Sponsor:	Institute of Blood Transfusion, Chinese Academy of Medical Sciences
Protocol Identifier:	ChiCTR2000029757
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Title:	A Randomized Controlled Clinical Trial of Using Collected Convalescent Plasma for the Treatment of Severe and Critical/life threatening COVID-19 Patients
SAP Date/Version:	03-30-2020/V0.2

Description:

- The SAP aims at describing the analysis planned for protocol ChiCTR2000029757 and the information to be reported for the clinical study.
- The SAP will describe the analysis on safety, efficacy and immunogenicity for the present study.

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1. INTRODUCTION

This document is a statistical analysis plan (SAP) of ChiCTR2000029757, a randomized controlled clinical trial of using collected convalescent plasma for the treatment of severe and critical/life threatening COVID-19 patients (Protocol Identifier: ChiCTR2000029757). The SAP summarizes the study design and objectives and aims at providing a detailed description of the statistical analyses planned for the protocol. Final SAP will be signed off before the dataset is locked.

2. KEY INFORMATION AND SUMMARY

2.1. SAP and Protocol Deviations

The changes to the analysis plan previously specified in the protocol are outlined in the following table.

Protocol	**SAP**	
Protocol Statistical Descriptions	SAP Statistical Descriptions	Reasons for the Changes
• In 5.4 of the protocol, the stratification variables in randomization include study sites and disease severity (severe vs critical/life threatening/life threatening). Study sites compete and are selected through IWRS.	• Randomized stratification by disease severity (severe or critical/life threatening)	• Consistent with the actual trial operations
• In 7.4.4 Safety Analyses of the protocol, the incidence (frequency) and severity distribution of adverse events (AE) related to blood transfusion, AEs leading to early withdrawal from treatment and study, and death, level 3 and above AEs, serious AEs, and adverse reactions of plasma transfusion are summarized.	• The number and percentage of subjects with transfusion-related Treatment Emergent Adverse Events (TEAE), TEAEs and SAEs (Serious Adverse Events) related to the suspension of transfusion, and TEAEs leading to death during the treatment period are summarized.	• Consistent with the actual collectable information

2.2. Study Design

This study is a multi-center, randomized, open, parallel controlled trial that aims at studying the efficacy and safety of convalescent plasma therapy combined with conventional treatment in patients with severe and critical/life threatening/life threatening covid-19, compared with a conventional-treatment-only approach.

In the study, the investigators plan to recruit 200 patients with severe or critical/life threatening COVID-19, who will be randomly assigned to the trial group or the control group with a proportion of 1:1 according to their disease severity (severe or critical/life threatening). The experimental group will be treated with the conventional treatment combined with convalescent plasma, and the control group, with only conventional treatment. The subjects who pass the screening and meet the eligibility criteria will join the study and be treated. All patients will receive the conventional treatment, including symptomatic control, antiviral and antibacterial treatments. If disease progress is reported at any time, the treatment can be stopped or combined with other interventions. The treatment group will be transfused with convalescent plasma within 24 hours of randomization, and being evaluated for the efficacy and safety outcomes. All subjects will be followed up to the 28th day of the study or to the clinical endpoints, when the patients are discharged or dead. Patients who complete the 28-day observation or reach the clinical endpoints will be considered to have completed the study.

2.3. Study Objectives

The investigators aim at collecting the convalescent plasma from recovered COVID-19 patients and exploring the use of such plasma in the clinical treatment of COVID-19 patients to proactively control COVID-19 infected pneumonia.

Primary Objective: To evaluate the therapeutic effects of convalescent plasma therapy in treating severe and critical/life threatening patients with COVID-19.

Secondary Objectives:

- To evaluate the safety and tolerance of convalescent plasma therapy in severe and critical/life threatening COVID-19 cases.
- To analyze the variables that may affect the efficacy of convalescent plasma therapy.

2.4. Statistical Hypotheses

The study is a superiority design. The main efficacy endpoint is the 28-day time to clinical improvement (TTCI) in the enrolled patients. The superiority hypothesis is that the TTCI of subjects is shortened in the treatment group, compared to the control group.

If the P value of the group comparison between groups ≤ the preset value α (one sided α of 0.025 and two sided α of 0.05), it can be considered that the treatment group is superior to the control group and the TTCI of the subjects is shortened.

The main purpose of this study is to verify if the conventional treatment combined with convalescence plasma therapy can shorten TTCI of subjects compared with the conventional treatment. The statistical hypothesis testing of the endpoint TTCI will be tested as follows:

Null hypothesis (H0): TTCI (experimental group) = TTCI (control group)

Alternative hypothesis (H1): TTCI (experimental group) < TTCI (control group)

2.5. Sample Size Determination

The original total sample size was set at least 100, since more than 80% power could be provided for the trial to detect a difference, with a two-sided significance level of α=0.05, of 8 days in the median TTCI between the two groups, supposing that 60% of the patients would reach clinical improvement and the median time in the control group was 20 days. Consequently, the sample size was set to be 100 for each group, which was also the maximum sample size that the resource could support.

2.6 Randomization process

The study is a multicenter, randomized, open, parallel and controlled trial.

A randomization schedule will be generated by the SAS statistical software. The randomization procedure of this study is as follows. Subjects will be randomly assigned to either the treatment group (conventional treatment combined with convalescent plasma therapy) or the control group (conventional treatment) in a 1:1 ratio and will be treated according to the treatment regimens of that group. Stratification will be used in this study. The stratification variable will be disease severity (severe vs critical/life threatening). Subjects will be assigned a random number upon completion of all screening evaluation and meeting the inclusion criteria. They will then be assigned to the designated treatment group (experimental group or control group). The random number of a randomized subject will be retained even if the subject withdraws from the study for whatever reason.

3. PLANNED ANALYSES

3.1. Interim Analysis

Due to the global pandemic, the efficacy of convalescent plasma in the treatment of COVID-19 has attracted much attention. Therefore, the interim analysis will be carried out according to the actual progress of research. The results of the interim analysis (one sided α of 0.025 and two sided α of 0.05) will be nominal test and will not be corrected.

3.2. Final analyses

Upon completion of the trial and the necessary data cleaning, the dataset will be locked for final analysis.

4. ANALYSIS POPULATIONS

Analysis Sets	Definition/Standard	Objects of Analysis
Full Analysis Set (FAS)	• The set of all randomized subjects who receive at least one treatment specified in the trial. • Statistical analysis will be performed on randomly assigned treatment groups.	Demographic and baseline variables, and efficacy analysis of subjects
Safety Analysis Set (SAF)	• The set of all randomized subjects who receive at least one treatment specified in the trial. • The statistical analysis will be performed on the actual treatment groups. If a subject does receive the convalescent plasma treatment in the whole course, then he/she will be included in the control group, otherwise, in the experimental group.	Safety and study drug exposure analysis
Per Protocol Set （PPS）	• The set of all randomized subjects who receive at least one treatment specified in the trial, and who have no significant protocol violations that affect the efficacy evaluation. • The final analysis population definition will be decided before lock-in. • Statistical analysis will be performed on randomly assigned treatment groups.	Efficacy analysis

Before the database is locked, the impact of protocol violation on the analysis will be evaluated and discussed, and the subjects of each analysis population will be finally determined.

5. DEFINITION OF ENDPOINT S

5.1. Efficacy Endpoints

5.1.1. Primary Endpoints

The time to clinical improvement (TTCI) within a 28-day period, that is, the days from randomized grouping to clinical improvement.

Clinical improvement was characterized by patient discharge or a reduction of 2 points on a 6-point disease severity scale from baseline. The 6-point scale was defined as:

- 6 points: Death;
- 5 points: Hospitalization, requiring extracorporeal membrane oxygenation (ECMO) and/or invasive mechanical ventilation;
- 4 points: Hospitalization, requiring non-invasive ventilation and /or high-flow supplemental oxygen;
- 3 points: Hospitalization, requiring supplemental oxygen (not high-flow or non-invasive ventilation);
- 2 points: Hospitalization, not requiring supplemental oxygen;
- 1 point: Discharge.

5.1.2. Secondary Endpoints

(1) 28-day mortality

(2) Duration of hospitalization (The criteria for release from quarantine or discharge include the body temperature being normal for more than 3 days, improved respiratory symptoms, and the respiratory pathogenic PCR being negative for two consecutive times [at least 1-day sampling interval], improvement of any other condition necessary for release from quarantine or discharge or transfer to other departments.)

(3) Ratio of negative viral nucleic acid testing results (within 3 days after transfusion)

5.1.3. Exploratory Endpoints

(1) Assessment of the moderating factors of convalescent plasma therapeutic efficacy

According to the outcome and discharge time, the age, gender, medical history, viral load, and antibody titer of the patients, changes of various cytokines and chemokines (such as IL-6, IL-10, TNF - α, IL-1 β, etc.) in the blood samples before and after the treatment are compared, and the moderating factors of convalescent plasma therapeutic efficacy analyzed.

(2) Antiviral mechanism in convalescent plasma therapy

5.2. Safety Endpoints

(1) Adverse Events

(2) Critical/life threatening Laboratory Evaluations

- Routine blood test: complete blood count (CBC) and leukocyte count (including percentage of neutrophils and lymphocytes), hemoglobin, hematocrit and platelets
- Biochemical profile: creatinine, glucose, total protein, ALT / GPT, AST / GOT, and total bilirubin
- Routine coagulation test: prothrombin time (PT) / international normalized ratio (INR)

(3) Vital signs: body temperature, heart rate, respiration rates, blood pressure, blood oxygen saturation

5.3. General Definitions

Age

Age is calculated from the date of birth to the date of signing the informed consent. The formula is the integral part of [(signing date of informed consent – date of birth) / 365.25].

Baseline

For the endpoint of interest, unless otherwise specified, baseline is defined as the non-missing value closest to the start time of the assigned treatment, and it should be earlier than the start time.

Baseline Deviations

Baseline deviations will be calculated for each patient at a given time point by subtracting the baseline from the value at that specific time point. If either the baseline or the value at that particular point in time is missing, then the difference from the baseline will be the deviation.

Study Duration

The duration of the study is defined as the time interval from the date of the subject event to the date of the first treatment, which will be calculated as follows:

Study duration = (date of event – date of first treatment), when the event occurs before the first treatment;

Study duration = (date of event – date of first treatment + 1), when the event occurs after the first treatment.

6. STATISTICAL ANALYSIS

6.1. General rules

All statistical analyses will be performed using SAS ® statistical software package 9.4 or later version.

All statistical analyses will mirror those of the treatment groups. The treatment group description and presentation are presented in the following table.

Treatment Group Description	Final Statistical Presentation
Conventional therapy + convalescent plasma group	Convalescent plasma group
Conventional therapy group	Conventional therapy group

Without any other specification, all statistical tests will be significant at the level of 0.05 (2-tailed/sided).

Without any other specification, the efficacy analyses will be performed on FAS and the safety analyses on SAS. The measurements will be statistically described by means ± standard deviations or medians and quartile deviations, and the counts by frequency (percentage).

6.2. Missing Data

In the analysis, the missing data will be processed as follows.

- The absence of adverse event time is checked item by item. If the exact time is not available, it will not be filled.
- The assessment of the severity and causal relationship of adverse events should not be missing. If missing, it will be checked item by item. If still unavailable, the severity of missing will be marked as severe, and the cause of missing data will be logged as treatment-related.
- If missing values for other individual data points remain missing, then they will not be imputed. Only observed values will be used for data analysis and presentation.

The padding values will be used for calculation only, and the actual values collected will be presented in the tables.

6.3. Subject Disposition

The summary statistics of the subjects will include the following information:

- The number and percentage of subjects who complete the trials and who drop out in advance according to the treatment groups;
- The number and percentage of subjects per analysis population according to the treatment groups.

6.4. Protocol Deviation

All protocol violations will be listed. The sponsor and/or its designee will review the protocol violations before the database is locked, and determine whether the involved subjects will be excluded from the per protocol set.

6.5. Demographic and Baseline Variables

The following demographic and baseline variable collected prior to the start of study medication will be summarized by treatment group.

- Sex
- Age

6.6. Medical History and Prior Conditions

- Allergy history

- Cardiovascular disease
- Liver disease
- Renal insufficiency
- Tumor/cancer

6.7. Concurrent Medications

After group inclusion, information on the concurrent medications taken by subjects will be recorded in detail, including the application of antiviral, antibacterial, antifungal, and hormonal drugs, and immunoglobulins. Each subject's generic drug name will be counted only once.

6.8. Efficacy Analyses

6.8.1. Primary Efficacy Analysis

The time to clinical improvement (TTCI) within a 28-day period, that is, the days from randomized grouping to clinical improvement. For the primary endpoint of TTCI, death, withdrawal, and switching groups before day 28 were considered to be right-censored at day 28, and otherwise would be considered to be right-censored at last observation date.

Clinical improvement was characterized by patient discharge or a reduction of 2 points on a 6-point disease severity scale from baseline. The 6-point scale was defined as:

- 6 points: Death;
- 5 points: Hospitalization, requiring extracorporeal membrane oxygenation (ECMO) and/or invasive mechanical ventilation;
- 4 points: Hospitalization, requiring non-invasive ventilation and /or high-flow supplemental oxygen;
- 3 points: Hospitalization, requiring supplemental oxygen (not high-flow or non-invasive ventilation);
- 2 points: Hospitalization, not requiring supplemental oxygen;
- 1 point: Discharge.

The Kaplan-Meier method will be used to estimate and map the survival curve of each treatment group. The median TTCI and a 95% confidence interval will be estimated according to the curve. The log-rank test will used for comparison between treatment groups. In addition, the survival curve of severe and critical/life threatening patients of each treatment group will be drawn separately.

The Cox proportional risk model will be built to estimate a hazard ratio (HR) and a 95% confidence interval of the treatment group effect with clinical improvement as the end event. The model will include study sites, disease severity (severe or critical/life threatening) and treatment group. On this basis, an interaction model between treatment group and disease severity will be built to evaluate the interaction between the two.

Additional Primary Efficacy Analysis

Sensitivity Analysis

The same method will be used in PPS population.

6.8.2. Secondary Efficacy Analysis

(1) 28-day mortality. The percentage of deaths in the analysis population at the 28th day of inclusion. P values will be calculated by the CMH method. For each treatment group, the binomial distribution ignoring randomization will be used to calculate the overall 28-day mortality rate. The 95% confidence interval will be estimated with the Clopper Pearson method. The odds ratio with 95% confidence interval of the convalescent plasma treatment, as compared to the conventional treatment, will calculated with the Wald method. A logistic regression model will be constructed with death on the 28th day as the dependent variable. The independent variables will include study sites, disease severity (severe or critical/life threatening) and treatment group. The odds ratio with 95% confidence interval of death on the 28th day, after adjusting for the study site effect and disease severity, will be calculated. In addition, a model will be established to estimate the interaction between disease severity and treatment group.

(2) Duration of hospitalization will be analyzed in two types.

- Duration of hospitalization 1: days from randomization to discharge or end of trial, whichever occurs first.
- Duration of hospitalization 2: days from the date of admission to the date of discharge or the end of the trial, whichever occurs first.

For failure of discharge or transfer, and death at the end of the trial, subjects will be censored. The date of withdrawal of the subject from the treatment will be the censor date. The Cox proportional risk model will be constructed, including study sites, disease severity (severe or critical/life threatening) and treatment group. The hazard ratio (HR) with 95% confidence interval of death on the 28th day, after adjusting for the study site effect and disease severity, will be calculated. In addition, a model will be established to estimate the interaction between disease severity and treatment group.

(3) The ratio of negative virus nucleic acid test results. The analysis will be divided into the following two types, with the same methodology as in 28-day mortality.

- The ratio of negative viral nucleic acid test results on the first day (24 h): the proportion of subjects with negative viral nucleic acid test results on the first

day (24 h). If the results on the first day are missing, the record will be positive.

- The ratio of negative viral nucleic acid test results on the second day (48 h): the proportion of subjects with negative viral nucleic acid test results on the second day (48 h). If the results on the second day are missing, the record will be the results of the first day.
- The ratio of negative viral nucleic acid test results on the third day (72 h): the proportion of subjects with negative viral nucleic acid test results on the third day (72 h). If the results on the third day are missing,
- the record will be the results of the second day. The results on the second day are derived with the method described in "the ratio of negative viral nucleic acid test results on the second day".

6.8.3. Exploratory Efficacy Analysis

(1) Assessment of the moderating factors of convalescent plasma therapeutic efficacy

According to the outcome and discharge time of the patients, their age, sex, medical history, viral load, antibody titer, changes of various cytokines and chemokines (such as IL-6, IL-10, TNF - α, IL-1 β, etc.) in the blood samples before and after the treatment will be compared and analysed. The factors that may moderate the efficacy of convalescent plasma therapy will be analyzed.

(2) Antiviral mechanism in convalescent plasma therapy

6.9. Safety Analyses

All safety analyses will be conducted in the safety analysis population. The subjects will be analyzed based on the actual treatment received.

6.9.1. Adverse Events

Adverse Reactions of Blood Transfusion

Adverse reactions of transfusion include acute hemolysis, delayed hemolysis, non hemolytic fever, allergic/anaphylaxis, transfusion-related acute lung injury, transfusion-related circulatory overload, transfusion-related dyspnea, transfusion-related hypotension, post-transfusion purpura and transfusion-related infection, among others. The percentage of adverse reactions of transfusion in total and stratified (by disease type) population will be calculated.

6.9.2. Clinical Laboratory Evaluations

The summary of laboratory data will include critical/life threatening laboratory evaluations such as routine blood test, biochemical profile and routine coagulation test. The changes of critical/life threatening laboratory evaluations before and after treatment will be summarized according to the treatment group and visit time.

The critical/life threatening laboratory evaluations include

- Routine blood test: complete blood count (CBC) and leukocyte count (including percentage of neutrophils and lymphocytes), hemoglobin, hematocrit and platelets
- Biochemical profile: creatinine, glucose, total protein, ALT / GPT, AST /GOT, and total bilirubin
- Routine coagulation test: prothrombin time (PT) / international normalized ratio (INR)

6.9.3. Vital Signs

Each vital sign (body temperature, heart rate, respiration rate, blood pressure, blood oxygen saturation) will be summarized with descriptive statistics according to the visit and treatment group. The descriptive statistics of deviations relative to the baseline at each time point will also be provided.

7. REFERENCES

1. A Randomized and Controlled Clinical Trial of Collecting Convalescent Plasma for the Treatment of Severe and Critical/life threatening COVID-19 Patients, February 22, 2020