A Randomized, Double-Blind Placebo Controlled Clinical Trial of Ivermectin plus Doxycycline for the Treatment of Confirmed Covid -19 Infection

STATISTICAL ANALYSIS PLAN

Study Title: A Randomized, Double-Blind Placebo Controlled Clinical Trial of Ivermectin plus Doxycycline for the Treatment of Confirmed Covid -19 Infection

Name of Test Drug: Ivermectin plus Doxycycline

Study Number: ERC-DMC/ECC/2020/117

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Analysis Plan Author(s): Dr. Reaz Mehmud

CONFIDENTIAL AND PROPRIETARY INFORMATION

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LIST OF ABBREVIATIONS

- AE- adverse event
- ALT- alanine aminotransferase
- AST- aspartate aminotransferase
- CI- confidence interval
- CRF- case report form
- CSR- clinical study report
- DMC- data monitoring committee
- FAS- Full Analysis Set
- Hb- Hemoglobin
- HLT high-level term
- ICH International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
- ITT- intent to treat
- LTT- lower-level term
- LOQ limit of quantitation
- MedDRA- Medical Dictionary for Regulatory Activities
- PCR-polymerase chain reaction
- PP - per protocol
- PT- preferred term
- SAP- statistical analysis plan
- SD- standard deviation
- SOC- standard of care
- TEAE- treatment-emergent adverse event
- WHO World Health Organization

1. INTRODUCTION

This Phase 3 Randomized controlled trial was conducted in Dhaka Medical College Hospital, Dhaka, Bangladesh. Total 400 participant who met all eligibility criteria randomized to 1:1 into treatment group (Ivermectin plus Doxycycline plus standard care) and placebo plus standard care.
This statistical analysis plan (SAP) describes the statistical analysis methods and data presentations for the final analysis.

1.1 Study Objectives

The purpose of this study is to provide Ivermectin and Doxycycline in Confirmed mild and moderate severity Covid 19 cases.

The primary objective of this study is as follows:

To observe the benefit (clinical and microbiological) of Ivermectin and Doxycycline in Confirmed mild to moderate Covid 19 cases.

The secondary objective of this study is as follows:

To evaluate the safety and tolerability of Ivermectin and Doxycycline to standard care.

1.2 Study Design

This is a randomized double blind placebo controlled trial in participants with mild to moderate covid-19 infection. 400 participants who meet all eligibility criteria randomized to 1:1 into one of the following treatment group

Treatment group1: Ivermectin plus Doxycycline plus standard care
Treatment group 2: Placebo plus standard care.

Key Eligibility Criteria

Inclusion Criteria:

- At least 18 years of age
- COVID-19 infection, confirmed by polymerase chain reaction (PCR) test within 3 days from enrollment
- Only mild and moderate COVID-19 infected cases
- Able to provide informed consent

Exclusion Criteria:

- Unable to take oral medication
- Pregnant or breast feeding lady
- Patients with severe COVID symptoms or admission in ICU/HDU

- Alanine Aminotransferase (ALT) or aspartate aminotransferase (AST) > 5 X upper limit of normal (ULN)
- On non-invasive positive pressure ventilation or mechanical ventilation at time of study entry
- Known hypersensitivity to Doxycycline or Ivermectin or its components.

Schedule of Assessments

The date of randomization was considered Day 1 and all participants randomized to receive the drug received their initial dose on Day 1.

On Days 1 through 14 or until discharge, or clinical improvement, whichever was later, clinical status, vital signs including respiratory status was measured and adverse events (AEs) was documented.

Laboratory testing was advised at day 1, which included complete blood count, random blood glucose, creatinine, ALT, D- dimer, CRP, Ferritin and chest X-ray or CT chest which ever was feasible.

If performed the patient who was treated as outpatient test results were documented in next visit or by online visit.

In Hospitalized patient tests was advised at Days 1, 7 and according to treating physician's need.

RT-PCR for covid-19 was done 14 days after the initial positivity.

Randomization

Participants who meet eligibility criteria was randomized in a 1:1 ratio to one of the two treatment groups on Day one. A computer generated code number was used to level the drugs and the patient. The allocation was concealed by using sealed envelope. Randomization was not stratified.

Site:

Triage and inpatient of Covid dedicated unit of Dhaka Medical College Hospital

Duration of Treatment

Participants received 12 mg of Ivermectin at day 1 and 100 mg Doxycycline twice daily for 5 days plus standard care as Active drug or placebo.

Standard care: both the experimental and placebo will receive the available standard of care, like-

- Paracetamol, Antihistamine, Cough suppressant, Vitamins
- Oxygen therapy according to indication and need
- Low molecular weight heparin according to indication
- Appropriate other broad spectrum antibiotics
- Other drugs for associated co- morbid condition

Discontinuation Criteria

The drugs were discontinued if patient developed any serious adverse effect. In this trial two patient developed erosive esophagitis and discontinued from the study.

End of Study

The end of the study was the last participant's last observation (or visit).

1.3 Sample Size and Power of the trial

Total 400 patients were randomized in a 1:1 ratio to two treatment group. (200 participants per group).

For superior trial, the formula is:

$$N=1/2\times [(Z_{\alpha/2}+Z_{\beta})/ (\arcsin\sqrt{p}-\arcsin\sqrt{P_0}]^2$$

N=size per group; P=the response rate of standard treatment group; P_0= the response rate of new drug treatment group; Z_x= the standard normal deviate for a one or two sided x; d= the real difference between two treatment effect; δ_0= a clinically acceptable margin; S^2= Polled standard deviation of both comparison groups.

All parameters were assumed as follows: P=0.40; P_0=0.58; α=0.05; β=0.20

Result for the superiority trial was 121.

However, we are assuming that lost to follow up or refuse to include in trial will be 20%, that means 24. So at least 150 patients was required to be allocated randomly with the power of the study 80%.

We had decided to enroll 200 patient for each arm.

2. Type of Planned Analysis

Primary Analysis: It was done when more than 2/3 patients were finished follow up. The DSM board reviewed the results on 25 August, 2020.

Final Analysis:

The final analysis for this study will be performed after all participants have completed the follow up, outstanding data queries have been resolved or adjudicated as unresolvable, and the data have been cleaned and finalized

3. General Consideration for Data analysis

Analysis results will be presented using descriptive statistics.
For categorical variables, the number and percentage of participants in each category will be presented; for continuous variables, the number of participants (n), mean, standard deviation (SD) will be presented.
All statistical tests will be 2-sided and performed at the 5% significance level unless otherwise specified.
By-subject listings will be presented for all participants in the All Randomized Analysis Set unless otherwise specified, and sorted by subject ID number, visit date, and time (if applicable).

3.1. Analysis Sets

Analysis sets define the participants to be included in an analysis. Analysis sets and their definitions are provided in this section. Participants included in each analysis set will be determined before database finalization. The analysis set will be included as a subtitle of each table, figure, and listing. A summary of the number and percentage of participants in each analysis set will be provided by treatment group, placebo group and in total.

3.1.1. All Randomized Analysis Set

The All Randomized Analysis Set will include all participants who are randomized into the study. This is the final analysis set for by-subject listings.

3.1.2 Full Analysis Set

The analysis set for efficacy analysis is defined as the Full Analysis Set (FAS), which will include all participants who (1) are randomized into the study and (2) have received at least 1 dose of study treatment if randomized to treatment groups. Participants in the placebo arm who have had protocol Day 1 visit will be included in the FAS. Participants will be grouped according to the treatment to which they were randomized

3.1.3. Safety Analysis Set

The analysis set for safety analyses is defined as the Safety Analysis Set, which will include all participants who (1) are randomized into the study and (2) have received at least 1 dose of study treatment if randomized to the treatment groups. Participants in the placebo arm who have had protocol Day 1 visit will be included in the safety analysis set.

3.2. Subject Grouping

Participants will be grouped by randomized treatment (Active treatment and placebo) regardless of the actual number of days of treatment.

3.2.1. Subject Subgroups for Efficacy Analyses

The primary endpoint will be analyzed for the following participant subgroups:

- Age (years): (a) < 40 and (b) 40-60 (c) >60
- Sex at birth: (a) male and (b) female
- Severity of illness (Mild and Moderate)

3.2.2. Subject Subgroups for Safety Analyses

Incidence of all treatment-emergent AEs (TEAEs) will be summarized for the following participant subgroups:

- Age (years): (a) < 40 and (b) 40-60 (c) >60
- Sex at birth: (a) male and (b) female

3.4. Missing Data and Outliers

3.4.1. Missing Data

Missing data can have an impact upon the interpretation of trial data. In general, values for missing data will not be imputed, unless methods for handling missing data are specified. In this study, a missing pre-treatment laboratory result would be treated as normal.

3.4.2 Outliers

Outliers will be identified during the data management and data analysis process, but no sensitivity analyses will be done to evaluate the impact of outliers on efficacy or safety outcomes, unless specified otherwise. All data will be included in the analyses.

3.5. Data Handling

In general, age collected at Day 1 (in years) will be used for analyses and presented in listings.

Laboratory data that are continuous in nature but are less than the lower limit of quantitation or above the upper limit of quantitation will be imputed as follows:

• A value that is 1 unit less than the LOQ will be used to calculate descriptive statistics if the datum is reported in the form of "< x" (where x is considered the LOQ). For example, if the values are reported as < 50 and < 5.0, values of 49 and 4.9, respectively, will be used to calculate summary statistics. An exception to this rule is any value reported as < 1 or < 0.1, etc. For values reported as < 1 or < 0.1, a value of 0.9 or 0.09, respectively, will be used to calculate summary statistics.

• A value that is 1 unit above the LOQ will be used to calculate descriptive statistics if the datum is reported in the form of "> x" (where x is considered the LOQ). Values with decimal points will follow the same logic as above. • The LOQ will be used to calculate descriptive statistics if the datum is reported in the form of "≤ x" or "≥ x" (where x is considered the LOQ).

3.6. Analysis Visit Windows

3.6.1. Definition of Study Day

Study Day 1/First Dose Date is defined as Participants randomized to any group as the day when the first dose of the drugs was taken as recorded on the Case record form.
Recovery day is defined as the day from which patient's symptoms improved for 3 successive day as defined by WHO improvement criteria**.**
Total duration of illness was calculated from the onset of any symptoms related to COVID-19 up to the day from which patient is improved clinically for 3 successive days**.**

Last Study Date is last days at which patient followed up for the last time

Covid positive negative date: The date when sample was given. If it come positive it recorded as missing data.

Baseline value is defined as the last value obtained on or prior to the first dose date (and time, if available) unless otherwise specified.

3.6.2. Analysis Visit Windows

Subject visits might not occur on protocol-specified days. As telephonic interview was taken for the outpatient participant patient might not available on a specific day. Therefore, for the purposes of analysis, observations will be assigned to analysis windows. The study day as defined in Section 3.6.2 will be used when data are summarized by visit.
Vital signs were to be collected daily; therefore, windows are not assigned and results will be summarized for each Study Day.

3.6.3. Selection of Data in the Event of Multiple Records for an Analysis Visit Day

Depending on the statistical analysis method, single values may be required for each Study Day/ analysis window.
If multiple valid, non-missing measurements exist for a Study Day/analysis window, records will be chosen based on the following rules if a single value is needed:

• For baseline, the last non-missing value on or prior to the first dosing date of study drug will be selected, unless specified differently. If there are multiple records with the same time or no time recorded on the same day, the baseline value will be the average of the measurements for continuous data, or the measurement with the lowest severity for categorical data.
• For post baseline values:
For windows spanning multiple days, the record(s) collected on the day closest to the nominal day will be selected. If there are 2 days equidistant from the nominal day, the later day will be selected.
If there is more than 1 record on the selected day, values will be selected for analysis as follows:

- For PCR, if there is more than 1 record on the selected day, the latest value will be selected. If there are multiple records with the same time or no time recorded on the same day, the selected value will be the highest severity (ie, highest value or positive result).
- for laboratory values (other than PCR) and SpO2 and PaO2, if there is more than 1 record on the selected day, the worst value will be selected.
- for other parameters, if there is more than 1 record on the selected day, the average will be taken for continuous data and the worst severity will be taken for categorical data, unless otherwise specified.

4. SUBJECT DISPOSITION

4.1. Subject Enrollment and Disposition

The number and percentage of participants randomized at each investigator site will be summarized by treatment group (Active drug and placebo) and overall using the full Analysis Set. The denominator for this calculation will be the number of participants in the full Analysis Set.
In addition, the number and percentage of the participants in the following categories will be summarized:

- Completed study drug as recorded on the case record form
- Prematurely discontinued study drug prior to completion of 5 days of dosing with summary of reasons for discontinuing study drug as recorded on the on the case record form .
- Completed study
- Prematurely discontinued from study prior to the data cut date (with summary of reasons for discontinuing study) as recorded on the on the case record form.

4.2. Extent of Study Drug Exposure

4.2.1. Exposure to Study Drug

Number of doses received will be summarized by treatment group for participants randomized to both groups in the Safety Analysis Set.
Time to premature discontinuation of study drug will be analyzed using the Kaplan-Meier method by treatment group for participants randomized to both treatment groups. They will be censored at the last visit date.

5. BASELINE CHARACTERISTICS

5.1. Demographics and Baseline Characteristics

Subject demographic data (e.g., sex, and age) will be summarized by treatment group and overall using descriptive statistics (n, mean, SD) for continuous data and by the number and percentage of participants for categorical data. The summaries of demographic data and baseline subject characteristics will be provided for the Safety Analysis Set.

Age group (< 40, 40 to 60, > 60) will be summarized by treatment group and overall. For categorical data, the chi square test (i.e., general association statistic for nominal data and row mean scores for ordinal data [age group]) will be used to compare the 2 treatment groups.

For continuous data, the 2-sided independed t test will be used to compare the 2 treatment groups.

5.2. Other Baseline Characteristics

The following baseline disease characteristics will be summarized by treatment group and overall using descriptive statistics:

- Clinical features
- Duration of illness prior to prior to Study Day 1
- Initial severity of the patients

For categorical data, the chi square will be used to compare the 2 treatment groups. For clinical status and continuous data, 2-sided independed t test will be used to compare the 2 treatment groups.

5.3. Medical History

Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is considered to be preexisting and should be documented as medical history. General medical history data will be collected at screening. The summary table will present the percentages of participants reporting each medical history.

6. EFFICACY ANALYSES

6.1. Primary Efficacy Endpoint

The analysis of primary efficacy endpoint was Final Analysis.

6.1.1. Definition of the Primary Efficacy Endpoint

Primary Outcome Measure:
Primary Outcome was to see the number of days required for clinical improvement.

1. Early Clinical improvement of the patients
   How many days it requires to become the patient completely symptoms free
   [Time Frame: 7 days]
2. Late clinical recovery
   How many days the symptoms persist
   [Time Frame: 12 days]

If a participant dies while hospitalized (as recorded on the Death CRF and Hospitalization CRF), the endpoint on the day of death

If the participant is discharged alive and dies in home within 1 month the end point on the patient dies.

6.1.2. Statistical Hypothesis for the Primary Efficacy Endpoint

Null hypothesis: The odds of improvement for active drug group is the same as the odds of improvement for the placebo group with respect to clinical improvement a day 7 and day 12.

Alternative hypothesis: The odds of improvement for active drug group is different the odds of improvement for the placebo group with respect to clinical improvement a day 7 and day 12.

6.1.3. Primary Analysis of the Primary Efficacy Endpoint

Comparing drug outcome in between two group survival analysis by Kaplan-Meier curve will be done for the duration of recovery. Odds ratio will be formulated from linear logistic regression for early and late clinical recovery. Crude odds ratio will be adjusted in cox proportional hazard ratio model. Each Ivermectin-Doxycycline plus standard of care group will compared with the placebo plus standard care group using the log-rank test, hazard ratio and 95% CI. The FAS will be the primary analysis set for efficacy endpoint evaluation. The primary endpoint will be analyzed for each of the subgroups defined in Section 3.2.1

6.1.4. Secondary Analysis of the Primary Efficacy Endpoint

As supportive analyses of the primary endpoint, the following will be conducted for treatment group compared to the placebo arm:

Secondary Outcome Measure:

1. Percentage of patients having clinical deterioration.
   Patients deteriorating to next level of severity, like moderate, severe and death [Time Frame: 1 month]
2. Persistently positive for RT-PCR.
   Percentage of Covid-19 Patients repeatedly become positive for RT-PCR for Covid-19 [Time Frame: 14 days]

Hazard ratio will formulated from the cox regression analysis for severity conversion and persistence of the positivity. The hazard ratio, 95% confidence interval, and p-value for comparing treatments will be provided.

7. SAFETY ANALYSES

Safety data will be summarized for the participants in the safety analysis set.

7.1 Secondary Endpoint

The secondary endpoint of the proportion of participants with any treatment emergent adverse events will be compared between two group using a Fisher's Exact test. The point estimate of the treatment difference and the associated 95% confidence intervals will be provided

7.2. Adverse Events and Deaths

7.2.1Adverse Event Dictionary

Clinical and laboratory adverse events (AEs) will be coded in SPSS using numeric value.

7.2.2. Adverse Event Severity

The severity of AEs will be graded using the Division of AIDS (DAIDS), Version 2.1 dated July 2017. For each episode, the highest grade attained should be reported as defined in the grading

scale. The DAIDS scale is available at the following location:
https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

Adverse events are graded by the investigator as Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life threatening) or Grade 5 (fatal) according to toxicity criteria specified in the document above. The severity grade of events for which the investigator did not record severity will be left as "missing" for data listings.

7.2.3. Relationship of Adverse Events to Study Drug

Related AEs are those for which the investigator selected "Related" on the AE CRF to the question of "Related to Study Treatment." Events for which the investigator did not record relationship to study drug will be considered related to study drug for summary purposes. However, by-subject data listings will show the relationship as missing

7.2.4. Serious Adverse Events

Serious adverse events (SAEs) will be identified and captured as SAEs if AEs met the definitions of SAE specified in the study protocol.

7.2.5. Treatment-Emergent Adverse Events

Definition of Treatment-Emergent Adverse Events

For participants randomized to either groups, treatment-emergent adverse events (TEAEs) are defined as 1 or both of the following:

- Any AEs with an onset date on or after the study drug start date and no later than 30 days after permanent discontinuation of study drug
- Any AEs leading to premature discontinuation of study drug.

For participants randomized to the placebo group, all AEs reported on or after protocol Day 1 visit will be considered as treatment-emergent

7.2.6. Summaries of Adverse Events and Deaths

The number and percentage of participants who experienced at least 1 TEAE will be provided and summarized by system organ class, HLT, PT, and treatment group. Comparison will be done by Fisher-exact test.

Data listings will be provided for the following:

- All AEs
- Study-Drug-Related AEs
- AEs with severity of Grade 3 or higher
- SAEs
- Study-Drug-Related SAEs (active treatment groups only)
- Deaths
- AEs leading to premature discontinuation of study drug (active treatment groups only)

7.3. Laboratory Evaluations

Laboratory data collected during the study will be analyzed and summarized using both quantitative and qualitative methods. Summaries of laboratory data will be provided for the Safety Analysis Set and will include data collected up to the last dose of study drug plus 30 days for the active groups if possible. For the placebo group all laboratory data will be included. The analysis will be based on values reported in conventional units. When values are below the LOQ, they will be listed as such, and the closest imputed value will be used for the purpose of calculating summary statistics as specified in Section 3.2.2.

A by-subject listing for laboratory test results will be provided by subject ID number and visit in chronological order for hematology, serum chemistry, and PCR separately. Values falling outside of the reference range and/or having a severity grade of 1 or higher on the DAIDS Grading Scale will be flagged in the data listings, as appropriate.

7.3.1. Summaries of Numeric Laboratory Results

Descriptive statistics will be provided by treatment group for each laboratory test as follows:

- Baseline values
- Values at each post baseline analysis window
- Change from baseline at each post baseline analysis window
- Percentage change from baseline to each post baseline analysis window (if specified

A baseline laboratory value will be defined as the last non missing value obtained on or prior to the date (and time, if applicable) of first dose of active drug groups. For the placebo group a baseline laboratory value will be defined as the last non missing value obtained on or prior to the protocol Day 1 visit date. Change from baseline to a post baseline visit will be defined as the post baseline value minus the baseline value. The mean, median and SD will be displayed. Baseline and change from baseline will be compared between active group and the placebo group using the 2-sided Wilcoxon Rank sum test.

7.3.2. Graded Laboratory Values

The DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Event, Version 2.1 (July 2017) will be used for assigning toxicity grades (0 to 4) to laboratory results for analysis. Grade 0 includes all values that do not meet the criteria for an abnormality of at least Grade 1. For laboratory tests with criteria for both increased and decreased levels, analyses for each direction (ie, increased, decreased) will be presented separately.

7.3.2.1. Treatment-Emergent Laboratory Abnormalities

Treatment-emergent laboratory abnormalities are defined as values that increase at least 1 toxicity grade from baseline at any post baseline time point, up to and including the date of last dose of study drug plus 30 days for either of the active drugs groups. For participants randomized

to the placebo group, all post baseline laboratory abnormalities in this study will be considered as treatment-emergent.

If the relevant baseline laboratory value is missing, any abnormality of at least Grade 1 observed within the time frame specified above will be considered treatment emergent.

Laboratory data that are categorical will be summarized using the number and percentage of participants in the study with the given response at baseline and each scheduled post baseline visit. The following summaries (number and percentage of participants) for treatment-emergent laboratory abnormalities will be provided by laboratory test and treatment group; participants will be categorized according to the most severe post baseline abnormality grade for a given laboratory test:

- Treatment-emergent laboratory abnormalities
- Treatment-emergent Grade 3 and 4 laboratory abnormalities

For all summaries of laboratory abnormalities, the denominator is the number of participants with non-missing post baseline values up to 30 days after last dosing date for the active drugs groups and the number of participants with non-missing post baseline values for the SOC group. A by-subject listing of laboratory abnormalities and Grade 3 or 4 laboratory abnormalities will be provided by subject ID number and visit in chronological order.

7.4. Subject Subgroup for Safety Endpoints

Incidence of all treatment-emergent AEs will be repeated within each subgroup defined in Section 3.2.2 using the safety analysis set

7.5. Changes from Protocol-Specified Safety Analyses

No change from the protocol-specified safety analysis is planned.

8. SOFTWARE

SPSS version 20.