



## Early View

Original research  
article

### **Azithromycin and hydroxychloroquine in hospitalised patients with confirmed COVID-19—a randomised double-blinded placebo-controlled trial**

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# **Azithromycin and hydroxychloroquine in hospitalised patients with confirmed COVID-19 - a randomised double-blinded placebo-controlled trial**

by the  
ProPAC-COVID study group\*

\*A complete list of members in the Proactive Protection with Azithromycin and hydroxy-Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID) Study Group is provided in Supplementary Appendix 3. The ProPAC-COVID study is an initiative by the independent research network COP:TRIN ([www.coptrin.dk](http://www.coptrin.dk)).

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## **TAKE HOME MESSAGE:**

**There are no beneficial or harmful effects from the combined intervention of hydroxychloroquine and azithromycin for hospitalised patients with confirmed coronavirus disease 2019 (COVID-19).**

## BACKGROUND

Combining the antibiotic azithromycin and hydroxychloroquine induces airway immunomodulatory effects, with the latter also having *in vitro* antiviral properties. This may improve outcomes in patients hospitalised for COVID-19.

## METHODS

Placebo-controlled double-blind randomised multicentre trial. Patients  $\geq 18$  years, admitted to hospital for  $\leq 48$  h (not intensive care) with a positive SARS-CoV-2 RT-PCR test, were recruited. The intervention was 500 mg daily azithromycin for 3 days followed by 250 mg daily azithromycin for 12 days combined with 200 mg twice daily hydroxychloroquine for all 15 days. The control group received placebo/placebo. The primary outcome was days alive and discharged from hospital within 14 days (DAOH14).

## RESULTS

After randomisation of 117 patients, at the first planned interim analysis, the data and safety monitoring board recommended stopping enrolment due to futility, based on pre-specified criteria. Consequently, the trial was terminated on 1 February 2021. A total of 61 patients received the combined intervention and 56 patients received placebo. In the intervention group, patients had a median of 9.0 DAOH14 (IQR, 3–11) vs. 9.0 DAOH14 (IQR, 7–10) in the placebo group ( $p = 0.90$ ). The primary safety outcome, death from all causes on day 30, occurred for 1 patient in the intervention group vs. 2 patients receiving placebo ( $p = 0.52$ ), and readmittance or death within 30 days occurred for 9 patients in the intervention group vs. 6 patients receiving placebo ( $p = 0.57$ ).

## CONCLUSIONS

The combination of azithromycin and hydroxychloroquine did not improve survival or length of hospitalisation in patients with COVID-19.

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## Introduction

Early in the coronavirus disease 2019 (COVID-19) pandemic, some evidence, mainly from laboratory studies, suggested that chloroquine and its less toxic derivative hydroxychloroquine, often used as an antirheumatic drug, had an antiviral effect on coronaviridae by inhibiting several pH-dependent steps in replication and endosomal viral uptake into human cells [1]. These findings have been confirmed in laboratory studies of primate cells infected with severe acute respiratory syndrome coronavirus (SARS)-1 [2]. Hydroxychloroquine may also bind to host cell sialic acids and gangliosides with high affinity, thus protecting the cell against binding to SARS-Corona virus-2 via its spike (S) protein [3]. Administered at recommended doses, in most countries up to 400–500 mg daily, hydroxychloroquine seems safe, even when used for longer periods and costs are low [4].

Azithromycin is a macrolide antibiotic, which has proven effective in reducing airway inflammation and consequent hospitalization-requiring exacerbations of Chronic Obstructive Pulmonary Disease (COPD), asthma and bronchiectasis [5-7]. Recently, a strong association was found in critically ill patients with acute respiratory distress syndrome (ARDS) between treatment with azithromycin and improved survival [8] as summarized with greater power in systematic meta-analyses [9, 10]. Further, hydroxychloroquine and azithromycin may act synergistically to prevent the coronavirus from binding to ganglioside receptors on human cells [11].

Important trials show positive outcomes for agents like remdesivir, anti-IL6 and convalescent plasma in milder cases and early disease stages [12-14] but these interventions seem less effective in severely ill patients [15]. On the other hand, in more severe cases, immunosuppressive pharmaceuticals like corticosteroids do show some effect [16]. Thus, antiviral treatment in the early, and less severe disease stages appears to be the window of opportunity for these drugs [17].

The present trial assessed whether a combination of azithromycin and hydroxychloroquine, both in moderate and approved (for rheumatic indications) dosing regimens, would increase the number of days alive and discharged from hospital among hospitalised patients with COVID-19.

## **Methods**

The trial protocol and statistical analysis plan are available in Supplementary Information sections 1 and 2 and have been published previously [18, 19]. The study was approved by the ethics committees of all participating sites (H-20022574), the Danish Medicines Agency (EudraCT no 2020-001198-55) and the Danish Data Protection Agency. It was monitored in accordance with good clinical practice (GCP) by the GCP units of the participating regions in Denmark. The study was conducted in accordance with the Declaration of Helsinki [20] No financial incentive was provided to the investigators or participants. There was an independent data and safety monitoring board (DSMB), consisting of three clinicians and researchers who are experts in performing large randomised studies. Additionally, the DSMB had access to the trial statistician, Mr. Tobias Wirenfeldt Klausen, a highly skilled biostatistician, who also supervised the interim analyses. Mr. Klausen was available any time the DSMB wanted his input. He was also blinded to treatment allocation, as only the trial pharmacist had the key to unblind.

This DSMB reviewed the trial's progress and performed safety, efficacy, and data completeness evaluations during the trial. It was not possible (in the interest of timeliness) to involve patients or the public in the design, conduct, reporting, or dissemination of our research. This study is a primary analysis and is described in accordance with the consolidated standards of reporting of randomised trials (CONSORT) guidelines.

### *Study design and sites*

The Proactive Protection with Azithromycin and hydroxyChloroquine in hospitalised patients with COVID-19 (ProPAC-COVID) study was a multicentre, double-blinded placebo-controlled, randomised clinical trial investigating whether adding 15-day treatment with azithromycin and hydroxychloroquine to standard of care could decrease the period of hospitalisation and reduce the risks of non-invasive ventilation (NIV), admittance to an intensive care unit (ICU), and death. Patients were enrolled between 6 April 2020 and 21 December 2020 at six hospitals in Denmark within the COP:TRIN collaboration ([www.coptrin.dk](http://www.coptrin.dk)). The dosages selected were based on well-tolerated doses used to treat other diseases (e.g. rheumatological diseases), while lowering risk of cardiac side effects. The durations were selected to ensure coverage of patients with prolonged admissions for a relatively large part of the admissions and to securely cover the entire observation period of the primary outcome. Also, durations were chosen to protect against secondary infections from Gram positive microorganisms.

### *Participants*

Eligible patients had to be (1) at least 18 years of age, (2) admitted to hospital with a confirmed positive test for SARS-CoV-2 infection by reverse transcription polymerase chain reaction (RT-PCR), and (3) hospitalised for  $\leq 48$  h. Each patient provided signed informed consent to participate. Patients were excluded if they met any of the following criteria: (1) received  $> 5$  L oxygen supply; (2) known intolerance/allergy to the study drugs; (3) neurogenic hearing loss; (4) psoriasis; (5) retinopathy; (6) maculopathy; (7) visual field changes; (8) were breastfeeding/pregnant; (9) severe liver disease (international normalised ratio  $> 1.5$  spontaneously); (10) severe gastrointestinal disease (investigator-assessed liver disease, severe ulcerative colitis or Crohn's disease, peptic ulcer disease, or cancer); (11) neurological or haematological disorder; (12) estimated glomerular filtration rate (eGFR)  $< 45$  mL/min/1.73 m<sup>2</sup>; (13) clinically significant cardiac conduction disorder/arrhythmia or a prolonged corrected QT interval (QTc; i.e., F  $> 480$  ms for males or  $> 470$  ms for females); (14) myasthenia gravis; (15) were receiving treatment with digoxin; (16) glucose-6-phosphate dehydrogenase deficiency; (17) porphyria; (18) hypoglycaemia (blood glucose  $< 3.0$  mmol/L); (19) unable to give informed consent; (20) severe linguistic problems that significantly hindered cooperation; or (21) were

receiving treatment with ergot alkaloids. The investigator evaluated patient eligibility based on these criteria.

### *Randomisation and masking*

The study pharmacist generated the randomisation sequence, which was then entered into the online platform REDCap electronic data-capture tools hosted by the participating Danish regions. Patients were randomised 1:1 to azithromycin plus hydroxychloroquine or matching placebo capsules. Randomisation was performed in blocks of unknown and varying size, and the final allocation was blinded and stratified for age ( $> 70$  vs.  $\leq 70$  years), site of recruitment, and whether the patient had any of the following chronic lung diseases (yes vs. no): COPD, asthma, bronchiectasis, or interstitial lung disease. All patients and study staff were blinded to participant treatment assignments. This included outcome assessors, investigators and study nurses, as well as research and clinical staff. The DSMB remained blinded throughout and made all recommendations blinded to treatment allocations. Only the trial's chief pharmacist held the key for unblinding. Formal unblinding took place on 1 February 2021 after the DSMB recommendation had been received and acknowledged.

### *Intervention*

Patients were randomised to one of two treatment arms: (1) 500 mg azithromycin once daily plus 200 mg hydroxychloroquine twice daily on days 1–3 and then 250 mg azithromycin once daily plus 200 mg hydroxychloroquine twice daily on days 4–15; (2) placebo instead of both types of intervention medication. Medication (both arms) was marked with neutral labels: e.g., "Azithromycin group A" and "Azithromycin group B". An important safety consideration for both study drugs was QTc prolongation [21, 22] Therefore, trial personnel measured the QTc at least twice during the period of hospitalisation.

### *Primary and secondary endpoints*

The primary endpoint was the number of days alive and out of hospital (DAOH) within 14 days from randomisation. This outcome measure was developed by trialists to be both sensitive and

clinically relevant, and it provides a method for counting days with sustained recovery without lead-time bias [23-25]. For the first secondary endpoint, each patient was placed in one of the following eight categories on day 5 and day 15, as described in our previous research [12]: 1) discharged from hospital with no restrictions on activities; 2) discharged from hospital but with restrictions on activities (may/may not be receiving long-term oxygen therapy at home); 3) hospitalised and under observation but not receiving supplemental oxygen or any other treatment; 4) hospitalised and not receiving supplemental oxygen, but receiving other treatment (which may/may not be related to COVID-19); 5) hospitalised and receiving supplemental oxygen by a method other than those described in (2) or (3), such as from a nasal catheter; 6) hospitalised and receiving NIV or oxygen from a high-flow device; 7) hospitalised and receiving mechanical ventilation or extra corporeal membrane oxygenation; or 8) dead. The trial included eight other secondary outcomes: (1) number of days in an ICU (time frame: 14 days); (2) number of days NIV was required during hospitalisation (time frame: 14 days); (3) mortality rates (time frames: 30, 90, and 365 days); (4) length of hospitalisation (time frame: 14 days); (5) DAOH (time frame: 30 days); (6) time to readmission for any reason (time frame: 30 days); (7) change in patient's pH, PaO<sub>2</sub>, or PCO<sub>2</sub> partial pressure measurements (time frame: 4 days); and (8) time until no supplementary oxygen was required or until the patient was given "long-term oxygen therapy" (time frame: 14 days). Outcomes with follow-up >30 days will be reported later. All outcomes and analyses were conducted in strict concordance with the SAP.

### *Sample size calculation*

The sample size for the primary outcome (DAOH within 14 days from randomisation) was calculated assuming a two-sided significance level of 5% and power ( $1 - \beta$ ) of 80%. A group-sequential study design with one planned interim analysis at half-target recruitment was used. The standard deviation was set at 4 days [26] and the detection limit was set at 1.5 days (both directions). StudySize software (ver. 3.0; CreoStat HB, Gothenburg, Sweden) was used to calculate the sample size of 226 participants.



### *Statistical analysis*

We compared outcomes using *t*-tests or Mann–Whitney U tests for continuous variables (depending on distribution),  $\chi^2$  tests or Fisher’s exact test for nominal variables, and log-rank tests to compare Kaplan–Meier survival curves. Cumulative event estimates were generated using hazard ratios (HRs) with 95% confidence intervals (CIs) in Cox proportional hazards models. Adjustment for continuous data was performed using multiple effects models. The primary analysis was based on intention-to-treat (ITT), and a secondary per protocol analysis was performed for both primary and secondary outcomes. A *p*-value < 0.05 was considered statistically significant and all analyses were two-sided. We originally planned to perform an interim analysis between the groups when the study had reached 50% of the total sample size. However, in response to a subsequently retracted article by Mehra *et al.* [27], the Danish Medicines Agency demanded that we performed an extraordinary acute interim analysis (without unmasking) on the first 75 patients who had been recruited. This was reviewed by the DSMB, who recommended continuing to accrue patients (May 2020). The first planned interim analysis was conducted at 117 patients (50% recruited), and the trial was stopped due to futility (February 2021). Sensitivity analyses for the primary outcome included: (1) a modified ITT population of patients who received part or complete treatment with the intervention (all days); (2) a per protocol population who received both interventional drugs for all planned days; and (3) a multiple effects adjusted model for the primary outcome, in which adjustment was made for the following parameters: *i*) age (per year increase), *ii*) sex (male vs. female), *iii*) body mass index (per unit increase), *iv*) oxygen therapy at inclusion (yes vs. no), *v*) remdesivir (yes vs. no), *vi*) any pre-existing lung disease (obstructive, interstitial or bronchiectasis: yes vs. no), *vii*) diabetes mellitus (yes vs. no), and *viii*) QTc across median (yes vs. no). Statistical analyses were performed using SAS software (ver. 9.4; SAS Institute, Inc., Cary, NC, USA) and R software (ver. 3.4.3; R Development Core Team, Vienna, Austria).

### *Stopping the trial*

On 1 February 2021, the trial was stopped for futility based on recommendations from the DSMB who met on 29 January 2021 and discussed the report from the first planned interim analysis. The maximum post conditional power to cross any boundary in the O’Brien–Fleming

plot [28] was 0.064, which was below the threshold of 0.2 communicated from the steering committee to the DSMB prior to the meeting. The interim analyses were performed in accordance with the trial monitoring guidelines. After reviewing the post-conditional power, the remaining data in the interim analysis and the available published data, the DSMB recommended stopping the trial on grounds of futility (the DSMB recommendation is included in Supplementary Information section 4).

## **Results**

Of the 664 patients screened, 117 were eligible for study inclusion (Figure 1). Reasons for exclusion included: unable to give informed consent (18.8% of exclusions), eGFR < 45 mL/min/1.73 m<sup>2</sup> (17.9% of exclusions) and declined to participate (16.3% exclusions). Of the patients enrolled, 61 patients were randomised to the azithromycin plus hydroxychloroquine arm and 56 to the placebo arm. Participants had a median age of 65 years (interquartile range [IQR], 52–77), and 65 (56%) of them were men. The median time since symptom onset was 8 days (IQR, 4–10). Baseline characteristics of patients randomised to the intervention and placebo groups are presented in Table 1, and in eTable 1 and eTable 2 in Supplementary Information section 3.

| <b>Table 1. Baseline patient demographic and clinical characteristics</b> |                  |   |                  |
|---|------------------|---|------------------|
|   | <b>No. (%)</b>   | <b>61</b>                                   | <b>56</b>        |
| <b>Characteristics</b>  | <b>All</b>       | <b>Hydroxychloroquine plus azithromycin</b> | <b>Placebo</b>   |
| Age, median (IQR), years  | 65 (52–77)       | 68 (52–80)                                  | 63 (52–74)       |
| Male sex  | 65 (56)          | 36 (59)                                     | 29 (52)          |
| Race/ethnicity  |                  |   |                  |
| Caucasian   | 98 (84)          | 53 (87)                                     | 45 (80)          |
| African (including Afro-American)   | 1 (1)            | 0 (0)                                       | 1 (2)            |
| Asian   | 12 (10)          | 6 (10)                                      | 6 (11)           |
| Unknown/other   | 6 (5)            | 2 (3)                                       | 4 (7)            |
| Body mass index, median (IQR), kg/m <sup>2</sup>                          | 27.2 (24.8–32.2) | 29.0 (24.9–33.3)                            | 26.8 (24.6–31)   |
| Smoking characteristics   |                  |   |                  |
| Current smoker  | 6 (5)            | 4 (7)                                       | 2 (4)            |
| Ex-smoker   | 48 (41)          | 29 (48)                                     | 19 (34)          |
| Never smoker  | 63 (54)          | 28 (46)                                     | 35 (62)          |
| Pack-years (current and ex-smokers), median (IQR), years                  | 20 (8–35)        | 20 (8–35)                                   | 25 (10–35)       |
| <b>Comorbidities</b>  |                  |   |                  |
| Asthma  | 26 (22)          | 12 (20)                                     | 14 (25)          |
| COPD  | 10 (9)           | 8 (13)                                      | 2 (4)            |
| <b>Bronchiectasis</b>   | 4 (3)            | 3 (5)                                       | 1 (2)            |
| <b>Previous myocardial infarction</b>                                     | 6 (5)            | 3 (5)                                       | 3 (5)            |
| <b>Heart failure</b>  | 8 (7)            | 5 (8)                                       | 3 (5)            |
| <b>Diabetes</b>   | 28 (24)          | 17 (15)                                     | 11 (9)           |
| <b>Chronic renal insufficiency</b>  | 8 (7)            | 5 (8)                                       | 3 (5)            |
| Time since symptom onset, median (IQR)                                    | 8 (4–10)         | 7 (4–10)                                    | 8 (5–11)         |
| Use of oxygen therapy   | 69 (59)          | 34 (56)                                     | 35 (62)          |
| Use of continuous positive airway pressure                                | 23 (20)          | 12 (20)                                     | 11 (20)          |
| Use of non-invasive mechanical ventilation                                | 4 (3)            | 3 (5)                                       | 1 (2)            |
| Infiltrate(s) on chest X-ray  | 85 (73)          | 43 (70)                                     | 42 (75)          |
| Oxygen use, median (IQR), L/min   | 1 (0–2)          | 0 (0–2)                                     | 1 (0–2)          |
| Clinical findings   |                  |   |                  |
| Systolic blood pressure, median (IQR), mm Hg                              | 125 (115–137)    | 127 (118–141)                               | 122 (111–134)    |
| Diastolic blood pressure, median (IQR), mm Hg                             | 74 (65–82)       | 76 (68–87)                                  | 72 (63–79)       |
| Heart rate, median (IQR), beats/min                                       | 77 (69–86)       | 78 (71–89)                                  | 74 (68–84)       |
| Oxygen saturation with nasal oxygen, median (IQR), %                      | 95 (94–97)       | 95 (94–97)                                  | 95 (94–97)       |
| Respiratory rate, median (IQR), breaths/min                               | 19 (18–20)       | 19 (18–20)                                  | 18 (18–20)       |
| Temperature, median (IQR), °C   | 37.2 (36.8–37.8) | 37.2 (36.8–37.8)                            | 37.2 (36.9–37.7) |
| Laboratory measurements   |                  |   |                  |
| Leukocyte count, median (IQR), ×10 <sup>9</sup> cells/L                   | 5.9 (4.6–8.0)    | 5.8 (4.6–7.9)                               | 5.9 (4.5–9.0)    |
| Blood eosinophil count, median (IQR), ×10 <sup>9</sup> cells/L            | 0.01 (0.00–0.04) | 0.02 (0.01–0.05)                            | 0.01 (0.00–0.02) |
| C-reactive protein, median (IQR), mg/L                                    | 62 (36–130)      | 58 (37–120)                                 | 71 (34–132)      |
| Fibrin D-dimer, median (IQR), mg/L  | 0.60 (0.37–1.20) | 0.55 (0.37–1.35)                            | 0.68 (0.36–1.10) |
| Ferritin, median (IQR), µg/L  | 458 (221–1100)   | 410 (176–1060)                              | 504 (262–1102)   |
| Lactate dehydrogenase, median (IQR), U/L                                  | 278 (218–346)    | 270 (214–342)                               | 278 (233–351)    |
| Arterial blood gas  |                  |   |                  |
| pCO <sub>2</sub> (baseline), mean ± SD, mmHg                              | 35.0 ± 6.8       | 35.9 ± 8.3                                  | 34.1 ± 4.9       |
| pO <sub>2</sub> (baseline), mean ± SD, mmHg                               | 72.8 ± 22.5      | 72.5 ± 26.0                                 | 73.2 ± 18.5      |
| HCO <sub>3</sub> <sup>-</sup> (baseline), mean ± SD, mmHg                 | 23.93 ± 3.74     | 24.39 ± 4.44                                | 23.46 ± 2.86     |
| pH (baseline), mean ± SD  | 7.46 ± 0.04      | 7.46 ± 0.04                                 | 7.46 ± 0.03      |
| QTc (F), median (IQR)   | 417 (401–436)    | 414 (400–436)                               | 420 (404–434)    |
| Remdesivir use  | 28 (25)          | 13 (22)                                     | 15 (28)          |
| Dexamethasone use   | 36 (32)          | 17 (28)                                     | 19 (35)          |

**Abbreviations:** IQR, interquartile range; SD, standard deviation; QTc, corrected QT interval.

*Primary outcome*

Primary outcome assessment after randomisation was completed for 117 patients (100%). We observed no significant difference between the two randomised groups for the primary outcome of DAOH within 14 days after recruitment: median of 9.0 DAOH14 (IQR, 3–11) in the hydroxychloroquine plus azithromycin group vs. 9.0 DAOH14 (IQR, 7–10) in the placebo group,  $p = 0.91$  (Table 2, Figure 2).

| <b>Table 2. Primary and secondary outcomes</b>   |   |                  |                |
|--|---|------------------|----------------|
| <b>Outcome</b>   | <b>Hydroxychloroquine plus azithromycin</b> | <b>Placebo</b>   | <b>P-value</b> |
| <b>Primary outcome</b>   |   |                  |                |
| <i>Intention to treat</i>  |   |                  |                |
| Days alive and out of hospital at 14 days, median (IQR), days  | 9.0 (3.0–11.0)                              | 9.0 (7.0–10.0)   | 0.90           |
| Adjusted* days alive and out of hospital at 14 days, <b>estimated mean difference (95% CI), days</b> | -0.7 (-2.2–0.8)                             | Ref              | 0.36           |
| <i>Modified Intention to treat</i>   |   |                  |                |
| Days alive and out of hospital at 14 days, median (IQR), days  | 9.0 (3.0–11.0)                              | 9.0 (7.0–10.0)   | 0.94           |
| <i>Per Protocol</i>  |   |                  |                |
| Days alive and out of hospital at 14 days, median (IQR), days  | 10 (9.0–11.0)                               | 10 (7.0–10.0)    | 0.11           |
| <b>Secondary outcomes</b>  |   |                  |                |
| Admitted to ICU, <i>n</i> (%)  | 4 (6.6)                                     | 3 (5.4)          | 0.78           |
| Days at ICU or dead within 14 days, median (IQR), days   | 14 (9.5–14)                                 | 11 (4–14)        | 0.46           |
| NIV, <i>n</i> (%)  | 3 (4.9)                                     | 1 (1.8)          | 0.35           |
| Days on NIV or death < 14 days, mean (95% CI), days  | 6.7 (-9.1–22.4)                             | 9.0 (NA)         | 0.78           |
| Mortality at 30 days, <i>n</i> (%)   | 1 (1.6)                                     | 2 (3.6)          |                |
| Mortality at 30 days, unadjusted HR  | 0.5 (0.0–5.0)                               | Ref              | 0.52           |
| Mortality at 30 days, adjusted* HR   | 0.08 (0.001–11.7)                           | Ref              | 0.32           |
| Duration of hospitalisation, median (IQR), days  | 4 (2–8)                                     | 4 (3–6)          | 0.73           |
| Days alive and out of hospital at 30 days, median (IQR), days  | 26 (21–28)                                  | 26 (23–27)       | 0.88           |
| Readmission or death within 30 days, <i>n</i> (%)  | 9 (7.7)                                     | 6 (5.1)          |                |
| Time to readmission or death < 30 days, HR   | 1.4 (0.5–3.8)                               | Ref              | 0.57           |
| Time to readmission or death < 30 days, adjusted* HR   | 1.2 (0.3–4.2)                               | Ref              | 0.76           |
| Change in pH (day 1–day 4), mean (95% CI),   | 0.0 (-0.03–0.01)                            | 0.0 (-0.02–0.01) | 0.44           |
| Change in pO <sub>2</sub> (day 1–day 4), mean (95% CI), mmHg   | -3.0 (-9.8–3.9)                             | -0.2 (-8.3–7.8)  | 0.70           |
| Change in pCO <sub>2</sub> (day 1–day 4), mean (95% CI), mmHg  | 1.7 (-0.7–4.0)                              | 1.4 (-0.4–3.3)   | 0.86           |
| Time to no oxygen, unadjusted HR   | 0.8 (0.5–1.5)                               | Ref              | 0.52           |
| Time to no oxygen, adjusted* HR  | 0.4 (0.2–1.0)                               | Ref              | 0.04           |
| QTc (F) > 500 ms, <i>n</i> (%)   | 0 (0)                                       | 2 (4.0)          | 0.23           |

**Abbreviations:** CI, confidence interval; HR, hazard ratio; ICU, intensive care unit; IQR, interquartile range; ms, millisecond; NIV, non-invasive ventilation; NA, not applicable; QTc, corrected QT interval. \*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),

### *Secondary outcomes*

At 15 days after randomisation, there was no significant difference between the hydroxychloroquine plus azithromycin group and the placebo group in COVID Outcomes Scale score (OR, 1.0 [95% CI, 0.5–2.2];  $p = 0.91$ ; Figure 3 and eTable 6 in Supplementary Information section 3). A *post-hoc* analysis of the ordinal outcome at day 5 was requested by the steering committee after unblinding to provide a time-updated assessment of clinical status; this analysis also suggested that the two groups were similar (OR, 0.9 [95% CI, 0.4–1.8]; Figure 3 and eTable 7 in Supplementary Information section 3). We also found no differences between the groups in the prespecified subsidiary clinical outcomes (Table 2, Figure 2). We tested for an interaction between the trial intervention and symptom duration (< 8 days vs. 8 days or above) and found no interaction ( $p = 0.79$ ).

| <b>Table 3. Adverse events</b>                  |                      |                                |  |                                    |
|---|----------------------|--------------------------------|--|------------------------------------|
| <b>Organ system</b>                             | <b>Adverse event</b> | <b>All Adverse events, no.</b> | <b>Hydroxychloroquine and azithromycin adverse events, no.</b> | <b>Placebo adverse events, no.</b> |
| Cardiac disorders                               | Prolonged QTc        | 11                             | 4  | 7                                  |
|   | Chest pain           | 7                              | 3  | 4                                  |
| 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                                  |
|   | Dizziness            | 13                             | 10   | 3                                  |
| Respiratory, thoracic and mediastinal disorders | Bronchospasm         | 5                              | 3  | 2                                  |
| Skin and subcutaneous tissue disorders          | Itching<br>Rash      | 3                              | 3  | 0                                  |
| Vascular disorders                              | Bleeding             | 2                              | 2  | 0                                  |
| Any serious adverse events                      |                      | 2                              | 0  | 2                                  |

Abbreviation: QTc, corrected QT interval.

Adverse event data are presented in Table 3 and eTable 8 in Supplementary Information section 3. During follow-up, 1 of 61 patients (1.64%) in the hydroxychloroquine plus azithromycin group and 2 of 56 patients (3.6%) in the placebo group had a recorded QTc greater than 500 ms (Table 2). Adverse events involving diarrhoea (12 vs. 3), nausea (11 vs. 6) and dizziness (10 vs. 3) were more frequent in the hydroxychloroquine plus azithromycin patient group than in the placebo group. Conversely, adverse events involving a prolonged QTc (> 470 ms for females and > 480 ms for males) were more frequent in the placebo group (4 vs. 7). Only 2 serious adverse events were reported, both in the placebo group (eTable 8 in Supplementary Information section 3).

## Discussion

The ProPAC-COVID trial was stopped at half recruitment based on prespecified futility criteria after a recommendation from the DSMB, in agreement with monitoring guidelines. Compared to placebo, the combination of azithromycin and hydroxychloroquine did not seem to have any effect on the measured outcomes. The primary outcome, DAOH within 14 days from randomisation, was similar in both arms, as was the ordinal outcome measure and the rates of death from all causes and readmissions.

Our trial is the first to report on this combination of hydroxychloroquine plus azithromycin administered in normal recommended doses for 15 days vs. placebo. Other trials have reported either a mono-drug intervention vs. placebo or higher doses of hydroxychloroquine plus azithromycin vs. one of these drugs.

One previous trial has reported on a dosing regimen of hydroxychloroquine similar to ours [29], albeit for a period of 5 days and without azithromycin; that trial was also stopped for futility and reported neutral results. In our trial, the study participants were generally not severely ill, which was congruent with the intention and rationale of the trial: to reduce viral replication (hydroxychloroquine) and hyperinflammation (azithromycin) before organ failure was evident. Some of the reasons that this combination of drugs failed to benefit patients with COVID-19 may include the drugs being unable to penetrate into the airway epithelium, lower potency *in vivo* than *in vitro*, and neutralisation of beneficial and harmful effects.

Although we are aware that the trial may have had insufficient power to analyse all the prespecified outcome measures, the uniform neutrality of all the analysed outcomes strongly suggests that the intervention resulted in no benefit or harm. Of special interest, we used the recommended doses of the two drugs and respected the contraindications of hydroxychloroquine and azithromycin when recruiting participants, and we did not observe changes in cardiac rhythm nor the QTc (F). Other trials investigating hydroxychloroquine/chloroquine have reported such changes, but in those trials, substantially higher doses than are recommended for other indications were used [30, 31].



Our results are consistent with those from other trials investigating the effects of hydroxychloroquine and azithromycin separately. A possibility of “neutralising” harm from drug toxicity and potential benefits against COVID-19 exists, although this is not considered to be likely, since we did not observe a higher incidence of serious adverse effects in the intervention arm. A recent placebo-controlled trial by Self *et al.*, investigating the effects of a 5-day treatment course of hydroxychloroquine at a similar dose to our trial, was also stopped for futility (close to the target sample size) and was neutral with regards to all outcome measures [29]. In the open-label RECOVERY trial [32], 500 mg of daily azithromycin for 10 days produced no benefit or harm, which was consistent with results from the COALITION II trial in which an identical azithromycin regimen was compared to placebo when added to high dose (800 mg/day) hydroxychloroquine. In the COALITION I trial, patients with suspected or confirmed COVID-19 were randomised to open-label treatment with *i*) standard care, *ii*) high dose hydroxychloroquine for 7 days or *iii*) a combination of high-dose hydroxychloroquine (800 mg daily) and high-dose azithromycin (500 mg daily) also for 7 days. The results were neutral on all outcomes, except for QTc, which was significantly longer in the two actively treated groups. Taken together, all of these trials that tested hydroxychloroquine vs. standard care, azithromycin vs. standard care, or azithromycin plus hydroxychloroquine have produced neutral results, except with regard to the QTc, which has been somewhat higher in patients who received high-dose hydroxychloroquine/chloroquine. Trial patients who received normal recommended doses of hydroxychloroquine/chloroquine did not exhibit prolonged QTcs.

One strength of the present study is that all enrolled patients had RT-PCR-confirmed COVID-19; in other trials exploring these drugs, patients with suspected but not necessarily confirmed COVID-19 were enrolled [30, 32, 33]. Additionally, the double-blind and placebo-controlled design is an important strength, especially when comparing outcomes such as the ordinal outcome and length of hospitalisation, which are heavily influenced by physician decisions. The discontinuation of the present study before full recruitment may be considered a limitation. However, we did use a relatively sensitive primary outcome. For the current study with admitted patients with lower respiratory tract infection, the SD is 3.5–4.0 [23, 34]. Using this, and setting the detection limit at 1.5 days change (both ways) in DAOH, we reached the sample size, the

trial was planned for. It can be discussed whether 1.5 days change is sensitive enough, however, the study group decided that if DAOH could not change at least 1.5 days, we would consider the effect to be of limited clinical value. At the time of trial termination, the chance of crossing a boundary of efficacy or harm was very low and when considered in the context of the evidence currently available, it seems unlikely that further recruitment would have demonstrated any effect. As the median time from onset of symptoms was 8 days, the study intervention could potentially have an effect if administered earlier in the course of the disease. However, this has not been studied in other trials. Our trial can not answer this question directly, however, such an effect in patients with a shorter duration of symptoms seems unlikely, as this had no effect on our results since there was no interaction between the study intervention and symptom duration regarding the primary outcome. Thus, we conclude that our trial results were neutral. The combination of azithromycin and hydroxychloroquine did not increase the likelihood of survival or discharge from hospital of patients with COVID-19. This conclusion is consistent with recent European Respiratory Society COVID-19 guidelines[35], which reported no clinical benefits associated with using hydroxychloroquine and/or azithromycin to treat patients hospitalised with COVID-19 (in the absence of bacterial infection).

**Author contributions:** Concept and design: Pradeesh Sivapalan, Charlotte Suppli Ulrik and Jens-Ulrik Jensen; acquisition, analysis and interpretation of data: Pradeesh Sivapalan, Charlotte Suppli Ulrik, Josefin Eklöf, Alexander Jordan, Therese Lapperre, Rasmus Dahlin Bojesen, Andrea Browatzki, Jon Torgny Wilcke, Vibeke Gottlieb, Kjell Erik Julius Håkansson, Casper Tidemandsen, Oliver Djurhuus Tupper, Howraman Meteran, Christina Marisa Bergsøe, Uffe Christian Steinholtz Bødtger, Daniel Bech Rasmussen, Sidse Graff Jensen, Lars Pedersen, Helene Priemé, Christian Søborg, Ida Elisabeth Steffensen, Dorthe Sandbæk Høgsberg, Martin Steen Frydland, Peter Lange, Asger Sverrild, Muzhda Ghanizada and Jens-Ulrik Jensen; drafting the manuscript: Pradeesh Sivapalan and Jens-Ulrik Jensen; critical revision of the manuscript for important intellectual content: all the authors; statistical analysis: Pradeesh Sivapalan, Jens-Ulrik Stæhr Jensen, Alexander Jordan, Tobias Wirefeldt Klausen and Josefin Eklöf; obtaining funding: Jens-Ulrik Jensen; administrative, technical and material support: Jens-Ulrik Jensen,

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**Role of the corresponding author:** Initiator and study director.

**Conflicts of interest/disclosure:** PS reports fees from Boehringer Ingelheim, outside the submitted work. CSU reports fees from Boehringer-Ingelheim, AZ, GSK, TEVA, Novartis, ALK-Abello, Mundipharma, Sanofi Genzyme, Orion Pharma and Actelion, outside the submitted work. KEJH reports personal fees from AstraZeneca, Chiesi and TEVA, outside the submitted work. TBS has received research grants from GE healthcare and Sanofi Pasteur, as well as personal fees from Sanofi Pasteur, Novartis and Amgen, outside the submitted work. None of the authors have any conflicts of interest.

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**Data sharing statement:** It is the opinion of the COP:TRIN steering committee that knowledge sharing increases the quantity and quality of scientific results. Requests for trial information can be submitted to the project management team (Jens-Ulrik Jensen, Charlotte Ulrik and Pradeesh Sivapalan) who will consider the request. Any reasonable requests will then be discussed with the COP:TRIN Steering Committee.

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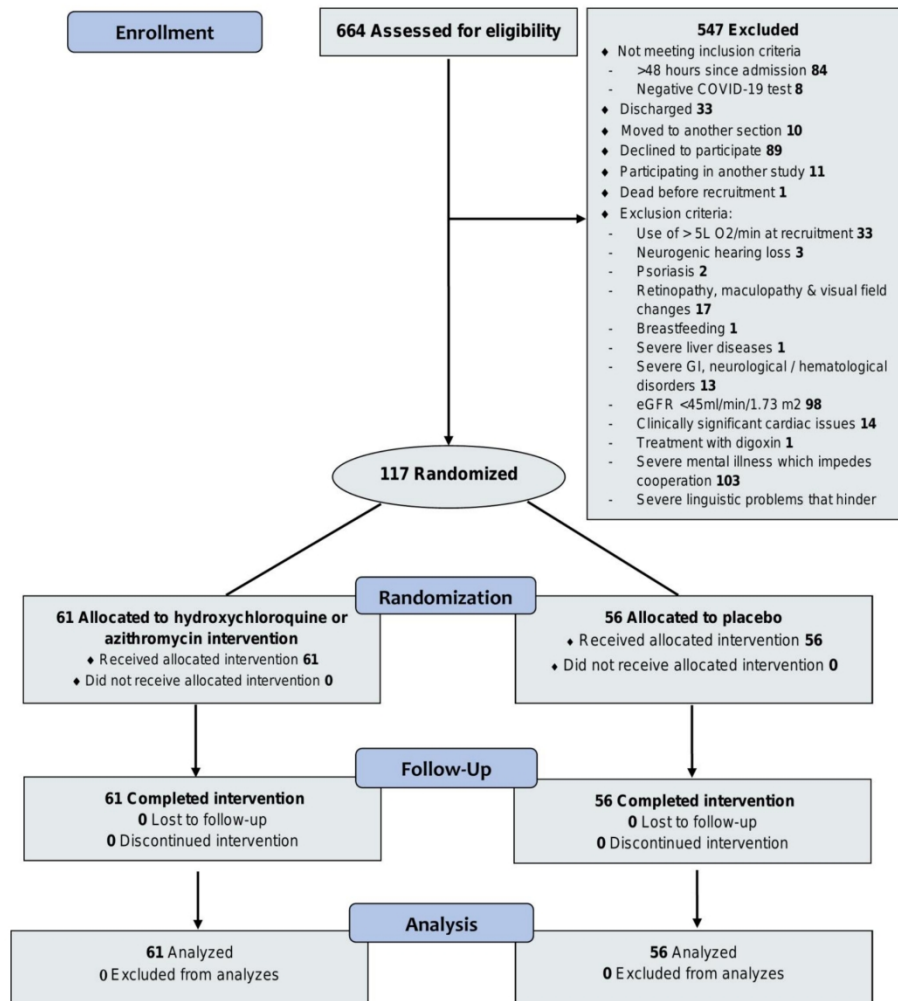
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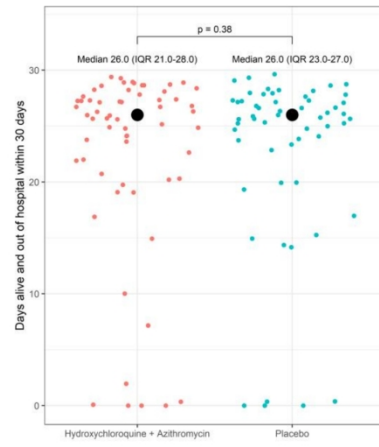
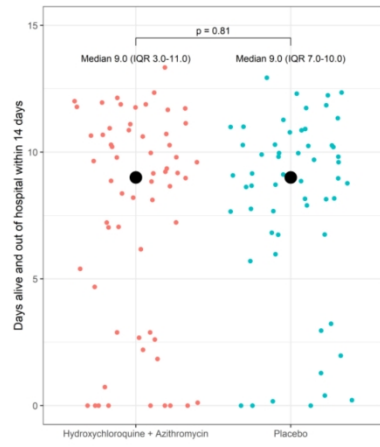
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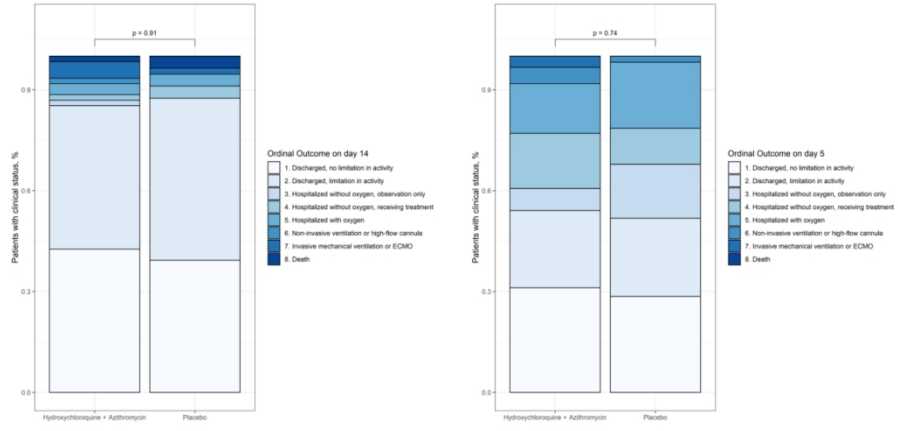
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CONSORT Diagram



Days alive and out of hospital at 14 days and 30 days, Median (IQR), days



Clinical status (COVID Outcomes Scale category) on day 5 and day 15

## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

16APR2020

V.1.8

### Study Protocol - Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

*A Randomised, Good-Clinical-Practice-monitored, Placebo-controlled, double-blinded study to clarify whether hospital length and risk of intensive care stay may be reduced in hospitalized patients who have COVID-19 treated with azithromycin and hydroxychloroquine for 15 days after inclusion.*

**Organisation: COP:TRIN – Chronic Obstructive Pulmonary Disease Trial Network:**

[www.coptrin.dk](http://www.coptrin.dk)

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This study is part of the Danish national non-commercial lung research network COP:TRIN ([www.coptrin.dk](http://www.coptrin.dk)) and is conducted as a randomised controlled trial.

Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19  
(ProPAC-COVID)

**Hypothesis:**

→ In patients with urgent hospital admission and a positive test SARS-CoV-2 treatment with hydroxychloroquine azithromycin leads to shorter hospitalisation and fewer admissions to the intensive care unit.

**EUdraCT-no.: 2020-001198-55**

**ClinicalTrials.gov Identifier: NCT04322396**

**The Danish Medicines Agency case no.: 2020033414**

**The Danish National Committee on Health Research Ethics approval no.: H-20022574**

**The Danish Data Protection Agency journal no.: P-2020-258**

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Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19  
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Associate Professor, Doctor, Ph.D. Tonny Studsgaard Petersen

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Ø Professor, Jens Kastrup, DMSc

GCP monitoring:

## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

The GCP units by GCP coordinator Kristina Devantier

COP:TRIN, Steering Committee: Please see: <http://coptrin.dk/steering-committee-members/>

Background information on the medication used in this trial:

### **Azithromycin**

Categorized as an antibacterial agent. The drug is an approved, and marketed in Denmark, for treatment of upper and lower respiratory tract infection. Azithromycin is also, according to available evidence and current guidelines, used for treatment of asthma, COPD and bronchiectasis in order to improve disease control and reduce exacerbation rate. Please see enclosed product summaries for further information.

### **Hydroxychloroquine:**

Categorized as an anti-inflammatory and anti-malaria agent.

The drug is approved and marketed in Denmark for the prevention and treatment of malaria, for the treatment of rheumatoid arthritis, discoid and systemic lupus erythematosus and juvenile idiopathic arthritis.

Manufacturing, packaging and labelling of IMP:

The trial drugs (IMP) are manufactured by Glostrup pharmacy by pharmacist Kristian Østergaard Nielsen. Placebo capsules are thus made similar to the intervention medicine. The drug (and Placebo) are labelled, according to Appendix 13. Glostrup pharmacy has a key for blinding. Therefore, it will always be possible to unblind a patient if indicated. Investigator must notify sponsor on grounds if a patient is unblinded.

Medical professionals dispense the IMP daily during hospitalization, except for patients preferring to handle their medication themselves. In the latter case, patient will fill in a medication diary (provided at randomisation). For all patients who are discharged during the intervention period, a medication diary will be provided. The medication diary is subsequently collected.

Exactly the amount of medication or placebo that the patient is required to take during the study periode will be provided, but for patients who do not take all the medication (protocol deviation), the remaining medication will be collected by the study staff. The patient will receive a follow-up phone call to check whether they have adhered to the medication schedule according to the trial protocol.



# Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

## 1. Hypothesis and aims:

### 1.1 Hypothesis:

In patients with acute hospital admission, a positive test for 2019-nCoV and symptoms of COVID-19 disease, treatment with virus-modifying agent hydroxychloroquine as well as virus-immunomodulatory and antibacterial drug azithromycin can lead to shorter hospitalisation and fewer admissions to the intensive care unit.

### 1.2 Aims:

The aim of this randomised GCP-controlled trial is to clarify whether combination therapy with macrolide azithromycin and hydroxychloroquine via anti-inflammation/immune modulation, antiviral efficacy and pre-emptive treatment of supra-infections can shorten hospitalisation duration (measured as "days alive and out of hospital" as the primary outcome), reduce the risk of non-invasive ventilation, treatment in the intensive care unit and death.

## 2. Background and scientific perspective:

### 2.1 Background - rationale

#### *Coronavirus - COVID-19*

In the ongoing coronavirus pandemic, COVID-19, with its origin in Wuhan, China, there is still sparse data on the course, risk of various complications, and the best possible treatment of patients admitted to hospital to ensure best possible survival and reducing length of stay at hospital. The most frequent symptoms are fever (> 80%) and cough (70-80%), together with radiologically findings of "ground-glass infiltrates" or "patchy infiltrates" in the patients with the most severely ill patients (86%), compatible with severe viral pneumonitis (1, 2). The length of hospitalisation is observed to be relatively long, 10-15 days (3), which in itself is a problem as hospitals can quickly reach the maximum capacity for hospitalisation and the proportion of patients who become critically ill have, based on the observations reported so far, had a mortality rate of > 60% (4), and overall mortality for admitted patients in China with COVID-19 infection is apparently unusually high for viral respiratory tract infections with an estimate of 25% (2).

#### *COVID-19 and lung disease*

Only specific data on patients with Chronic Obstructive Pulmonary Disease (COPD) have been reported in a few studies, but the risk of in-hospital death appears to be very high (OR 5.4 [95% CI 0.96-30.40]) (2).

#### *Lack of specific treatment*

## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

Despite the rapid spread of the disease globally, there is no solid data yet to recommend any specific treatments, and by that, symptomatic, organ supportive therapy is recommended, and in case of progression to severe acute respiratory failure mechanical ventilation (5). A high incidence of bacterial super-infections has been reported in patients with COVID-19 who died (50%) compared to survivors (1%) ( $p < 0.0001$ ), and likewise, an incidence of septic shock of 70% and 0%, respectively (2). Thus, there is an urgent need for treatments that can improve the course of the diseases in the individual patients, including positive impact on risk for hospital admission, duration of hospitalisation, risk of secondary infections and death.

### *Macrolide azithromycin as a possible treatment for patients with COVID-19*

Azithromycin is a macrolide antibiotic that has shown convincing efficacy in several studies in recent years to reduce hospitalisation-related exacerbations in COPD (6, 7), and to reduce exacerbation rate in asthma (8) and non-cystic fibrosis bronchiectasis (9). At the same time, it has been shown that azithromycin has a distinct effect by down-regulating airway inflammation by reducing CXCL1, TNF-alpha, IL-13 and IL-12p40 (10). Furthermore, a strong association has also been reported between survival from Acute Respiratory Distress Syndrome (ARDS) and administration of azithromycin (HR for 90 days of death for all causes: 0.49 [95% CI 0.27 - 0.87] in a well-conducted study (11).

Furthermore, it has been consistently observed in several recent publications that azithromycin itself appears to have an antiviral effect on a number of several viruses causing respiratory tract infections, such as Respiratory Syncytial Virus (RSV) (12), Rhinovirus (13) and Zika virus (14).

### *Hydroxychloroquine as immunomodulatory and antiviral agent by COVID-19*

Hydroxychloroquine has been marketed since 1934, originally developed for prophylaxis and treatment of malaria, but has for years also been used as an anti-inflammatory agent for rheumatic diseases. Large daily doses (up to 400 mg a day) of hydroxychloroquine are prescribed over many years to patients with arthritis such as systemic lupus erythematosus and rheumatoid arthritis for anti-inflammatory purposes, which is generally well-tolerated (16). but in addition to these effects, it is well described that the drug has an antiviral effect especially against flavivirus, retrovirus and coronavirus by inhibiting a number of low-pH-dependent steps in virus replication, as well as by inhibiting the pH-dependent endosomal mediated viral uptake in cells (15). The drug is well tolerated even with high dosage, for up to five years and there is no signal for birth defects with usage of the drug summarised by Savarino et al. (15).

Cell studies with primate cells infected with the coronavirus that induced SARS-1 (formerly called SARS) have shown that chloroquine, in a dose-dependent manner, inhibits the ability of the corona virus to infect cells and to spread among cells (17). Thus, several researchers and health care professionals have, during the present SARS-CoV-2 pandemic, have proposed studies examining hydroxychloroquine/chloroquine as treatment for patients with COVID-19 disease (18, 19).

# Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

## 2.2 Scientific perspective for this study

The study will clarify whether treatment with azithromycin in combination with hydroxychloroquine for 15 days from the time of hospital admission with diagnosed COVID-19 disease may reduce the length of hospitalisation, the risk of admission to the intensive care unit, treatment with non-invasive ventilation and death. The study will also clarify whether this treatment can reduce the need for oxygen supplementation (time for breathing on its own without oxygen supplementation) or for regular Long-Term Oxygen Therapy oxygen supplementation ("home oxygen").

If the treatment also improves the course of COVID-19 disease in patients with pre-existing lung disease, a very large number of patients could benefit from the treatment immediately.

The study originates from the Danish national non-commercial lung research network COP:TRIN (Chronic Obstructive Pulmonary Disease: Trial Network).

## 3. Method:

### 3.1 Design:

Randomised, Good-Clinical-Practice-monitored, placebo-controlled, double-blind study.

### 3.2. Recruitment and inclusion:

See point 12.

### 3.3 Inclusion:

#### Inclusion criteria:

- Patient admitted to Danish emergency departments, respiratory medicine departments or internal medicine departments
- Age  $\geq$  18 years
- Hospitalized  $\leq$  48 hours
- Positive SARS-CoV-2 test / diagnosis during the hospitalization (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<sub>2</sub>/min (equivalent to 40% FiO<sub>2</sub> if measured)
- Known intolerance/allergy to azithromycin or hydroxychloroquine or hypersensitivity to quinine or 4-aminoquinoline derivatives

## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

- Neurogenic hearing loss
- Psoriasis
- Retinopathy
- Maculopathy
- Visual field changes
- Breastfeeding
- Severe liver diseases other than amoebiasis (spontaneous INR > 1.5)
- Severe gastrointestinal, neurological and hematological disorders (investigator-assessed)
- eGFR <45 ml/min/1.73 m<sup>2</sup>
- 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 hospitalization 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.

### Drug Interactions

Interactions should be taken into account if the patient is taking other medications. For azithromycin, this includes antacids, ergotamine derivatives, colchicine and cyclosporine. For hydroxychloroquine, these include antidiabetic agents, tricyclic antidepressants, antipsychotics, halofantrine, cyclosporine, mefloquine, antiepileptic drugs, praziquantel and agalsidase.

### Standard Treatment

As there is no specific treatment for COVID-19, standard assessment and treatment is based on organ supportive therapy such as oxygen therapy (central), fluid therapy, antibiotic therapy for secondary infections. If the disease progress to severe acute respiratory failure, the patients will often require referral to an intensive care unit for mechanical ventilation.

## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

In addition, Danish national guidelines for handling of in-hospital Covid-19 patients can be obtained from [www.lungemedicin.dk](http://www.lungemedicin.dk) or from the open access journal European Respiratory Clinical Journal (at present in press).

### 3.3.2. Allocation:

The sponsor generates a randomisation sequence. Randomisation will be in blocks of unknown size and the final allocation will be via an encrypted website (REDCap), where also inclusion and exclusion criteria are required to be filled in correctly in order to randomise a patient.

Allocation will be stratified for age (>70 years vs. ≤70 years), site of recruitment and whether the patient has any of the following chronic lung diseases: COPD, asthma, bronchiectasis, interstitial lung disease (Yes vs. No).

### 3.3.3. Data collection, surveys and follow-up:

The primary daily project management is carried out by the project manager. In addition, a project group (investigators), consisting of doctors from the departments involved, is trained to assist the project manager with the recruitment, sampling and follow-up of patients. All medical decisions regarding patients will be taken by a physician. Data is collected in Case Report Forms (CRF) for each individual patient.

Prior to consent to participate in the trial, we will only assess the specific information needed to assess inclusion and exclusion criteria. No other information will be accessed.

It is the attending physician who asks if patients are interested in hearing more about the trial. If yes, an investigator is contacted, who will inform the patient about the trial.

As part of the study, all patients will be regularly monitored for oxygen saturation, heart rate, blood pressure, respiratory rate and temperature during hospitalisation.

The following information will also be obtained:

- Date of birth, age and gender
- Height, weight and BMI
- Use of medicine
- For patients with chronic obstructive pulmonary disease (COPD) classification in GOLD 1-4 and GOLD A-D (symptoms assessed via MRC degree)
- Systematic screening for co-morbidities
- Smoking history with pack years, current smoking status and alcohol consumption
- Information from the chest x-ray
- Arterial-blood gas analysis (pH, pCO<sub>2</sub>, pO<sub>2</sub>, Base Excess, oxygen supplementation)
- Hb, leucocytes + differential count, CRP, kidney function, liver parameters, electrolytes, LDH
- ECG, vital status, and adverse events

And from the patients' medical journal:



## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

\*The blood samples include haemoglobin (Hb), leukocytes + differential count, thrombocytes, C-reactive protein (CRP), Na+, K+, albumin, creatinine, urea, amylase, alkaline phosphatase, beta-2-Microglobulin, fibrinogen, glucose, TSH, INR, bilirubin, D-dimer, APTT, calcium, triglycerides, ferritin and lactate dehydrogenase (LDH). These blood tests will also be recommended daily for COVID patients outside studies in the recommendation of the Danish lung medicine association. \*\*When screening for the study, any ECG from within the last 3 days can be used. \*\*\*A follow-up ECG can be recorded during any remaining days of the hospital admission. \*\*\*\*Only in patients with COPD.

### 3.3.4 Intervention

Neither patients nor study staff will know which group the patient is allocated to. The medicine will be marked neutral, e.g. "Azithromycin group A" and "Azithromycin group B" and the same for Hydroxychloroquine.

NOTE: If the patient is receiving azithromycin prophylaxis, common practice is followed: the prophylaxis is paused and then restarted as usual.

#### Control group:

The control group will receive the standard treatment + placebo for both types of intervention IMP at all times. If part or all the intervention therapy being investigated becomes standard treatment during the study, this may also be offered to the control group.

#### Intervention group:

The patients in the intervention group will also receive standard care. Immediately after randomisation to the intervention group, the patient will begin treatment with :

#### Azithromycin:

Day 1-3: 500 mg x 1

Day 4-15: 250 mg x 1

If the patient is unable to take the medication orally by themselves, the medication will, if possible, be administered by either stomach-probe, or alternatively, temporary be changed to clarithromycin 500 mg x 2 (this only in agreement with either study coordinator Pradeesh Sivapalan or principal investigator Jens-Ulrik Stæhr Jensen). This will also be done in the control group if necessary. The patient will switch back to azithromycin when possible.

#### Hydroxychloroquine:

Furthermore, the patient will be treated with hydroxychloroquine as follows:

Day 1-15: 200 mg x 2

## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

Follow-up is done on days 14, 29, 90 and 365 days, by accessing the electronic medical record system. The specific information obtained, and its purpose can be found in section 3.3.3.

### Regarding dosage

The Summary of Product Characteristics for Azithromycin suggests 500mg/day for three days or 500mg/day for one day and then 250mg daily for four days. However, other clinical studies have found a positive effect of a daily dose of 250 mg for prolonged periods as prophylactic treatment. Mortality among hospitalised patients with COVID-19 is quite high and the median time for hospitalisation is 10-15 days, so it seems reasonable to give patients prophylactic dose for 12 days.

The dosing of hydroxychloroquine follows the summary of product characteristics.

### Regarding other treatment with antibiotics:

If antibiotic therapy is deemed indicated to the patient due to e.g. pneumonia or if it becomes standard therapy, piperacillin-tazobactam should be given as an empirical treatment at a dose adjusted to renal function. In case of penicillin allergy, cefuroxime is also given at a dose appropriate to renal function, weight and age. When positive microbiology is available, immediately switch to targeted treatment.

If *specific suspicion of atypical pneumonia* is raised, ciprofloxacin is administered at a dose of corresponding to kidney function and concomitant examination for atypical pneumonia will be performed. If negative, ciprofloxacin is discontinued. If positive, ciprofloxacin treatment is continued for the duration of treatment corresponding to the microorganism detected. If there is a specific need for treatment with macrolide and where other options are not available (e.g. allergy to fluoroquinolones, or when there is an estimated need for combination treatment of e.g. legionella pneumonia), consult with an investigator, and in this case it may be decided to discontinue azithromycin (active) or azithromycin placebo. In this case, treatment stops without unblinding.

Furthermore, ECG recordings during the treatment period will be analysed with focus on QTc. At QTc (F) > 480/470 ms for respectively women and men, IMP will be discontinued for safety reasons (but the patient remains in the study).

### 3.3.5. Statistical analyses:

#### Primary endpoint:

- Number of days alive and discharged from hospital within 14 days (summarises both whether the patient is alive and discharged from hospital) ("Days alive and out of hospital")

#### Secondary endpoints:



## Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID)

Secondary endpoint no. 1:

Ordinate outcome. The patient is categorized into one of the following 8 categories on day 15:

1. Dead
2. Hospitalised and mechanical ventilation or ExtraCorporalMembraneOxygenation (ECMO)
3. Hospitalised and Non-invasive ventilation or high-flow oxygen device
4. Hospitalised and given oxygen supplements that do not live up to oxygen supplements in (2) or (3) - e.g. oxygen on nasal catheter
5. Hospitalised and do not receive oxygen supplementation but need treatment (COVID-19 related or other)
6. Hospitalised and do not receive oxygen supplements and do not need treatment (just observed)
7. Discharged with restriction on activities, may be free of oxygen depletion or use LTOT ("home oxygen")
8. Discharged, no restrictions on activities

Other secondary endpoints:

- Admitted to the intensive care unit in the two groups (0 vs. 1) during the index admission
  - o For patients admitted to intensive care unit: number of days on intensive care (Length of stay, ICU)
- Have been on Non-Invasive Ventilation (NIV) (0 vs. 1) during index hospitalisation
- Dead at day 30
- Duration (days) of index hospital admission
- Days alive and discharged from hospital within 30 days (summarises both whether the patient is alive and discharged from hospital)
- Dead at day 90 (reported later)
- Dead within 12 months (reported later)
- Number of readmissions for all causes within 30 days
- Number of days on NIV or mechanical ventilation during index admission
- Delta PaO<sub>2</sub> day 1 (baseline) to day 4 (72 hours). At the same time oxygen supplements and oxygen systems are registered
- Delta PaCO<sub>2</sub> day 1 (baseline) to day 4 (72 hours)
- pH day 4 (72 hours)

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- Time to no oxygen supplement (or regular LTOT oxygen supplement)

In addition, several explorative endpoints.

Data is processed and analysed in SAS v.9.4 and graphs are generated in Microsoft Excel and SigmaPlot.

### 3.3.6. Sample size:

#### Randomised controlled study

Prerequisite: Type 1 error rate = 5%. Power = 80%. Two-sided statistics. Group sequential design with one scheduled interim analysis after randomisation of 113 patients (50% recruitment).

Analysis: T-test.

Sample size is calculated based on the following estimates and indicative figures:

Expected duration of hospitalisation with COVID-19: 10 days

SD for "Days alive and discharged within 14 days" in patients with lung disease and COVID-19: 4 days (up to 10 days hospitalization)

Estimated time for improvement/deterioration: 1.5 days.

This requires 226 patients randomised 1:1 with 113 in each group. This is a fixed sample size. It is assumed that most patients complete the intervention.

However, for interim analysis, the Data and Safety Monitoring Board (DSMB) may recommend the steering committee to expand sample size.

### 3.3.7 GCP Monitoring:

Frequency and depth are determined by the GCP units. Initiation visits and the first monitoring visits to all centres will be conducted off-site, i.e. without a physical meeting, due to the SARS-CoV-2 pandemic. Consent sheets will be scanned into an online system (REDCap or journal system) that can be accessed by GCP monitors.

## **4. Interim Analysis and Data and Safety Monitoring Board**

After recruiting half the sample size (approximately 113 patients), an interim analysis will be performed focusing on safety. An external Data and Safety Monitoring Board is appointed.

The interim analysis will be prepared and presented by physician Josefin Eklöf. The groups will be presented as "Group A" and "Group B" and DSMB will only be unblinded if they ask the steering committee for the study on this.

## **5. Blood samples:**

As part of usual care, blood samples are taken daily from the time of inclusion and as long as the patient is admitted. Blood samples include haemoglobin (Hb), leukocytes + differential count, thrombocytes, C-reactive protein (CRP), Na<sup>+</sup>, K<sup>+</sup>, albumin, creatinine, urea, amylase, alkaline phosphatase, beta-2-Microglobulin, fibrinogen, glucose, TSH, D-dimer, APTT, calcium, triglycerides, ferritin, bilirubin, ALAT, INR, and lactate dehydrogenase (LDH), see Table 1. These blood samples are analysed at the hospitals.

In addition, supplemental blood tests and material obtained with nasal swabs will be performed according to the sub-study protocols. Material from this will be included in a research biobank for the trial, and after completion of the trial in another regional biobank. The trial is expected to end in February 2021, and the material will then be transferred to the regional biobank.

For the present trial, the results of blood tests are collected from the patient record.

## **6. Side effects, risks and disadvantages:**

The treating physician may at any time discontinue intervention with IMP if, in clinical and/or paraclinical assessment, it is deemed contraindicated.

### Blood tests:

Serious side effects to regular blood sampling (venous puncture) are rare. Frequent (5-15%) can be seen transient discoloration of skin around the insertion site.

### X-ray:

Chest X-rays correspond to a radiation dose of approx. 0.1 millisievert (mSv). This should be compared with the average background radiation in Denmark of approx. 3 mSv per year. There are no documented adverse effects of the radiation dose received by Chest X-rays in the literature. Therefore, we believe that the study is not associated with any risks or side effects.

### Side effects of the trial medicine:

See [www.medicin.dk](http://www.medicin.dk)

### Azithromycin:

Very common (>10%) Abdominal pain, Diarrhoea, Flatulence, Nausea.

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Common (1-10%) Powerlessness, Decreased appetite. Vomiting, Taste Disorders. Decreased lymphocyte count. Decreased serum bicarbonate. Arthralgia. Headaches, Paraesthesia, Dizziness. Skin itching, rash. Visual disturbances.

Uncommon (0.1-1%) Pain. Hepatitis, Oral candidiasis. Dyspnea, Pneumonia, Oedema. Eosinophilia, Leukopenia, Neutropenia. Elevated serum bicarbonate, Hyperchloremia, Hyperglycaemia, Hyperkalaemia, Hyponatremia, Hypokalaemia, Hyponatraemia. Arthritis, Back Pain. Nervousness, Somnolence. Facial oedema, Dermatitis, Photosensitivity. Candidiasis, Infections. Metrorrhagia, Kidney Pain, Testicular Disease, Vaginitis. Hearing loss, Tinnitus.

Rare (0.01-0.1%) Cholestasis, Liver Impact. Agitation. Acute generalized exanthematous pustulosis\*, Allergic reactions\*, Angioedema\*, Hypersensitivity.

Not known Fulminant hepatitis, Hepatotoxicity, Hepatic insufficiency, Pancreatitis, Pseudomembranous colitis. Arrhythmias, Extended QT interval, Hypotension, Torsades de pointes tachycardia. Haemolytic anaemia, Thrombocytopenia. Aggravated myasthenia gravis. Aggressiveness, Anxiety, Delirium, Hallucinations, Hyperactivity, Hypesthesia, Seizures, Syncope. DRESS - drug reaction with eosinophilia and systemic symptoms, Erythema multiforme, Stevens-Johnson syndrome, Toxic epidermal necrolysis. Anaphylactic reaction. Acute renal failure, Interstitial nephritis.

\*In case of allergic reactions, including acute generalized exanthematous pustulosis and DRESS, azithromycin should be discontinued.

### Hydroxychloroquine:

Very common (> 10%) Abdominal pain, Nausea.

Common (1-10%) Eating refusals. Diarrhoea, Vomiting. Emotional lability, Headaches. Skin itching, rash. Accommodation difficulty.

Uncommon (0.1-1%) Liver effect. Nervousness, Sensomotor disorders, Dizziness. Alopecia. Corneal oedema, Double vision, Retinopathy \*, Tinnitus.

Not known Fulminant liver failure. Bronchospasm, prolonged QT interval\*\*, Cardiomyopathy. Aplastic Anaemia, Bone Marrow Depression, Influence of the Blood Image (agranulocytosis, anaemia, leukocytopenia, thrombocytopenia). Aggravation of Porphyria, Hypoglycaemia. Myopathy. Extrapyramidal genes, Cramps, Neuropathy, Palsy, Psychosis, Suicidal behaviour. Acute generalized exanthematous pustulosis\*\*\*, Exfoliative dermatitis, Erythema multiforme, Photosensitivity, Stevens-Johnson syndrome, Toxic epidermal necrolysis. Allergic reactions (including DRESS syndrome), Angioedema. Hearing loss, Macular and retinal degeneration, Maculopathy.

\*Retinopathy:

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- With pigment change requires careful dosing and careful control. When using rheumatologic doses, eye examination by an ophthalmologist is recommended before starting treatment and with follow-up to check for any eye manifestations that may arise. Annual monitoring after 5 years of treatment is recommended, however in risk patients initially annual control, see also Chloroquine derivatives (inflammatory rheumatic diseases), side effects.
- With cardiomyopathy can be fatal.
- With macular degeneration is seen and may be irreversible.
- With reversible corneal changes with oedema and blemishes can cause blurred vision or photophobia.
- With blurred vision accommodation is dose-dependent and reversible.
- In malaria treatment and prophylaxis, fewer and milder side effects occur.

\*\*Prolonged QT interval has been seen in patients with particular risk factors for it.

\*\*\*Acute generalized exanthematous pustulosis must be distinguished from psoriasis. Psoriasis exacerbation may occur. May be associated with fever and hyperleukocytosis.

An adverse reaction (AR) is defined as any adverse and undesirable reaction to a trial drug regardless of dose. An adverse event (AE) is defined as any adverse event in a patient or subject in a clinical trial following treatment with a drug, without necessarily linking this treatment to the adverse event.

Since the trial drugs are well known and have been used for many years, we will only record side effects not mentioned in the respective drug summary of the trial drug.

A severe adverse reaction or event (SAR/SAE) is defined as an event or adverse event that, regardless of dose, results in death, is life-threatening, results in hospitalization or prolongs hospitalization, results in significant or persistent disability or incapacity, or leading to a congenital anomaly or malformation.

Investigators must immediately (= within 24 hours) report serious incidents and serious adverse reactions (SAEs and SARs) to the sponsor regardless of whether they are described in the respective product summary. This allows the sponsor to assess the benefits and risks along the way in the study.

Events and adverse events recorded during the period from the patient have received the first dose of trial medication up to and including day 15.

Recording and reporting of all events and adverse events will end when the trial drug is stopped.

A high degree of comorbidity and death is seen in this patient group and therefore it is also expected that prolonged admissions, re-admissions, NIV, Respirator treatment and death will occur in this patient group. Therefore, these parameters will not be considered as a SAE.

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All incidents and registered side effects are reported at the end of the trial in a final report to the Danish Medicines Agency. All serious suspected adverse reactions must be reported annually together with a report on the safety of the subjects and sent to the the Danish Medicines Agency (LMST) and the Danish National Committee on Health Research Ethics (VEK).

The product summary of the trial drugs is used to assess whether a serious adverse event is unexpected and thus possibly a Suspected Unexpected Serious Adverse Reactions (SUSAR).

In the event of a fatal or life-threatening SUSAR, this must be registered and reported to LMST and VEK within 7 days of the sponsor becoming aware of it. No later than 8 days after the report, the sponsor must provide LMST and VEK with all relevant information about the sponsors and investigators' follow-up on the event. All other SUSARs are reported to LMST and VEK within 15 days of the sponsor becoming aware of them.

The report must be followed up by a detailed written report, and in both the immediate report and the subsequent report, the investigator must identify the subjects with a personal code number. When reporting deaths, the investigator must provide any additional information that the sponsor may request.

### **7. Economy:**

The research project is (investigator) initiated by COP:TRIN. Funding has been obtained from the Novo Nordisk Foundation of DKK 2.2 million for sponsor, remuneration of auxiliary personnel, payment of laboratory tests and equipment, as well as for manufacture of IMP treatment and placebo. The sponsors and investigators are not financially linked to private companies, foundations, etc. in this research project.

Medical expenses are covered, if not obtained from other sources, by the section for respiratory medicine research, Gentofte Hospital.

To the extent possible, the section for respiratory medicine research, Gentofte Hospital supports follow-up for endpoints and otherwise by appointment.

### **8. Remuneration:**

Patients are not paid for participation in the trial.

### **9. Availability of information and right to data:**

The consent gives the primary investigator, monitor and any control authority direct access to obtain information in the patient's medical record, etc., including electronic record, in order to see

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information about the subject's health conditions which are necessary as part of the implementation of the research project and for control purposes, including self-monitoring, quality-control etc.

The project group that has designed and conducted this study has the right to data and the right (and duty) to publish based on data. Project management manages data and invites members of the study group to publications. All sites that recruit patients are entitled to at least one authorship on the primary publication, and for every 10 patients recruited, the site is entitled to an extra authorship. Sites that have not participated in the design of the study are entitled to a maximum of 3 authorships. It is the opinion of the steering committee that knowledge sharing creates more and better scientific results. Requests for knowledge sharing from other groups may be submitted to Project Management (Jens-Ulrik Jensen, Charlotte Ulrik, Pradeesh Sivapalan) who will evaluate primarily and who, if the project is found suitable, will discuss it with the COP:TRIN Steering Committee.

Project Management has the first right to undertake sub-studies but may well assign projects to other contributors. In that case, the following considerations will be significant in the assessment: 1) Participation in the design phase of this RCT and at what level, and 2) Number of patients recruited at a site. If the hypothesis to be investigated is not planned to be examined by our group, we will allow the use of our data if the Steering Committee finds the project scientifically sound and, if appropriate, a collaboration with members of the COP:TRIN Steering Committee will be proposed. However, it should be emphasized that data is used for a specific purpose, not for future purposes in general. This becomes conditional by the steering committee for data to be used in a sound way to test hypotheses with relevant scientific content.

Information regarding subjects are processed and stored in accordance with the **Data Protection Regulation (GDPR)**, the Data Protection Act and the Health Act and the project is properly notified in accordance with applicable rules and laws to the appropriate authorities.

### **10. Publication of results:**

All project results will be sought published in scientific contexts, including international journals. This will happen regardless of whether the result is positive, negative or inconclusive.

### **11. Scientific Ethics Statement:**

The study is conducted in accordance with the Declaration of Helsinki and is carried out in accordance with the rules of the Personal Data Act and the Health Act. The study has been registered at the Danish Data Protection Agency.

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Recruitment and inclusion will take place as previously described (section 3.3.1). Participation requires a signed statement of consent. Patients can withdraw their participation consent and withdraw from the research project at any time without this having any effect on their right to current or future treatment. Furthermore, the patient is entitled to bring a bystander to the information interview and is entitled to reflection time before any declaration of consent is signed.

The important objective of the study is to investigate whether pro-active and *pre-emptive* treatment against COVID-19 can reduce the length of hospitalisation and the risk of intensive care and improve the survival of patients - an area that has so far been poorly researched and where the need for evidence-based guideline for handling and processing is large and very urgent.

Potential disadvantages and side effects are described in the separate section 5. Among other things, it appears that the likelihood of serious adverse reactions to both treatment and examinations is rare. In addition, the treating physician can always discontinue treatment if it is considered contraindicated.

Placebo is given patients allocated to the control group as no specific standard medical treatment is available.

The experimental method and statistical analyses have been carefully considered in order to be able to disseminate and apply relevant and secure research results to clinical practice.

Based on the above considerations, we believe that the experiment is ~~sound~~ ethically sound and can be conducted without exposing the test participant to unjustifiable risks.

### **12. Recruitment of subjects and informed consent**

At each trial centre, screening of patients admitted with a positive SARS-CoV-2 test is performed. Patients are assessed against the inclusion and exclusion criteria of the attending physician who receives the patient's consent to contact the investigator. The Investigator then contacts the patient for recruitment to the study. Disclosure of information about the study and obtaining informed consent may also be undertaken by other healthcare professionals. This includes research assistants (medical students), clinical nursing specialists and project nurses (See below for specific requirements). These are all separately trained in the task and have the opportunity to call a physician should any medical issues arise. They can also contact the coordinating investigator as well as a hotline team for the trial should any questions arise about informed consent. This hotline is available 24 hours a day. All patients are offered a consultation with a physician if they ask for it. For project nurses, the following applies specifically: i) must have at least 5 years of seniority. These requirements are verified by primary investigator from each site that afterwards creates a document for these individuals from which the above specific requirements are verified. This document is dated and signed.



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If a patient is considered suitable, the person will be invited to participate in the project. Participation in the trial is voluntary. Informed consent is obtained from the participants of the trial acc. to Executive Order No. 1149 of 30 September 2013 on information and consent for participation in health science research projects and on notification and supervision of health science research projects. The first contact with the the potential participant in the trial will be at admission to one of the participating departments. Participant information is provided both orally and in writing, and the patient is informed that they are entitled to 24 hours of reflection time before consent is given for participation in the trial. Participants who wish to do so themselves after the period of reflection time may give consent in connection with the information meeting.

The right to a bystander is ensured by the patient being able to bring a bystander, however, subject to COVID isolation rules. If no bystanders come to the first call, they are ensured afterwards to a bystander, when the patient is out of isolation. It is ensured that the conversations are undisturbed by using the patient's isolation room. If the doctor carries a "pager" or telephone, these are handed in prior to the call. The trial participant will be provided with the document "The research subject's rights in a health science research project", which contains information about confidentiality, access to documents and access to complaints. The subjects are protected under the Personal Data Processing Act. The trial has been reported to the Regional Science Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency.

It must be ensured at all times that subjects have consented to participate in clinical trials. If an isolated subject with COVID-19 can sign consent declaration via electronic tool, this can be used instead of consent with signature. This can be, for example, a mobile phone, an iPad, a laptop with secure identification, for example by an easy ID (or other solution that meets the OCES standard). If the above described solution is not possible, the following solutions can be used as temporary documentation for the consent:

- Copy of signed consent declaration – e.g. using camera: The subject can sign the consent form as usual. Since the signed form must not leave the isolation room, the signature can be documented in the form of a photograph of the signed form, for example through a window.
- If the test subject cannot sign the consent declaration himself, e.g. due to problems with having electronic equipment in the room, or obtaining documentation for the consent out of the room, the witness can sign on behalf of the subject: If the subject verbally consents, a witness can on behalf of the subject sign the consent form. For both of the above solutions, documentation (photo and witness signature) will be filed in the investigator's section of the Trial Master File (TMF). Furthermore, it is ensured that the Data Protection Regulation and the Data Protection Act are complied with, although documentation of the consent is temporarily different than it usually is. If the situation is normalised, the correct signed consent form must be obtained from the subject as soon as possible.

### **13. Exclusion and interruption of trials:**

Regular monitoring and quality control of the study will be carried out. If the physician responsible for the study deems it necessary, the physician may during treatment take the subject out of the study. The physician may also terminate the study at any time if there is a medical justification (such as the development of allergies to medicines), a safety risk or a requirement from the authorities. The test subject may also withdraw their informed consent and withdraw from the investigation at any time, as mentioned in the above paragraph.

### **14. Information on compensation and compensation schemes:**

Patients who participate in these studies and who believe they have suffered injury can seek compensation through the patient compensation (<http://patienterstatningen.dk/>) cf. Danish law.

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# **Statistical Analysis Plan - Proactive Prophylaxis with Azithromycin and Chloroquine in Hospitalized Patients with COVID-19 (ProPAC-COVID)**

19<sup>th</sup> May 2020

**A randomized, placebo-controlled, double-blinded multi-centre trial conducted in Departments of Internal Medicine, Emergency Medicine and Respiratory Medicine departments in Denmark**

**Estimated Primary Completion Date:** April 2021  
**Estimated Study Completion Date:** December 2021

ClinicalTrials.org identifier: NCT04322396. Registered on 26<sup>th</sup> of March 2020.

**Authors:** Pradeesh Sivapalan (PS) & Jens-Ulrik Stæhr Jensen (JUS)

## **Introduction**

In the ongoing coronavirus pandemic, COVID-19, that arose in Wuhan China, there is still sparse data in the course, risk of various complications, and how patients who are hospitalized are best treated to ensure high survival and short hospitalization. Despite the rapid spread of the disease globally, there is no robust data yet to recommend any specific treatments, which is why symptomatic, organ supportive therapy, including respiratory therapy in acute pulmonary failure, is recommended. There has been reported a high incidence of bacterial super-infections in patients with COVID-19. Patients with COVID-19 also have a higher risk of mortality because of septic shock. Thus, there is an urgent need for treatment that can improve the patient's chance of the shorter hospitalization and treatment that can lower the risk of secondary infection and death.

This is a randomized, placebo-controlled, double-blinded multi-center trial evaluating the effect of azithromycin and hydroxychloroquine treatment in patients with COVID-19 during hospitalization. The aim of the study is to investigate whether the therapy can shorten hospitalization, reduce the risk of non-invasive ventilation, admittance to intensive care units and death

The patients are enrolled in the trial only after obtaining informed consent. The trial is conducted at eight centers in Denmark.

1. Section of Respiratory Medicine, Herlev-Gentofte Hospital, Copenhagen, Denmark  
Principal investigator: Jens-Ulrik Jensen, Senior Consultant, Research Associate Professor, MD, PhD. Co-founder of COP:TRIN ([www.coptrin.dk](http://www.coptrin.dk)).
2. Department of Respiratory Medicine, Hvidovre Hospital, and University of Copenhagen  
PI: Charlotte Suppli Ulrik Professor DMSc. Head of the Respiratory Research Unit and the Management of Asthma during Pregnancy (MAP) program at Hvidovre Hospital.
3. University Hospital North Zealand Hospital, department of Pulmonary and Infectious diseases. PI: Andrea Browatzki Senior consultant, MD
4. Respiratory Medicine at the Bispebjerg Hospital, Copenhagen,  
PI: Therese Lapperre, Senior Consultant, Research associate professor
5. Respiratory medicine department, Zealand Hospital, Næstved and the University of Southern Denmark. PI: Uffe Bødtger, Research Associate Professor, Senior Consultant in Respiratory Medicine
6. Respiratory medicine department, Odense University Hospital, Odense, Denmark. pi: Christian Laursen, Research Associate Professor, Senior Consultant in Respiratory Medicine
7. Respiratory medicine department, Aarhus- and Aalborg University, PI: Ulla Weinreich, Research Associate Professor, Senior Consultant in Respiratory Medicine

Patients will be randomized to one of the two treatment arms:

- i) **Intervention group:** Azithromycin day 1-3: 500 mg x 1, day 4-15: 250 mg x 1  
Hydroxychloroquine: Day 1-15: 200 mg x 2
- ii) **Control group:** The control group will always receive the standard treatment and placebo for both types of intervention medication. If part or all the intervention therapy being investigated becomes standard treatment during the study, this may also be offered to the control group.

The analyses described in this document will be performed by Pradeesh Sivapalan, MD, PhD coordinating investigator, in cooperation with the sponsor and principal investigator Jens Ulrik Jensen, research associate professor, Respiratory Medicine Section, University of Gentofte Hospital, once the data have been entered, cleaned and released for use.

This document provides a detailed description of the statistical analyses that will be performed for the evaluation of the primary and secondary endpoints of protocolized for the ProPAC-COVID study.

The analyses described in this document are compatible with the recommendations of the CONSORT 2010 statement.

The International Conference on Harmonisation (ICH) of Good Clinical Practice (GCP)(1) and leading experts recommend that randomized clinical trials should be analyzed according to predefined outcomes and a predefined detailed statistical analysis plan (2). To prevent selective reporting of outcomes and data-driven analysis results and increase transparency this paper will in detail describe the detailed statistical analysis plan for the ProPAC-COVID trial while enrolment of patients and collection of data is still on-going and before the database is accessed for trial results.

### **Analysis population**

Data will be analyzed using intention-to-treat (ITT) principles and main analyses will also be subject to modified ITT analysis (started but not completed) and per protocol analysis (completed all intervention). When applying the ITT principle, all randomized patients will be analyzed in the groups to which they were originally allocated, regardless of whether they received the intended treatment or whether a protocol violation or protocol deviation occurred.

Patients who withdrew consent for the use of their data will not be included in any analysis. Only the facts that they were enrolled into the trial and withdrew consent, and the original study group to which they were allocated, will be reported.

A secondary analysis of the primary efficacy outcome will use a per protocol (PP) population.

A Consort diagram of participants will be presented in the study.

For the secondary ordinary outcome, we will use a Wilcoxon rank sum test. The primary outcome uses an ordinal severity scale with 8 categories, analyzed using the proportional odds model. The key parameter of interest is the “common odds ratio,” which quantifies the shift in the severity distribution resulting from treatment. For an efficacious treatment, an odds ratio greater than 1 quantifies an improvement in disease severity; a value of 2 indicates a bigger improvement than a value of 1.25.

### **Sample size**

The power to avoid type II error is 80% (1- $\beta$ ) at a two-sided 5% significance level, using a t-test for the primary outcome, and a group-sequential design allowing for one interim analysis at half target recruitment. This provides a sample size of 226 subjects. All confidence intervals reported will be 95% confidence intervals.

### **Analysis Software**

All analyses will be performed using SAS software version 9.4.

## **DATA ANALYSIS**

### **Descriptive analyses – Baseline characteristics at study enrollment (defined as day 1).**

The following baseline characteristics of the study population will be summarized separately within each randomized group:

- Age, median (IQR), y
- Male sex, n (%)
- Ethnicity (Caucasian, African (incl. Afro-American), Asian, Inuit, Unknown/other)
- Body mass index ( $\text{kg}/\text{m}^2$ , median, IQR)
- Current smoker, n (%)
- Ex-smoker, n (%)
- Nonsmoker, n (%)
- Pack-years history (median, IQR, y)
- Use of oxygen therapy, n (%)
- Use of CPAP, n (%)
- Use of noninvasive mechanical ventilation, n (%)
- Infiltrate on Chest X-ray, n (%)
- Oxygen consumption: L/min (median, IQR)
- Oxygen consumption:  $\text{FiO}_2$  (median, IQR)

### Clinical findings

- Systolic blood pressure (mm Hg)
- Diastolic blood pressure (mm Hg)
- Heart rate, beats/min
- Oxygen saturation with nasal oxygen, median, IQR
- Respiratory rate, breaths/min
- Temperature (°C)

### Biochemistry findings (daily measurements)

- Leukocyte count,  $\times 10^9$  cells/L
- Blood eosinophil count,  $\times 10^9$  cells/L
- CRP (mg/L)
- Fibrin D-dimer
- Ferritin
- Lactate Dehydrogenase (U/L)

### Arterial Blood Gas (mean $\pm$ SD) day 1 and day 4

- PCO<sub>2</sub>, mmHg
- PO<sub>2</sub>, mmHg
- HCO<sub>3</sub>
- pH

### Other Lab findings

- QTc (F) (via electronic measurement on ECG at baseline)

### Comorbid conditions, n (%)

- Asthma
- COPD
- Bronchiectasis
- Interstitial lung disease
- Allergy
- Diabetes mellitus
- Previous myocardial infarction
- Heart failure
- Atrial fibrillation
- Chronic renal insufficiency
- Essential hypertension,
- Osteoporosis
- Peripheral vascular disease



- Cerebrovascular disease
- Hematological diseases
- Depression
- Past or present lung cancer
- Previous cancer (which is not lung cancer)
- Former DVT or pulmonary embolism
- Liver failure
- Rheumatic diseases

### **Follow-up data /missing data**

% followed for each outcome data parameter will be reported for all predefined outcomes (primary and all secondary). If exploratory outcome analyses will be planned by the study group on suggestion from the reviewers/editors, % followed /missing data will also be reported for these outcomes.

### **Adherence data:**

N + % patients in both arms who:

- Started azithromycin
- Started hydroxychloroquine
- Completed azithromycin (all days)
- Completed hydroxychloroquine (all days)
- Completed both drugs (all days)

### **Medication during hospitalization**

- Type of antibiotics (non-study drugs)
  - Ciprofloxacin, Piperacillin/tazobactam, Ceftazidime, Meropenem, Colistin,
  - Gentamycin, Amoxicillin, Amoxicillin/Clavulanic, Surlid, Dicloxacillin, Penicillin, Azithromycin or other
- Days with antibiotics - any (Median days on any type)
- Days with corticosteroids (Median days)

For continuous variables, means and standard deviations will be presented, when normally distributed, otherwise as medians and interquartile ranges (IQR). For categorical variables, the number and percentage of participants within each category will be presented. For each variable, the percent of missing values will be reported. For categorical values, chi-square, Fisher's exact test. For time-to-event variables Cox regression and log-rank test will be used and for the latter, a corresponding Kaplan-Meier plot will be presented.

## **Primary objective and outcome**

The primary outcome is “days alive and out of hospital (DAOH) within 14 days after recruitment” defined as the time from hospital discharge and days without hospitalization up to 14 days from recruitment where the patient is alive. Data for the primary outcome analysis will be presented as mean [95%CI] and corresponding t-test and additionally for sensitivity analysis median [IQR] with corresponding non-parametric test, e.g. Mann-Whitney U-test

The estimation from the study group is that DAOH14 will be a number above or equal to 4. If DAOH14 is < 4, DAOH at 21 days will be presented instead. In this case, SD is estimated to not exceed 3.8 days, and the sample size should thus not be adjusted.

Apart from the main, unadjusted analysis, the primary outcome will be performed as an adjusted analysis using general linear models, while adjusting for the following variables: Age (per year increase), sex (M/F), COPD GOLD C/D (Yes vs. No), Heart failure NYHA III-IV (Yes vs. no), Active smoker (Yes vs. No).

## **Secondary objective and outcomes**

1. Categorization of hospitalization status [ Time Frame: 14 days]

The patient will be categorized into one of the following 8 categories depending on status of their hospitalization:

- a. Dead (yes/no)
- b. Hospitalized and receiving mechanical ventilation or Extra Corporal Membrane Oxygenation (ECMO) (yes/no)
- c. Hospitalized and receiving Non-invasive ventilation or "high-flow oxygen device" (yes/no)
- d. Hospitalized and given oxygen supplements different from (2) and (3) (yes/no)
- e. Hospitalized and without oxygen treatment, but receiving other treatment (both related to COVID-19 or other) (yes/no)
- f. Hospitalized for observation (yes/no)
- g. Discharged from hospital with restriction of activity level (yes/no)
- h. Discharged from hospital without any restrictions of activity level (yes/no)

For this analysis, the patient will be assigned a number between 1 and 8. Frequencies for the categories will be presented. Furthermore, the location on the scale for each group will be presented by median (IQR). Significance for differences will be calculated by a Wilcoxon-Sign-rank test (WSR).

Only one category can be "yes".

2. Admitted to intensive care unit, if admitted to ICU then length of stay [ Time Frame: 14 days]

Number of patients admitted to intensive care will be compared using chi-square test. Length of stay in ICU will be analyzed using a t-test. Days not alive within the time frame will be added to days at ICU. If days not alive are equal in the two treatment groups, we will further present days at ICU excluding days not alive.

3. Have used Non-invasive ventilation (NIV) during hospitalization [ Time Frame: 14 days]

Use of NIV will be compared by a chi-square test.

4. Mortality [Time Frame: 30 days ]

Differences in mortality will be displayed using the Kaplan-Meier plots method in combination with the log-rank test and as an adjusted analysis using Cox proportional hazards models, while adjusting for the following variables: Age (per year increase), sex (M/F), COPD GOLD C/D (Yes vs. No), Heart failure NYHA III-IV (Yes vs. no), Active smoker (Yes vs. No).

5. Length of hospitalization [ Time Frame: 14 days]

Length of hospitalization will be compared using t-test.

6. Days alive and discharged from hospital [ Time Frame: 30 days]

This is equal to the primary endpoint but with a longer time frame and will be analyzed like the that.

7. Mortality [ Time Frame: 90 days] – reported in secondary publication.

Differences in mortality will be displayed using the Kaplan-Meier plots method in combination with the log-rank test and as an adjusted analysis using Cox proportional hazards models, while adjusting for the following variables: Age (per year increase), sex (M/F), COPD GOLD C/D (Yes vs. No), Heart failure NYHA III-IV (Yes vs. no), Active smoker (Yes vs. No).

8. Mortality [ Time Frame: 365 days] – reported in secondary publication.

Differences in mortality will be displayed using the Kaplan-Meier plots method in combination with the log-rank test and as an adjusted analysis using Cox proportional hazards models, while adjusting for the following variables: Age (per year increase), sex (M/F), COPD GOLD C/D (Yes vs. No), Heart failure NYHA III-IV (Yes vs. no), Active smoker (Yes vs. No).

9. Number of readmissions (all causes) [ Time Frame: 30 days]

Number of readmission and compared using a Mann-Whitney test, treating death as a competing risk.

10. Number of days using non-invasive ventilation (NIV) [ Time Frame: 14 days]

Number of days using non-invasive ventilation will be compared using t-test, treating death as a competing risk.

11. Change in patient's oxygen partial pressure [ Time Frame: 4 days ]

Delta PaO<sub>2</sub> measured in arterial puncture

Changes will be calculated by an ANCOVA method adjusting for baseline values.

12. Change in patient's carbondioxid partial pressure [ Time Frame: 4 days ]

Delta PaCO<sub>2</sub> measured in arterial puncture

Changes will be calculated by an ANCOVA method adjusting for baseline values.

13. Level of pH in blood [ Time Frame: 4 days ]

pH measured in arterial puncture

Levels in pH will be compared using t-test

14. Time to no oxygen supplement (or regular oxygen supplement "LTOT") [ Time Frame: 14 days]

Time to no oxygen supplement will be presented by the Kaplan-Meier method and differences calculated by log-rank test.

For all analyses using parametric statistic (t-test, ANCOVA) the distribution will be inspected. Biochemical markers will be transformed if necessary whereas length of stays will not be transformed. If parametric statistics is considered inappropriate a non-parametric alternative will be used. For analyses with a dichotomous outcome Fisher's exact test will be used if the chi-square test is not considered appropriate.

Arrhythmias:

- ECG: Qtc: n (%) patients in both arms who at any time point after baseline had a Qtc (F) >500 ms
- N (%) ventricular arrhythmias (apart from ventricular extrasystoles and non-sustained VT).

### **Subgroup analyses (all according to baseline values)**

Scheduled to perform following stratified analyses for the primary outcome:

- stratified analyzes in the presence of chronic lung disease or not
- stratified analyzes for QTc across the median
- stratified analyzes for < 2L nasal oxygen or ≥ 2L nasal oxygen
- stratified analyzes CRP < 50 mg/L and CRP ≥ 50 mg/L
- stratified analyzes D-dimer > 0.8 mg/L or D-dimer ≥ 0.8 mg/L

## **Figures and tables**

The first figure will be a Consolidated Standards of Reporting of Randomized Trials (CONSORT) flow chart. The second figure will be a Kaplan-Meier plot to describe the process of death by treatment arms. The third figure will be a forest plot illustrating all the preplanned sub analyses.

The first table will be the baseline characteristics of the ITT population. The second table will be of the primary and secondary outcomes according to the two groups and pair-wise comparisons.

## **Blinding of the statistician**

The detailed analysis plan was written in strict concordance with the trial protocol approved by the regulatory authorities prior to recruitment initiation. The entire statistical analysis plan was published at [www.coptrin.dk](http://www.coptrin.dk) before the trial was finalized (while the database was closed). All analyses will be done prior to breaking of the randomization code (analysis comparisons between “arm A” and “arm B” (random names). The coordinating investigator (PS) and the study sponsor and principal investigator (JUU) will conjointly perform all the data analyses according to this plan, except the interim analyses which will be performed by Dr. Josefin Eklöf (who is not an investigator of this trial). An unblinding date will be chosen and published online at [www.coptrin.dk](http://www.coptrin.dk) and on this date, the allocation will be unblinded. After unblinding of the allocation, further analysis will not be done, except on reviewer/editor demand when submitted.

Interim Analysis:

The interim analysis will focus on reporting: selected baseline data (those readily available from the baseline data list above), primary outcome (in an O- Brien-Fleming Plot) and all-cause mortality at 30 days (Chi-square or Fisher’s Exact test, whichever appropriate).

## **Funding**

The trial was funding by Novo Nordisk Foundation with 2.2 million Dkr. No medical company is supporting this trial. The funding sources will have no influence on trial design, data collection, analysis, or reporting.

**Approved by:** Ethics Committees of all participating sides (H-20022574) and the Danish Medicines Agency (EudraCT no: 2020-001198-55) and the Danish Data Protection Agency (2020-256).

## **Acknowledgements**

The Steering Committee of COP:TRIN for input and advice during the trial recruitment.

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## **Competing interests**

The authors declare that they have no competing interests.

## **Abbreviations**

AECOPD Acute exacerbations of chronic obstructive pulmonary disease

ANOVA Analysis of variance

CAT COPD Assessment Test

CONSORT Consolidated Standards of Reporting of Randomised Trials

DAOH Days alive and out of hospital

FEV<sub>1</sub> Forced expiratory volume in 1 second

GCP Good Clinical Practice

ICH International Conference on Harmonisation

ITT Intention-to-treat

IQR Interquartile range

## References

1. ICH Harmonised Tripartite Guideline. Guideline for good clinical practice E6(R1). ICH Harmon Tripart Guidel. 1996;1996(4):i-53.
2. Finfer S, Bellomo R. Why publish statistical analysis plans? Crit Care Resusc [Internet]. 2009 Mar [cited 2018 Sep 20];11(1):5–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19281437>

## 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  $\geq$  18 years
- Hospitalised  $\leq$  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)

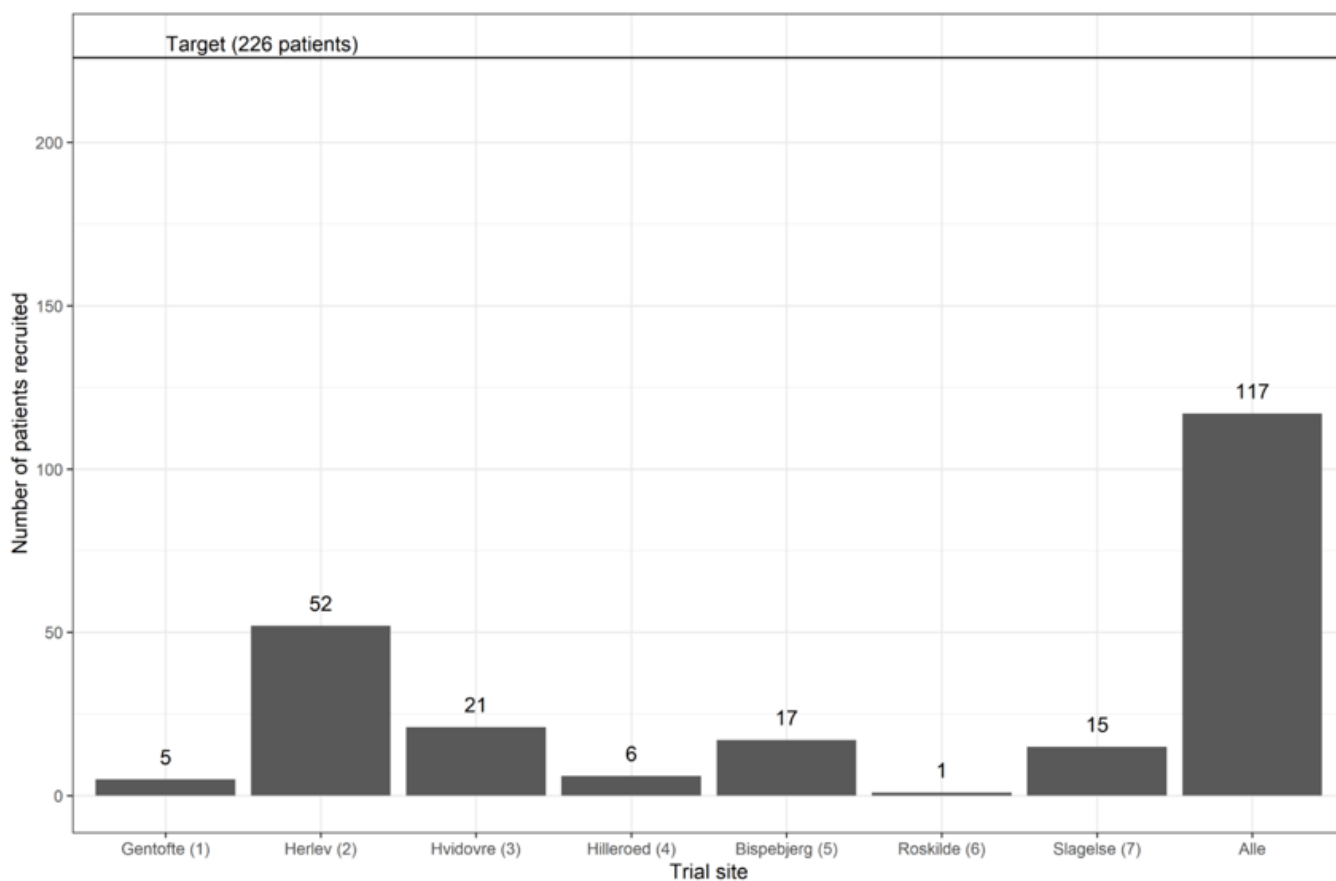
### Exclusion criteria

- At the time of recruitment, the patient uses  $> 5$  LO<sub>2</sub>/min (equivalent to 40% FiO<sub>2</sub> 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<sup>2</sup>
- 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.

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

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

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

Abbreviation: PDE4, phosphodiesterase-4.

**eTable 2. Chronic conditions**

(baseline characteristics in addition to those listed in Table 1)

| <b>Chronic condition – no. (%)</b>   | <b>All<br/>(<i>n</i> = 117)</b> | <b>Hydroxychloroquine<br/>and azithromycin<br/>(<i>n</i> = 61)</b> | <b>Placebo<br/>(<i>n</i> = 56)</b> |
|--|---------------------------------|--|------------------------------------|
| 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<br>(e.g., mature-onset diabetes of<br>the young) | 0 (0)                           | 0 (0)  | 0 (0)                              |
| Diabetes mellitus, unknown<br>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<br>lung cancer)                                  | 19 (16)                         | 11 (18)  | 8 (14)                             |
| Former deep vein thrombosis<br>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)                             |

**eTable 3. Medications received during hospital admission (before and after randomisation)**

| <b>Medication – no. (%)</b>                                      | <b>All<br/>(n = 117)</b> | <b>Hydroxychloroquine<br/>and azithromycin<br/>(n = 61)</b> | <b>Placebo<br/>(n = 56)</b> |
|--|--------------------------|---|-----------------------------|
| 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

**eTable 4. Adherence to trial drugs**

| <b>Adherence – no. (%)</b>                                     | <b>All<br/>(n = 117)</b> | <b>Hydroxychloroquine<br/>and azithromycin<br/>(n = 61)</b> | <b>Placebo<br/>(n = 56)</b> |
|--|--------------------------|---|-----------------------------|
| 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,<br>median (IQR) | 14 (3–15)                | 14 (2–15)   | 13 (7–15)                   |

Abbreviation: IQR, interquartile range



**eTable 5. Subgroup analyses, all according to baseline values: Treatment group difference (hydroxychloroquine and azithromycin minus placebo)**

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

Abbreviation: QTc, corrected QT interval

**eTable 6. Secondary outcomes – Ordinal day 15**

| <b>Clinical status (COVID Outcomes Scale category) – no. (%)</b>  | <b>Hydroxychloroquine and azithromycin (n = 61)</b> | <b>Placebo (n = 56)</b> | <b>P-value</b> |
|---|---|-------------------------|----------------|
| 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.

**eTable 7. Secondary outcomes – Ordinal day 5**

| <b>Clinical status (COVID Outcomes Scale category) – no. (%)</b>  | <b>Hydroxychloroquine and azithromycin (n = 61)</b> | <b>Placebo (n = 56)</b> |
|---|---|-------------------------|
| 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.

**eTable 8. 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.

| <b>Organ system</b>                             | <b>Adverse event</b>   | <b>All Adverse events, no.</b> | <b>Hydroxychloroquine and Azithromycin Adverse event, no.</b> | <b>Placebo Adverse event, no.</b> |
|---|------------------------|--------------------------------|---|-----------------------------------|
| 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                    | Itching<br>Rash        | 3                              | 3   | 0                                 |

|                            |          |            |           |           |
|----------------------------|----------|------------|-----------|-----------|
| disorders                  |          |            |           |           |
| Vascular disorders         | Bleeding | 2          | 2         | 0         |
| Any serious adverse events |          | 2          | 0         | 2         |
| <b>TOTAL</b>               |          | <b>103</b> | <b>60</b> | <b>43</b> |

Abbreviation: QTc, corrected QT interval

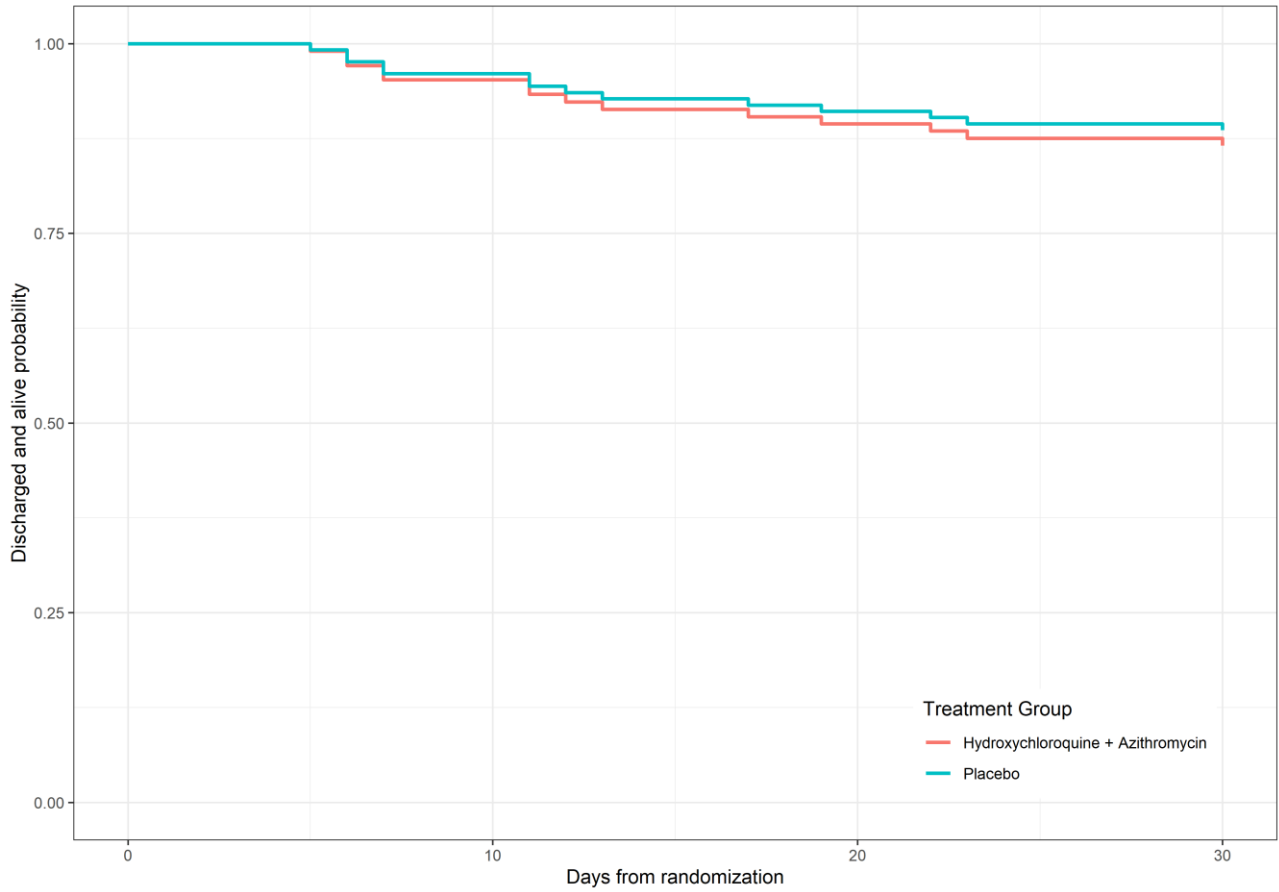
**eTable 9. Sensitivity analysis for primary outcome**

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

\*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).

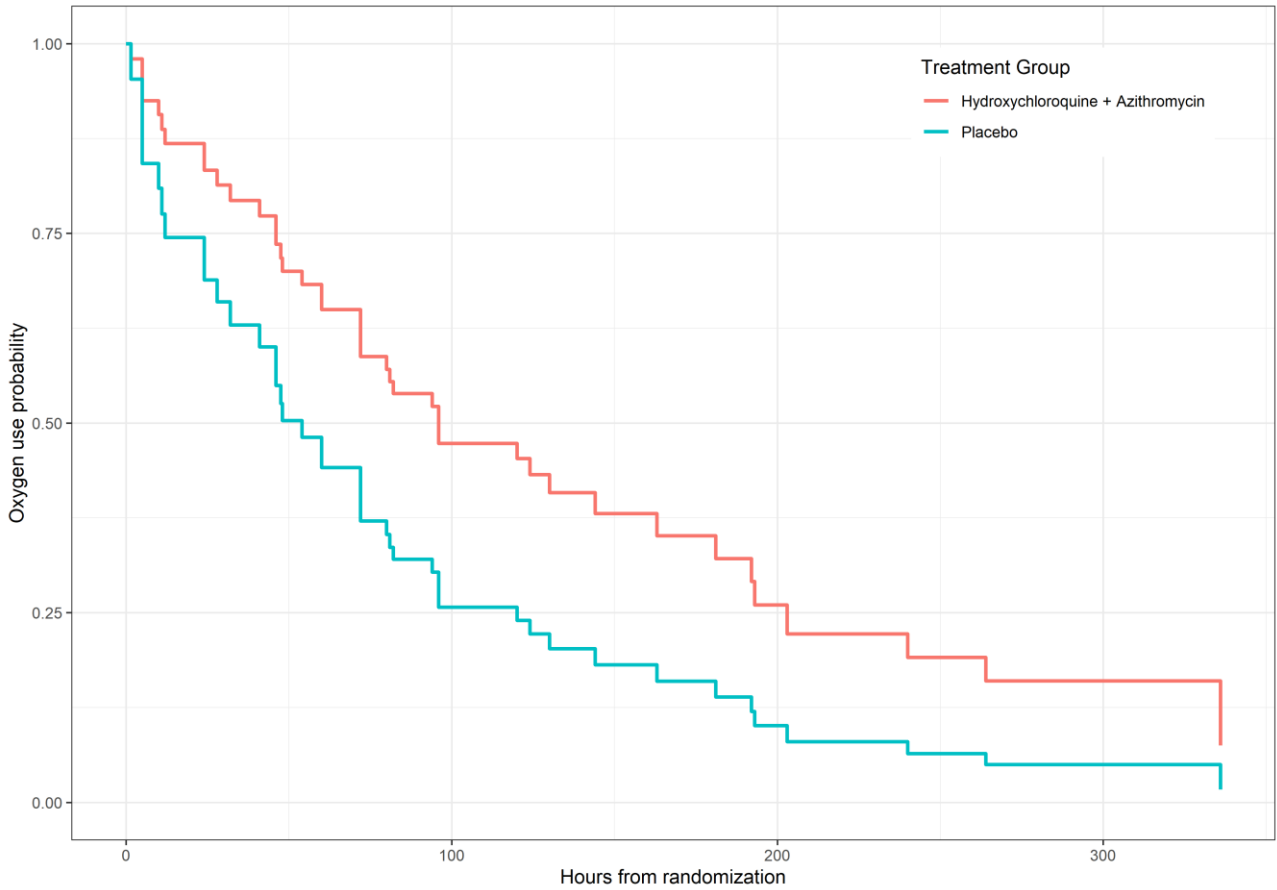
**Figure S2. Time to readmission or death within 30 days, adjusted analyses**

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).

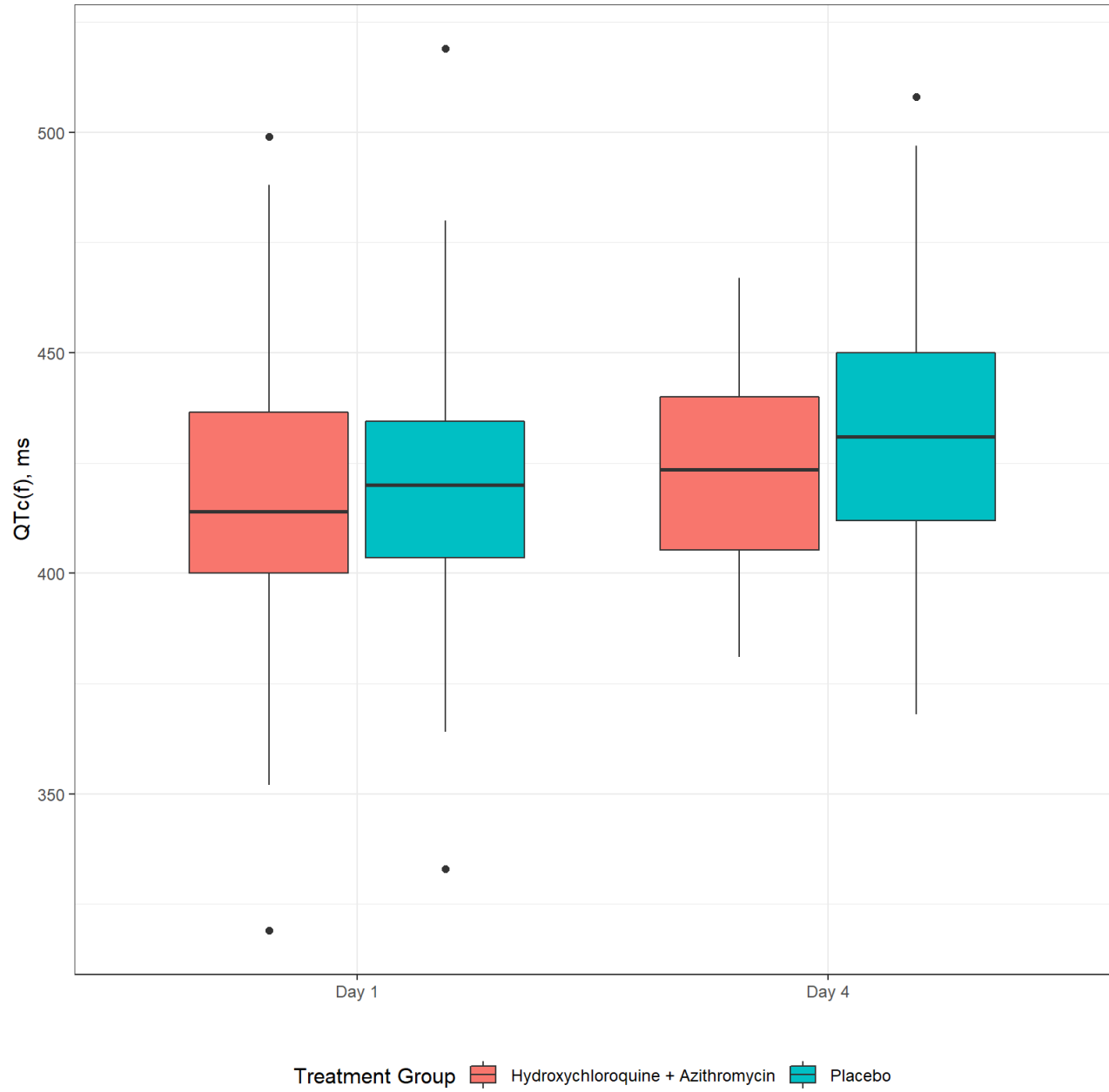


**Figure S3. Time to no oxygen within 14 days, adjusted analyses**

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**





## DSMB charter for the ProPAC-COVID-trial

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## I. Study Identification information

- A. Sponsors protocol code:** KronLungesyg\_COVID\_19\_protokol\_1\_4
- B. Study Title:** Proactive Protection with Azithromycin and hydroxyChloroquine in hospitalized patients with COVID-19 (ProPAC-COVID): A Randomized Clinical Trial
- C. Principal Investigator (PI):** Jens-Ulrik Jensen MD PhD, Research associate professor
- D. Study Coordinator:** Pradeesh Sivapalan MD PhD
- E. Study centre:** a) Department of Internal Medicine, Herlev and Gentofte University Hospital, Hellerup, Denmark. b) Department of Respiratory Medicine, Amager and Hvidovre University Hospital, Copenhagen, Denmark, c) Department of Internal Medicine, Zealand Hospital, University of Copenhagen, Roskilde, Denmark, d) Department of Respiratory Medicine, Bispebjerg University Hospital, Copenhagen, Denmark, e) Department of Respiratory and Infectious Diseases, Nordsjællands Hospital, Hillerød, f) Department of Respiratory Medicine, Aalborg University Hospital, Aalborg, Denmark; g) Department of Internal Medicine, Odense University Hospital, Odense, Denmark; h) Department of Medicine, Slagelse University hospital, Denmark.

## II. Study Overview

**A.** We are conducting a multicenter, randomized, placebo-controlled, blinded study in hospitalized patients with coronavirus infection (COVID-19). The aim is to determine for patients admitted to hospital with coronavirus infection and symptoms, whether the treatment with virus modifier agent Hydroxychloroquine as well as virus immunomodulatory and antibacterial drug Azithromycin may lead to better outcome (reduce the length of hospitalization, the risk of hospitalization, non-invasive treatment. ventilation and death).

**B.** The monitoring guideline outlined below for ProBe-COVID-trial will adhere to the protocol approved by the Ethics Committees of all participating sides (H-20022574), Danish Medicines Agency (EudraCT no: 2020-001198-55) and the Danish Data Protection Agency (journal-nr.: P-2020-256)

**C.** The independent Data and Safety Monitoring Board (DSMB) is established to ensure the safety of research participants and the integrity of the study data. It will periodically monitor progress, efficacy, safety and other confidential data from this trial. It is comprised of experts in relevant biomedical fields and biostatistics who have no direct relationship with the study. Outcome data will be privileged and shared only with members of the DSMB during the conduct of the trial.

### **III. Data Quality and Safety Review Plan and Monitoring**

#### **A. Subject Accrual and Compliance**

Review of the rate of subject accrual and compliance with inclusion/exclusion criteria will occur monthly during the first 3-month recruitment phase and then every 3 months to ensure that a sufficient number of participants are being enrolled and that they meet eligibility criteria.

Data on adherence to the treatment protocol will be collected for each patient by research staff and reviewed quarterly by the PI. Adherence of participants will be evaluated by performing pill counts and by monitoring the appropriate measures at each visit.

#### **B. Justification of Sample Size**

The probability that the study will detect a treatment difference is 80% at a two-sided 5% significance level. This provides a sample size of 226 subjects. Patients will be randomly assigned in a 1:1 fashion to either:

- i) Intervention group: Azithromycin day 1-3: 500 mg x 1, day 4-15: 250 mg x 1  
Hydroxychloroquine: Day 1-15: 200 mg x 2
- ii) Control group: The control group will always receive the standard treatment and placebo for both types of intervention medication. If part or all the intervention therapy being investigated becomes standard treatment during the study, this may also be offered to the control group.

#### **C. Stopping Rules**

This study will be stopped prior to its completion if the intervention is associated with adverse effects that call into question the safety of the intervention.

#### **D. Designation of an Independent Monitor**

The Independent Monitor for this study is the GCP unit at Bispebjerg University Hospital, Copenhagen, Denmark (Staff-monitor Kristina Devantier)

#### **E. Safety Review**

The DSMB review will be centered in systematic analysis of

1. Days alive and out of hospital within 14 days after recruitment
2. 30-day mortality rate
3. Readmission of all causes within 30 days

## **F. Membership:**

The PI and study staff may opt to attend the meetings for informational purposes but must be excused from portions of the meetings which involve voting and final decision-making. The following members have been requested to be part of DSMB:

- Dr John Hurst PhD FRCP  
Reader in Respiratory Medicine  
Royal Free Campus  
UCL Medical School
- Philipp Schuetz, Professor, Dr.Med. MPH  
Kantospital Aarau AG |  
KSA · Internal Medicine & Emergency Medicine  
Switzerland
- Bodil Steen Rasmussen  
Clinical Professor, Anaesthesia and Intensive Care Medicine, Aalborg University Hospital  
and President of EACTA - Aalborg Universitetshospital

These members have previously participated as DSMB members in previous trials, also for our group. They have a very high degree of knowledge on these patients and in-depth experience with trial management. The DSMB will be confirmed in good time before the interim analysis planned at half of full recruitment.

## **IV. Analyses**

The analyses described in this document will be performed by investigator, Josefin Eklöf MD Ph.d.-student, with guidance from the Principal investigator Jens Ulrik Jensen, Section of Respiratory Medicine, Department of Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Hellerup, Denmark, once the data have been entered, cleaned and released for use.

This document provides a description of the statistical analyses that will be performed for the evaluation of the primary and secondary endpoints of the ProBe-COVID-trial.

The analyses described in this document are compatible with the recommendations of the CONSORT 2010 statement ([www.consort-statement.org](http://www.consort-statement.org))<sup>1</sup>.

### **A. Analysis population**

Data will be analyzed using intention-to-treat (ITT) principles. All randomized patients will be analyzed in the groups to which they were originally allocated to, regardless of whether they actually received the intended treatment or whether a protocol violation or protocol deviation occurred[1].

Patients who withdrew consent for use of their data will not be included in any analysis. Only the facts that they were enrolled into the trial and withdrew consent, and the original study group to which they were allocated, will be reported.

Two-sided 5% significance levels will be used to identify statistically significant results. All confidence intervals reported will be 95% confidence intervals.

A secondary analysis of the primary efficacy outcome will use a per protocol (PP) population.

## **B. Definitions**

Baseline (during admission)

Follow-up is done on days 14, 29, 90 and 365 days. This is obtained via the journal system.

## **C. Analysis Software**

All analyses will be performed using SAS software version 9.4.

## **D. Descriptive analyses**

The following baseline characteristics of the study population will be summarized separately within each randomized group:

- Age, years (mean  $\pm$  SD)
- Male, n (%) / Female, n (%)
- Baseline, body mass index, kg/m<sup>2</sup> (mean 95% CI)
- Current smoker, n (%)
- Ex-smoker, n (%)
- Nonsmoker, n (%)
- Alcohol use, n (%)
- Pack-years history (mean 95% CI)
- GOLD classification 1-4 og A-D
- Baseline Arterial blood gas
- Systemic screening for comorbidities
- LTOT use and dosage
- Home NIV
- Baseline, systolic blood pressure, mm Hg, median (quartiles)
- Baseline, diastolic blood pressure, mm Hg, median (quartiles)
- Baseline, heart rate, beats/min, median(quartiles)
- Baseline, oxygen saturation, %
- Baseline, temperature (°C), median (quartiles)
- Baseline, Dyspnea mMRC, n (%)
- Baseline, leukocyte count, x10<sup>9</sup> cells/L (mean 95% CI)
- Baseline, CRP, mg/L

- Chest X-ray infiltrate, n (%)

For continuous variables, means and standard deviations will be presented, unless the variable has highly skewed distribution, in which case the median and interquartile range (IQR) will be presented. For categorical variables, the number and percentage of participants within each category will be presented. For each variable, the percent of missing values will be reported.

### **E. Primary objective and outcome**

The primary outcome is days alive and out of hospital within 14 days after recruitment. This is a very sensitive and specific outcome. Among other advantages, lead-time bias due to death was avoided using this endpoint measure (i.e., patients who died early would not be counted as a short length of stay). We will use student's t-test or Wilcoxon-Mann-Whitney test depending on the data distribution,

### **F. Secondary objective and outcomes**

The following endpoints will be included when assessing the clinical outcome:

1. Ordinary outcome.

The patient is categorized into one of the following 8 categories on day 15:

- a. Death
  - b. Inpatient and mechanical ventilation or ExtraCorporalMembraneOxygenation (ECMO)
  - c. Inpatient and Non-invasive ventilation or high-flow oxygen device
  - d. Hospitalized and given oxygen supplements that do not live up to oxygen supplements in (2) or (3) - e.g. oxygen on "nostrils"
  - e. Hospitalized and do not receive oxygen supplementation but need treatment (COVID-19 related or other)
  - f. Hospitalized and do not receive oxygen supplements and do not need treatment (just observed)
  - g. Discharged with restriction on activities, may be free of oxygen depletion or use LTOT ("home oxygen")
  - h. Discharged, no restrictions on activities
  - i. Number of readmissions for all causes within 30 days
2. 30-day mortality
3. Readmission of all causes within 30 days

### **G. COPD related hospital readmission within 30 days**

30-day hospital readmission rates will be analyzed by chi-squared tests or Fisher exact test.

### **H. All-cause mortality and time to next exacerbation**

Time to readmission of all causes or time to death will be calculated using the Kaplan-Meier method in combination with the log-rank test and Cox proportional hazards models.

### **I. Interim analysis**

We have planned the interim analysis when all the data from the first 113 patients have been entered into the database (half of the patients recruited (half of the patients recruited)). The DSMB may, apart from this planned interim analysis, decide to request an extra-ordinary interim analysis at any time point. This will be blinded to the investigators.

### **J. References**

1. **CONSORT STATEMENT** [<http://www.consort-statement.org>]

## Extra ordinary interim analysis:

### **Proactive Protection with Azithromycin and hydroxyChloroquine in hospitalized patients with COVID-19 (ProPAC-COVID):**

A Randomised, Good-Practice-monitored, Placebo-controlled, double-blind trial to clarify hospital length and risk of intensive care may reduce in hospitalized patients who have COVID-19 treated with azithromycin and hydroxychloroquine for 15 days after inclusion.

**Report date:** May 31, 2020

#### **Conducted and reported by:**

Alexander Svorre Jordan, Bach.Med, Respiratory Research Unit, Section of Pumonary Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark.

Josefin Eklöf, MD, PhD, Respiratory Research Unit, Section of Pumonary Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark.

#### **Principal investigator / Study director and scientific sponsor:**

Jens-Ulrik Stæhr Jensen, MD, PhD, Research Associate Professor: Respiratory Research Unit, Section of Pumonary Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark.

#### **Recruiting study centers:**

1. Pulmonary medicine section, Gentofte hospital, Kildegårdsvej 28, 2900 Hellerup, Denmark.
2. Pulmonary Medicine Department, Hvidovre Hospital, Kettegaard alle 30, Hvidovre, Denmark.
3. Pulmonary Medicine Section, Herlev Hospital, Herlev Ringvej, Herlev, Denmark.
4. Pulmonary and infectious medicine department, North Zealand Hospital, Dyrehavevej 29, 3400 Hillerød, Denmark.
5. Pulmonary Medicine Department, Aalborg Hospital, Hobrovej 18 -22, 9000 Aalborg, Denmark.
6. Pulmonary Medicine Section, Medical Department, Roskilde Hospital, Roskilde, Denmark.
7. Pulmonary Medicine Department, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark.
8. Medical Department, Slagelse Hospital, Denmark.



**Aim of the study:**

The aim of this randomised GCP-controlled trial is to clarify whether combination therapy with macrolide azithromycin and hydroxychloroquine via anti-inflammation/immune modulation, antiviral efficacy and pre-emptive treatment of supra-infections can shorten hospitalisation duration (measured as "days alive and out of hospital"; primary outcome), reduce the risk of non-invasive ventilation, intensive care and death.

**Analyses:**

Data is analyzed using intention-to-treat (ITT) principles, as stated in the trial protocol. All randomized patients were analyzed in the groups, to which they were originally allocated, regardless of whether they actually received the intended treatment or whether a protocol violation or protocol deviation occurred.

**Total number of patients that are planned to be recruited to the trial: 226**

**First patient in:** April 2020

**Total number of patients recruited:** 75 (33% of the planned patients. Treatment group A, n=41; Treatment group B, n=34)

**Following outcomes are included in the interim analyses:**

- Days alive and out of hospital (DAOH) within 14 days after recruitment (primary outcome)
- All-cause mortality rate 30 days after recruitment
- Readmission (any cause) or all-cause mortality within 30 days after recruitment
- ECG at baseline or day 2-5 with QTc (F) > 500 ms

Completed 14 days follow-up:

n=65 (treatment group A: 37/37, treatment group B: 28/28 patients)

Completed 30 days follow-up:

n=44 (treatment group A: 25/25, treatment group B: 19/19 patients)

## 1. Descriptive analyses:

Baseline characteristics of the population is described below (Table 1). The baseline characteristics are determined based on the population that completed 14 day follow-up (n=65), which is the same population for which the primary outcome was analyzed. The number of patients in each treatment group are noted in the first row of the table. In case of missing values, the total of number of patients are noted in the same row as the corresponding variable.

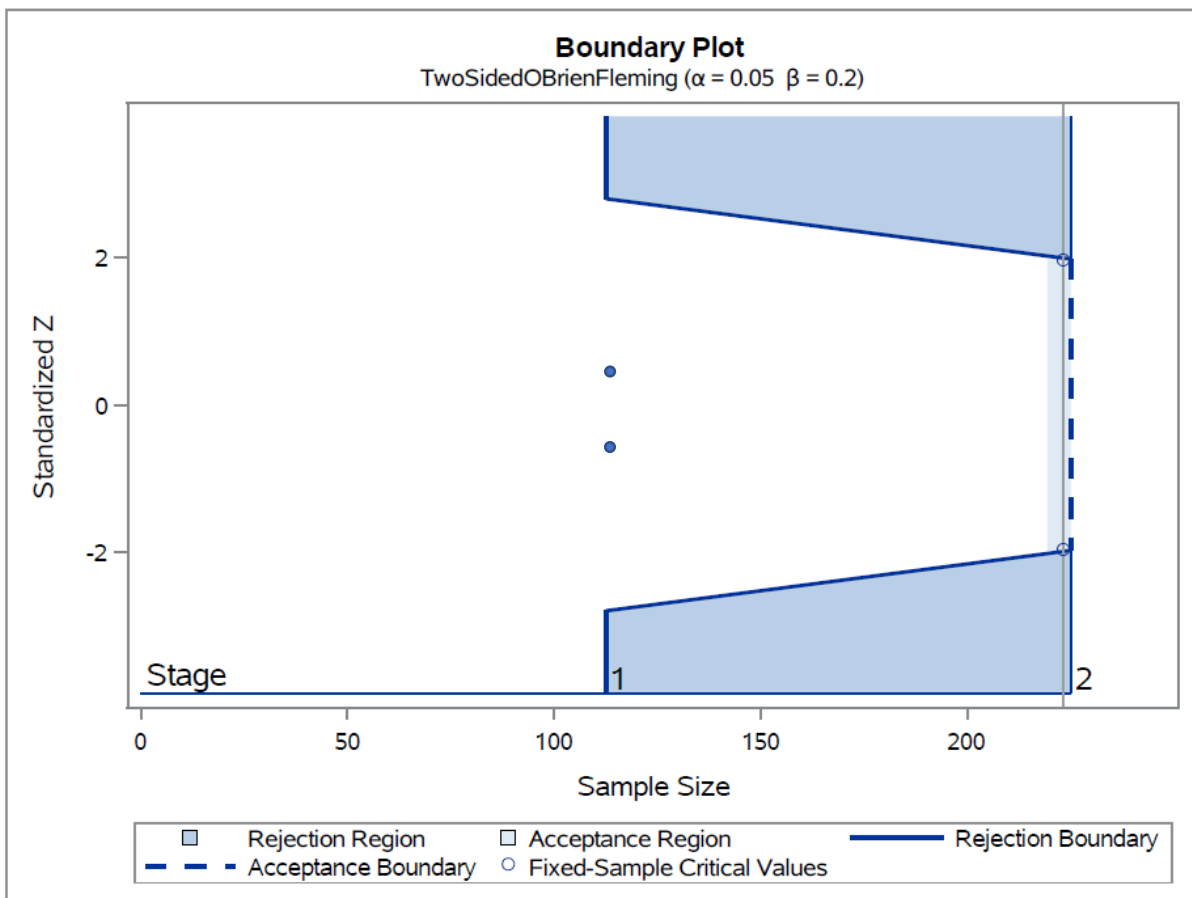
**Table 1.** Baseline characteristics of trial participants in both treatment groups (n=65).

| <b>Variables:</b>   | <b>Treatment group A:<br/>(n=37)</b> | <b>Treatment group B:<br/>(n=28)</b> |
|---|--------------------------------------|--------------------------------------|
| Age, years, median (IQR)  | 60 (51-82)                           | 62 (52-78)                           |
| Male, n (%)   | 23 (62,2)                            | 14 (50,0)                            |
| Pack-years tobacco, median (IQR)  | 5 (0-27)                             | 0 (0-25)                             |
| Oxygen consumption, L/min, median (IQR)<br>(Treatment group 1 n=36, Treatment group 2 n=27) | 0,5 (0-2)                            | 1 (0-2)                              |
| CRP, mg/L, median (IQR)<br>(Treatment group 1 n=35, Treatment group 2 n=27)                 | 59 (36-145)                          | 57 (33-118)                          |
| Diabetes mellitus, n (%)  | 9 (24,3)                             | 6 (21,4)                             |
| Heart failure, n (%)  | 4 (10,8)                             | 2 (7,1)                              |
| Atrial fibrillation, n (%)  | 8 (22,2)                             | 2 (7,1)                              |

## 2. Outcomes analysed in the interim analyses:

### 2.1 Days alive and out of hospital (DAOH) within 14 days after recruitment

|                     | Treatment group A<br>(n=37) | Treatment group B<br>(n=28) | P-value |
|---------------------|-----------------------------|-----------------------------|---------|
| DAOH, mean (95% CI) | 7.8 (6.4-9.3)               | 7.6 (5.9-9.3)               | 0.81    |
| DAOH, median (IQR)  | 9 (3-11)                    | 9.5 (5-10)                  | 0.62    |



**Figure 1. Primary outcome (DAOH within 14 days from recruitment):** Boundaries of an O’Brien-Fleming sequential design involving the ProPAC-COVID trial. Two Z-values have been plotted since arms are blinded. The actual Z-values are + 0.49 and - 0.49.

**2.2 All-cause mortality rate 30 days after recruitment**

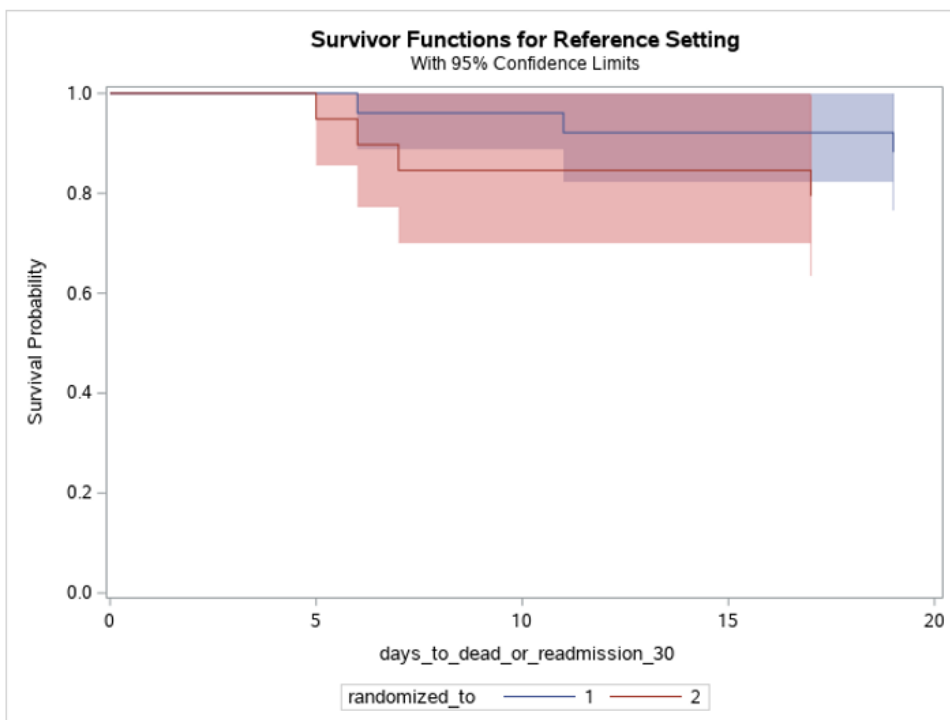
|                         | Treatment group A<br>(n=25) | Treatment group B<br>(n=19) |
|-------------------------|-----------------------------|-----------------------------|
| Dead (all-cause), n (%) | 1 (4,0)                     | 1 (5,3)                     |
| Alive, n (%)            | 24 (96,0)                   | 18 (94,7)                   |

P-value (Fischer Exact Test): 1.00

**2.3 Readmission (any cause) or all-cause mortality within 30 days after recruitment**

|                                 | Treatment group A<br>(n=25) | Treatment group B<br>(n=19) |
|---------------------------------|-----------------------------|-----------------------------|
| Readmitted or dead, n (%)       | 3 (12,0)                    | 4 (21,1)                    |
| Not readmitted and alive, n (%) | 22 (88,0)                   | 15 (78,9)                   |

P-value (Fischer Exact Test): 0.44



**Figure 2.** Kaplan-Meier plot: Time to readmission (any cause) or all-cause mortality within 30 days from recruitment. Log-rank test: p = 0.40 (randomized\_to=1: treatment group A; randomized\_to=2: treatment group B).

**2.4 Any ECG at baseline and day 2-5 withy QTc (F) > 500 ms**

|                                     | <b>Treatment group A<br/>(n=37)</b> | <b>Treatment group B<br/>(n=28)</b> |
|-------------------------------------|-------------------------------------|-------------------------------------|
| QTc (F) > 500 ms at baseline, n (%) | 0 (0,0)                             | 1 (3,6)                             |
| QTc (F) > 500 ms at day 2-5, n (%)  | 0 (0,0)                             | 0 (0,0)                             |

## **1<sup>st</sup> planned interim analysis:**

### **Proactive Protection with Azithromycin and hydroxyChloroquine in hospitalized patients with COVID-19 (ProPAC-COVID):**

A Randomised, Good-Practice-monitored, Placebo-controlled, double-blind trial to clarify hospital length and risk of intensive care may reduce in hospitalized patients who have COVID-19 treated with azithromycin and hydroxychloroquine for 15 days after inclusion.

**Report date:** January 28 ,2021

#### **Conducted and reported by:**

Tobias Wirenfelt Klausen, M.Sc., Statistician, Department of Hematology, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark.

Alexander Svorre Jordan, Bach.Med, Respiratory Research Unit, Section of Pumonary Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark.

Josefin Eklöf, MD, PhD, Respiratory Research Unit, Section of Pumonary Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark.

#### **Principal investigator / Study director and scientific sponsor:**

Jens-Ulrik Stæhr Jensen, MD, Professor: Respiratory Research Unit, Section of Pulmonary Medicine, Herlev and Gentofte Hospital, University of Copenhagen, Hellerup, Denmark.

#### **Recruiting study centers:**

1. Section of Pulmonary Medicine, Gentofte hospital, Kildegårdsvej 28, 2900 Hellerup, Denmark.
2. Department of Pulmonary Medicine, Hvidovre Hospital, Kettegaard alle 30, Hvidovre, Denmark.
3. Section of Pulmonary Medicine, Herlev Hospital, Herlev Ringvej, Herlev, Denmark.
4. Department of Pulmonary and Infectious Medicine, North Zealand Hospital, Dyrehavevej 29, 3400 Hillerød, Denmark.
5. Departmen of Pulmonary Medicine, Aalborg Hospital, Hobrovej 18 -22, 9000 Aalborg, Denmark.
6. Section of Pulmonary Medicine, Medical Department, Roskilde Hospital, Roskilde, Denmark.
7. Department of Pulmonary Medicine, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark.
8. Department of Internal Medicine, Slagelse Hospital, Denmark.

**Aim of the study:**

The aim of this randomised GCP-controlled trial is to clarify whether combination therapy with macrolide azithromycin and hydroxychloroquine via anti-inflammation/immune modulation, antiviral efficacy and pre-emptive treatment of supra-infections can shorten hospitalisation duration (measured as "days alive and out of hospital"; primary outcome), reduce the risk of non-invasive ventilation, intensive care and death.

**Analyses:**

Data is analyzed using intention-to-treat (ITT) principles, as stated in the trial protocol. All randomized patients were analyzed in the groups, to which they were originally allocated, regardless of whether they actually received the intended treatment or whether a protocol violation or protocol deviation occurred.

**Following outcomes are included in the interim analyses:**

1. Days alive and out of hospital (DAOH) within 14 days after recruitment (primary outcome)
2. All-cause mortality rate 30 days after recruitment
3. Readmission (any cause) or all-cause mortality within 30 days after recruitment

**Total number of patients that are planned to be recruited to the trial:** 226

**Total number of patients recruited:** 117 (52%)

**First patient in:** April 2020

**Total number of patients allocated to Treatment group A:** 61

**Total number of patients allocated to Treatment group B:** 56

**Number of patients who have completed 14 days follow-up:** n=117 (100%)

**Number of patients who completed 30 days follow-up:** n=117 (100%)

**Conditional power analysis (see page 4):**

This analysis was performed on the primary outcome (DAOH within 14 days after recruitment) of the trial (using t-test, non-equality design and two-sided significance level of 0.05).

A post-conditional power <0.20 for efficacy should be considered with the integrated impression of the entire report, as well as other available publications in the field, as a stopping guide for futility.

## Descriptive analyses of the study population:

Baseline characteristics of the study population (n=117) are described in Table 1.

In case of patients with missing values, the total of number of patients assessed are noted in the row of the corresponding missing variable.

**Table 1. Baseline characteristics of study population (n=116)**

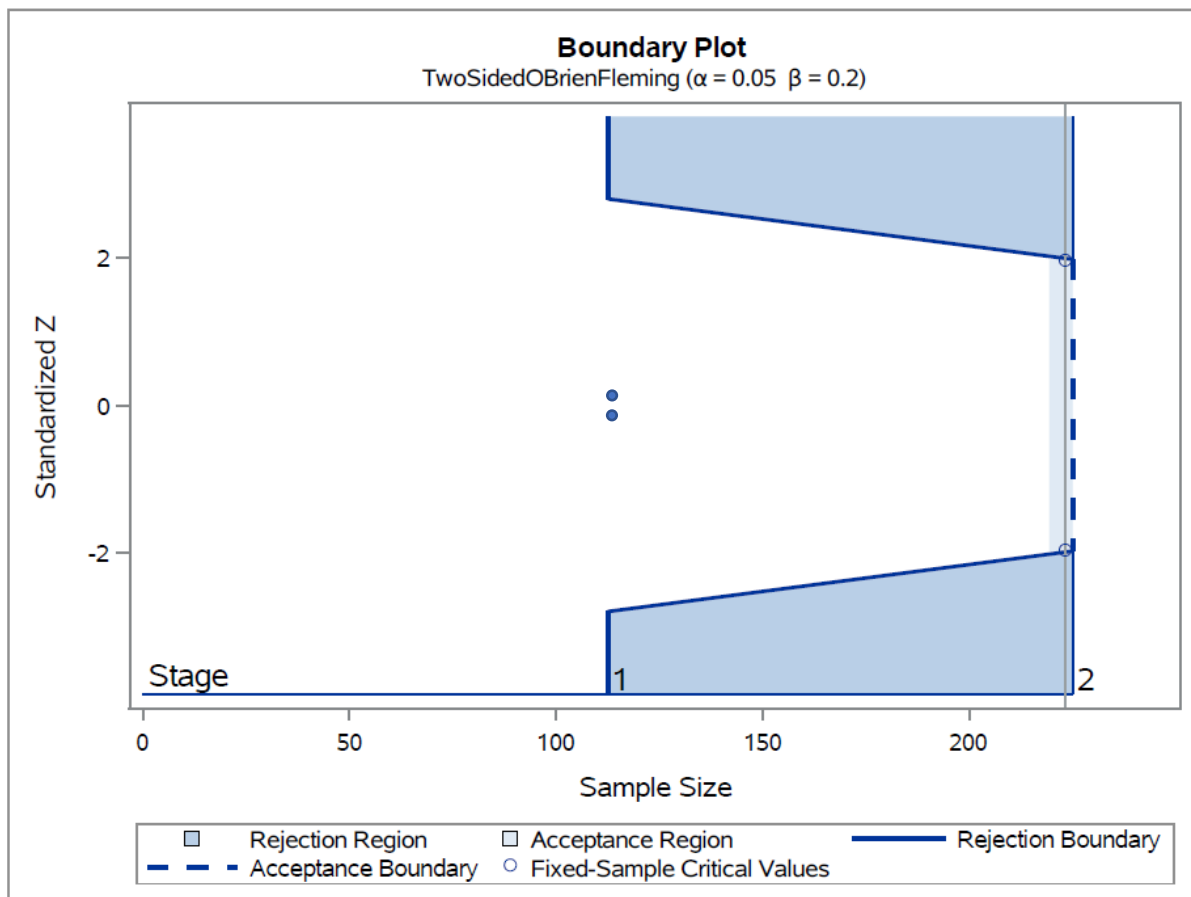
| <b>Variables:</b>   | <b>Treatment group A<br/>(n=61)</b> | <b>Treatment group B<br/>(n=56)</b> |
|---|-------------------------------------|-------------------------------------|
| Age, years, median (IQR)  | 67 (52-80)                          | 62 (52-74.5)                        |
| Male, n (%)   | 36 (59.0)                           | 29 (51.8)                           |
| Pack-years tobacco, median (IQR)  | 1 (0-20)                            | 0 (0-20)                            |
| Oxygen consumption, L/min, median (IQR)<br>(Treatment group 1: n=57, Treatment group 2: n=52) | 0 (0-2)                             | 1 (0-2)                             |
| CRP, mg/L, median (IQR)<br>(Treatment group 1: n=55, Treatment group 2: n=54)                 | 58 (36-101)                         | 81.5 (34-136)                       |
| Diabetes mellitus, n (%)  | 17 (27.9)                           | 11 (19.6)                           |
| Heart failure, n (%)  | 5 (8.2)                             | 3 (5.4)                             |
| Atrial fibrillation, n (%)  | 13 (21.7)                           | 6 (10.7)                            |



**Results - outcome analyses:**

**1. Days alive and out of hospital (DAOH) within 14 days after recruitment**

|                     | Treatment group A<br>(n=61) | Treatment group B<br>(n=56) | P-value           |
|---------------------|-----------------------------|-----------------------------|-------------------|
| DAOH, mean (95% CI) | 7,5 (6,4-8,6)               | 8,0 (7,0-9,0)               | 0,4922 (T-test)   |
| DAOH, median (IQR)  | 9,0 (3,0-11,0)              | 9,0 (7,0-10,0)              | 0,9093 (Wilcoxon) |



**Figure 1. Primary outcome (DAOH within 14 days from recruitment):** Boundaries of an O’Brien-Fleming sequential design involving the ProPAC-COVID trial. Two Z-values have been plotted since arms are blinded. The actual Z-values are + 0,1210 and - 0,1210.

**Post-conditional power analysis of DAOH within 14 days from recruitment:**  
0.064

**2. All-cause mortality rate 30 days after recruitment**

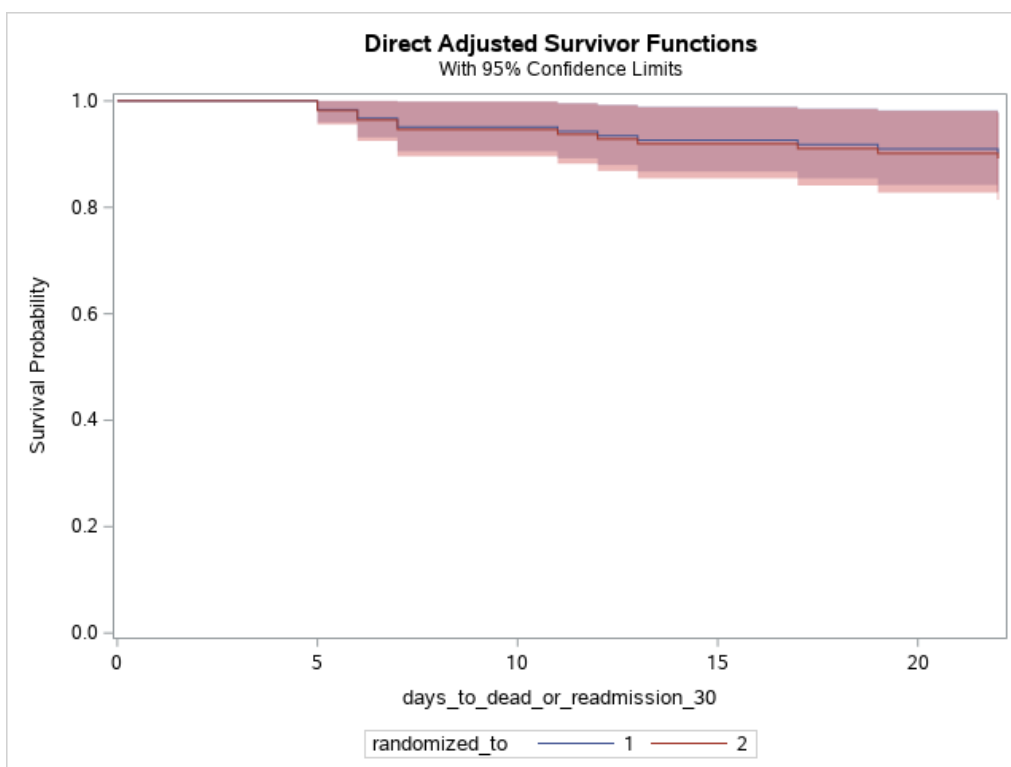
|                         | Treatment group A<br>(n=61) | Treatment group B<br>(n=56) |
|-------------------------|-----------------------------|-----------------------------|
| Dead (all-cause), n (%) | 1 (1,6)                     | 2 (3,6)                     |
| Alive, n (%)            | 60 (98,4)                   | 54 (96,4)                   |

P-value (Fischer Exact Test): 0.6060

**3. Readmission (any cause) or all-cause mortality within 30 days after recruitment**

|                                 | Treatment group A<br>(n=61) | Treatment group B<br>(n=56) |
|---------------------------------|-----------------------------|-----------------------------|
| Readmitted or dead, n (%)       | 8 (13,1)                    | 6 (10,7)                    |
| Not readmitted and alive, n (%) | 53 (86,9)                   | 50 (89,3)                   |

P-value (Fischer Exact Test): 0.7800



**Figure 2.** Kaplan-Meier plot: Time to readmission (any cause) or all-cauase mortality within 30 days from recruitment. Log-rank test: p = 0.9109 (randomized\_to 1= treatment group 1; randomized\_to 2= treatment group 2).

3<sup>rd</sup> June 2020

Dear Jens-Ulrik

This letter confirms that it was the unanimous decision of the ProPAC-COVID Data Safety and Monitoring Board to recommend continuation of the ProPAC-COVID study.

Data from 65 participants were reviewed at an Extraordinary Meeting of the DSMB today, June 3<sup>rd</sup> 2020. We saw no evidence of a difference in the primary outcome 'Days Alive and Out of Hospital' between the two groups, or in all cause mortality at thirty days. The event rate is low suggesting that there is no safety concern in either arm.

Recent controversy around observational data in relation to these drugs in COVID only serves to emphasise the importance of completing randomised trials such as ProPAC-COVID.

Yours sincerely,

Professor John Hurst, Chair of the DSMB, and on behalf of:  
Professor Dr. med. Philipp Schuetz, Medizinische Universitätsklinik, Aarau, Switzerland  
Professor Bodil Steen Rasmussen, Aalborg University Hospital, Denmark



**Professor John Hurst PhD FRCP FHEA**

Professor of Respiratory Medicine  
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<https://iris.ucl.ac.uk/iris/browse/profile?upi=JHURS15>

## Jens Ulrik Stæhr Jensen

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**Fra:** Hurst, John <j.hurst@ucl.ac.uk>  
**Sendt:** 1. februar 2021 11:14  
**Til:** Jens Ulrik Stæhr Jensen  
**Cc:** 'Schütz Philipp'; 'Bodil Steen Rasmussen'  
**Emne:** ProPAC-COVID DSMB Recommendaiton

1<sup>st</sup> February 2021

Dear Jens-Ulrik

**Re: Proactive Prophylaxis With Azithromycin and Chloroquine in Hospitalised Patients With COVID-19 (ProPAC-COVID) Study**

The DSMB met on Friday 29<sup>th</sup> January 2021 to discuss results of the pre-planned interim analysis of the ProPAC-COVID study, provided to us by Josefin Eklöf and Tobias Wirenfeldt Klausen.

The interim analysis was completed when 117/226 (52%) of patients had been recruited. We remained blinded to the treatment allocation.

The data were complete with 100% availability of primary outcome data, analysed using ITT principles.

We noted some minor differences between the two groups at baseline such that Group 1 were a little older, more likely to be male and had more frequent co-morbidities. In contrast, Group 2 appeared to have a higher median serum CRP concentration. We did not conclude that any differences here materially affected the interim analysis.

With regard to safety, we did not see any concerning safety signal for the variables available to us: the primary outcome of Days alive and out of hospital (DAOH) within 14 days after recruitment, all-cause mortality and re-admission rate.

With regard to efficacy, we also did not observe any meaningful difference between the groups in these outcomes. The pre-specified stopping criteria for futility was met with a post-conditional power analysis of DAOH within 14 days from recruitment of 0.064 (so pre-specified <0.20).

**In light of the rapidly expanding literature on the use of Azithromycin and Chloroquine in COVID, which your results are consistent with, and the stopping criteria for futility being met, it was the unanimous opinion of the DSMB to recommend stopping the trial at this point.**

We congratulate you and all the recruiting centres for the excellent data quality and rapid set-up of this important study during the initial phase of the COVID-19 pandemic.

Yours sincerely,

Professor John Hurst, UCL Respiratory, University College London, London, UK (Chair)

Bodil Steen Rasmussen, Department of Anaesthesia and Intensive Care, Aalborg University Hospital, Aalborg, Denmark

Prof. Dr. med. Philipp Schütz, Chefarzt Allgemeine Innere & Notfallmedizin, Titularprofessur an der Universität Basel

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