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

19th 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 26th of March 2020.

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

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).

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-β) 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 (kg/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: 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, $x10^9$ cells/L
- Blood eosinophil count, $x10^9$ cells/L
- CRP (mg/L)
- Fibrin D-dimer
- Ferritin
- Lactate Dehydrogenase (U/L)

Arterial Blood Gas (mean ± SD) day 1 and day 4

- PCO2, mmHg
- PO2, mmHg
- HCo3
- 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 form 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 PaO2 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 PaCO2 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.

Arrythmias:

- ECG: Qtc: n (%) patients in both arms who at any time point after baseline had a QTc (F) >500 ms
- N (%) ventricular arrythmias (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 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 (JUJ) 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 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.

Contributor Information

Pradeesh Sivapalan MD PhD Resp. trainee

Department of Internal Medicine, Zealand University Hospital, Roskilde &

Post Doc Respiratory Medicine Section, Herlev-Gentofte Hospital

Pradeesh Sivapalan, Email: pradeesh.sivapalan.02@regionh.dk

Jens-Ulrik Stæhr Jensen, PhD

Research Associate Professor, University of Copenhagen &

Research Leading senior consultant

Respiratory Medicine Section Herlev-Gentofte Hospital

Jens-Ulrik Jensen, Email: Jens.ulrik.jensen@regionh.dk

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_1	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