 (16)
Amoxicillin/Clavulanic	11 (9)	6 (10)	5 (9)
Surlid	0 (0)	0 (0)	0 (0)
Dicloxacillin	1 (1)	1 (2)	0 (0)
Penicillin	16 (14)	11 (18)	5 (9)
Azithromycin	0 (0)	0 (0)	0 (0)
Other antibiotics	23 (20)	10 (16)	13 (23)
Remdesivir	28 (25)	13 (22)	15 (28)
Dexamethasone	36 (32)	17 (28)	19 (35)
Corticosteroids other than dexamethasone	17 (15)	10 (16)	7 (12)
Days with antibiotics, median (IQR)	4.5 (3.0–6.0)	5.0 (3.0–6.0)	4.2 (3.0–6.0)
Days with dexamethasone, median (IQR)	6.0 (5.0–8.0)	6.0 (5.0–9.0)	6.0 (5.0–7.0)
Days with corticosteroids other than dexamethasone, median (IQR)	5.0 (1.0–6.0)	3.5 (1.2–5.8)	6.0 (2.5–7.0)

Abbreviation: IQR, interquartile range

Table E4. Adherence to trial drugs

Adherence – no. (%)	**All (*n* = 117)**	**Hydroxychloroquine and azithromycin (*n* = 61)**	**Placebo (*n* = 56)**
Started azithromycin	108 (92)	54 (89)	54 (96)
Started hydroxychloroquine	107 (91)	54 (89)	53 (95)
Days with azithromycin, median (IQR)	15 (4–15)	15 (2–15)	15 (7–15)
Days with hydroxychloroquine, median (IQR)	15 (3–15)	14 (2–15)	15 (7–15)
Days with azithromycin and hydroxychloroquine, median (IQR)	14 (3–15)	14 (2–15)	13 (7–15)

Abbreviation: IQR, interquartile range

Table E5. Subgroup analyses, all according to baseline values: Treatment group difference (hydroxychloroquine and azithromycin minus placebo)

Characteristics	Treatment group difference	*P*-value
Chronic obstructive pulmonary disease		
Yes	0.12 (–2.83–3.06)	0.9345
No	–0.79 (–2.54–0.96)	0.3714
QTc greater than the group median value		
QTc ≥ 417	–0.15 (–2.31–2.01)	0.8916
QTc < 417	–1.00 (–3.00–1.01)	0.3239
Nasal oxygen supply		
≥ 2 L/min	0.35 (–2.09–2.78)	0.7739
< 2 L/min	–1.35 (–3.12–0.42)	0.1337
C-reactive protein		
≥ 50	–0.57 (–2.60–1.46)	0.5766
< 50	–0.52 (–2.73–1.69)	0.6382
Fibrin d-dimer		
≥ 0.8	0.36 (–3.82–4.54)	0.5766
< 0.8	–0.63 (–2.20–0.94)	0.6382
Remdesivir as concomitant medication		
Yes	–1.74 (–4.52–1.04)	0.2063
No	–0.28 (–2.15–1.58)	0.7614

Abbreviation: QTc, corrected QT interval

Table E6. Secondary outcomes – Ordinal day 15

Clinical status (COVID Outcomes Scale category) – no. (%)	**Hydroxychloroquine and azithromycin (*n* = 61)**	**Placebo (*n* = 56)**	***P*-value**
1. Discharged with no limitations on activities	26 (43)	22 (39)	
2. Discharged with limitations on activities: may be free of oxygen therapy or be on LTOT	26 (43)	27 (48)	
3. Admitted and without oxygen but not receiving treatment (observation only)	1 (2)	0 (0)	
4. Admitted and without oxygen but receiving treatment (COVID-19-related or other)	1 (2)	2 (4)	
5. Admitted and on other oxygen supplement different from (2) or (3) such as oxygen through a nasal cannula	2 (3)	2 (4)	
6. Admitted and on non-invasive ventilation or "high-flow oxygen device"	1 (2)	0 (0)	
7. Admitted and on mechanical ventilation or ECMO	3 (5)	1 (2)	
8. Dead	1 (2)	2 (4)	
Proportional odds model, odds ratio	1.0 (0.5–2.2)	Ref	0.91

Abbreviations: LTOT, long-term oxygen therapy; ECMO, extra corporeal membrane oxygenation.

Table E7. Secondary outcomes – Ordinal day 5

Clinical status (COVID Outcomes Scale category) – no. (%)	**Hydroxychloroquine and azithromycin (*n* = 61)**	**Placebo (*n* = 56)**
1. Discharged with no limitations on activities	19 (31)	16 (29)
2. Discharged with limitations on activities: may be free of oxygen therapy or be on LTOT	14 (23)	13 (23)
3. Admitted and without oxygen but not receiving treatment (observation only)	4 (7)	9 (16)
4. Admitted and without oxygen but receiving treatment (COVID-19-related or other)	10 (16)	6 (11)
5. Admitted and on other oxygen supplement different from (2) or (3) such as oxygen through a nasal cannula	9 (15)	11 (20)
6. Admitted and on non-invasive ventilation or "high-flow oxygen device"	3 (5)	1 (2)
7. Admitted and on mechanical ventilation or ECMO	2 (3)	0 (0)
8. Dead	0 (0)	0 (0)
Proportional odds model, odds ratio	0.9 (0.4–1.8)	Ref

Abbreviations: LTOT, long-term oxygen therapy; ECMO, extra corporeal membrane oxygenation.

Table E8. Adverse events by organ system

Adverse events (AEs) were reported by site investigators, who were blinded to randomised groups, to the Clinical Coordinating Centre.

The site investigator who reported each adverse event classified it as serious or not serious and evaluated relatedness to study procedures. Individual patients could experience more than one adverse event. Adverse events were recorded during the period beginning when the patient received their first dose of trial medication up to and including day 15.

A serious adverse event (SAE) was defined as an event or adverse event that, regardless of dose, was life-threatening, resulted in significant or persistent disability or incapacity, or led to a congenital anomaly or malformation.

Because comorbidities and mortality are common in this patient group, prolonged admission, re-admission, non-invasive ventilation, invasive respiratory treatment and death were not considered SAEs.

This table displays all adverse events reported in the trial and whether each adverse event was classified as a serious adverse event.

Organ system	Adverse event	All Adverse events, no.	Hydroxychloroquine and Azithromycin Adverse event, no.	Placebo Adverse event, no.
Cardiac disorders	Prolonged QTc	11	4	7
	Chest pain	7	3	4
	Myocardial infarction	0	0	0
	Ventricular arrhythmia	1	0	1
Endocrine disorders	Hypoglycaemia	0	0	0
Gastrointestinal disorders	Diarrhoea	15	12	3
	Vomiting	4	2	2
	Nausea	17	11	6
	Abdominal pain	14	7	7
Nervous system and psychiatric disorders	Headache	8	3	5
	Photophobia	0	0	0
	Dizziness	13	10	3
	Hearing loss	1	0	1
	Seizure	0	0	0
	Stroke	0	0	0
Respiratory, thoracic and mediastinal disorders	Bronchospasm	5	3	2

Skin and subcutaneous tissue disorders	Itching Rash	3	3	0
Vascular disorders	Bleeding	2	2	0
Any serious adverse events		2	0	2
TOTAL		**103**	**60**	**43**

Abbreviation: QTc, corrected QT interval

Table E9. Sensitivity analysis for primary outcome

Outcome	**Hydroxychloroquine plus azithromycin**	**Placebo**	***P*-value**
Adjusted* days alive and out of hospital at 14 days, estimated mean difference (95% CI), days	**-0.43 (95% CI -3.77–2.92)**	**Reference**	**0.80**

*Adjusted for age (per year increase), sex, body mass index (per unit increase), oxygen supply at baseline (yes/no), pre-existing lung disease (yes/no), diabetes (yes/no), remdesivir (yes/no), QTc across median (yes/no) and pack-years (current and ex-smokers).

Figure S2. Time to readmission or death within 30 days, adjusted analyses
Adjusted for age (per year increase), sex (male/female), body mass index (per unit increase), oxygen supply at baseline (yes/no), pre-existing lung disease (yes/no), diabetes mellitus (yes/no), remdesivir (yes/no), corrected QT interval across median (yes/no), C-reactive protein > 50 (yes/no) and cancer (yes/no).

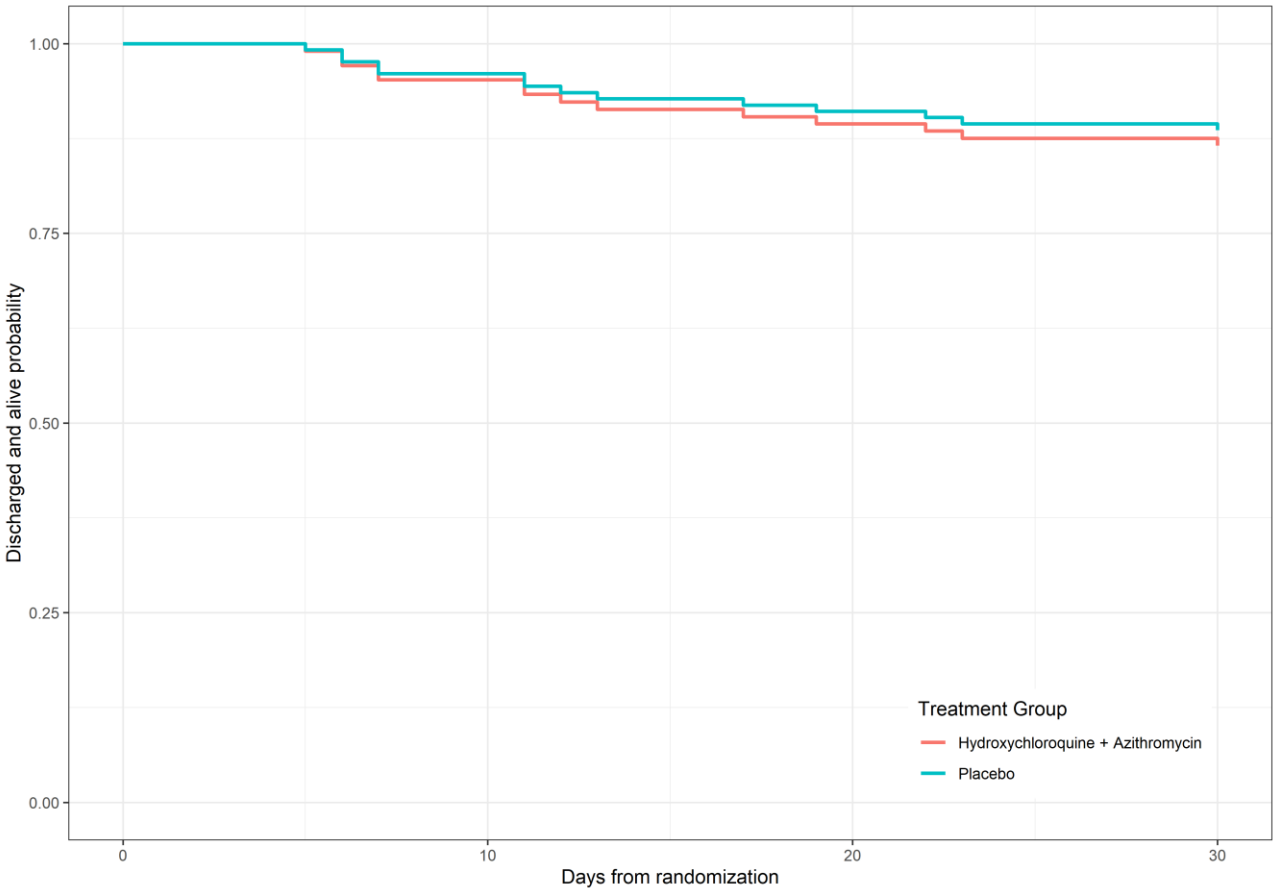


Figure S3. Time to no oxygen within 14 days, adjusted analyses
Adjusted for age (per year increase), sex (male/female), body mass index (per unit increase), oxygen supply at baseline (yes/no), pre-existing lung disease (yes/no), diabetes mellitus (yes/no), remdesivir (yes/no), corrected QT interval across median (yes/no), C-reactive protein > 50 (yes/no) and cancer (yes/no).

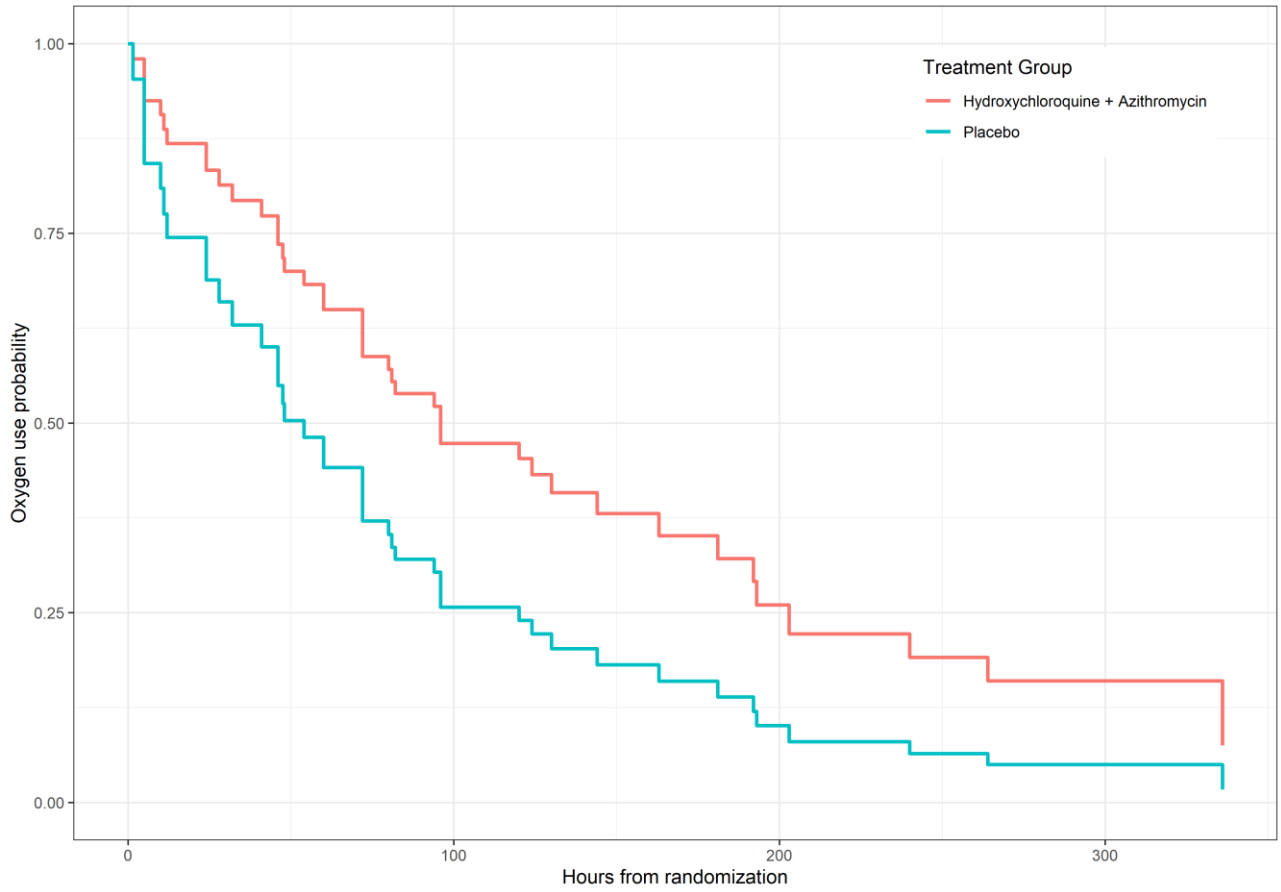


Figure S4. Change in corrected QT interval for hydroxychloroquine and azithromycin group vs. placebo group