

Rationale and design of tocilizumab in patients with moderate to severe COVID-19: an open label multicentre randomized controlled trial (TOCIBRAS)

Racionalidade e delineamento de tocilizumabe em pacientes com COVID-19 severo a moderado: estudo aberto, multicêntrico, randomizado, controlado (TOCIBRAS)

Danielle Leão Cordeiro de Farias¹, João Prats¹, Alexandre Biasi Cavalcanti^{2,3}, Regis Goulart Rosa^{2,4}, Flávia Ribeiro Machado², Otávio Berwanger⁵, Luciano César Pontes de Azevedo^{2,6}, Renato Delascio Lopes^{7,8}, Álvaro Avezum⁹, Leticia Kawano-Dourado³, Lucas Petri Damiani³, Salomón Soriano Ordinola Rojas¹, Cleyton Zanardo de Oliveira¹, Luis Eduardo Coelho Andrade¹⁰, Alex Freire Sandes¹⁰, Maria Carolina Pintão¹⁰, Claudio Galvão de Castro Jr^{5,11}, Phillip Scheinberg¹, Viviane Cordeiro Veiga^{1,2} for the COALITION COVID-19 BRAZIL VI

1. BP - A Beneficência Portuguesa de São Paulo - São Paulo (SP), Brazil.
2. Brazilian Research in Intensive Care Network (BRICNet) - São Paulo (SP), Brazil.
3. Research Institute, HCor-Hospital do Coração - São Paulo (SP), Brazil.
4. Hospital Moinhos de Vento - Porto Alegre (RS), Brazil.
5. Academic Research Organization, Hospital Israelita Albert Einstein - São Paulo (SP), Brazil.
6. Hospital Sírio-Libanês - São Paulo (SP), Brazil.
7. Duke Clinical Research Institute, Duke University Medical Center, Durham, NC, USA
8. Brazilian Clinical Research Institute - São Paulo (SP), Brazil.
9. International Research Center, Hospital Alemão Oswaldo Cruz - São Paulo (SP), Brazil.
10. Fleury Medicine and Health Laboratories - São Paulo (SP), Brazil.
11. Santa Casa de Porto Alegre - Porto Alegre (RS), Brazil.

Conflicts of interest: None.

Conflict of individual interest disclosures: Danielle Leão Cordeiro de Farias: Roche, Janssen, Amgen, Roche, Bristol-Myers Squibb, Libbs, Celgene - Speaker, Advisory. Phillip Scheinberg: Roche, Pfizer, BioCryst, Amgen, Merck, Eurofarma - Advisory; Novartis, Abbvie, Janssen, Alexion - Speaker, Advisory. Renato Delascio Lopes: Grants and personal fees from Bristol-Myers Squibb and Pfizer, personal fees from Boehringer Ingelheim and Bayer AG and grants from Amgen Inc, GlaxoSmithKline, Medtronic PLC, and Sanofi Aventis outside the submitted work.

Submitted on July 17, 2020

Accepted on July 22, 2020

Corresponding author:

Viviane Cordeiro Veiga

BP - A Beneficência Portuguesa de São Paulo.

Rua Maestro Cardim, 769 - Bela Vista

Zip code: 01323-001 - São Paulo (SP), Brazil

E-mail: viviane.veiga@bp.org.br

ABSTRACT

Introduction: Pro-inflammatory markers play a significant role in the disease severity of patients with the new coronavirus disease (COVID-19). Thus, anti-inflammatory therapies are attractive agents for potentially combating the uncontrolled inflammatory cascade in these patients. We designed a trial testing tocilizumab *versus* standard of care with the aim to improve outcomes through inhibiting interleukin-6, an important inflammatory mediator in COVID-19.

Methods and analysis: This open-label multicentre randomized controlled trial will compare clinical outcomes of tocilizumab plus standard of care versus standard of care alone in patients with moderate to severe COVID-19. Two of the following four criteria are required for protocol enrolment: D-dimer > 1,000ng/mL; C reactive protein > 5mg/dL, ferritin > 300mg/dL, and lactate dehydrogenase > upper limit of normal. The primary objective will be to compare

the clinical status on day 15, as measured by a 7-point ordinal scale applied in COVID-19 trials worldwide. The primary endpoint will be assessed by an ordinal logistic regression assuming proportional odds ratios adjusted for stratification variables (age and gender).

Ethics and dissemination: TOCIBRAS was approved by local and central (national) ethical committees in Brazil following current national and international guidelines/ directives. Each participating centre had the study approved at their institutional review boards before initiating protocol enrolment. The data derived from this trial will be published regardless of the results. If proven active, this strategy could alleviate the consequences of the inflammatory response in COVID-19 and improve clinical outcomes.

Keywords: Coronavirus; Tocilizumab; Covid-19; SARS-CoV-2

Clinicaltrials.gov identifier: NCT04403685

INTRODUCTION

At the end of 2019, a new coronavirus outbreak in Wuhan (China) made headlines as cases of severe pneumonia and deaths began to emerge.¹ The new virus was named 2019-nCoV, and little was known about its pathogenesis, transmissibility, and lethality rates.^(1,2) Cases started to spread within China and in 2020 beyond its borders to all continents (except Antarctica). In February 2020, the World Health Organization (WHO) adopted the official name SARS-CoV-2 (from 2019-nCoV) and the syndrome associated with the new coronavirus infection as COVID-19, which was declared a pandemic in mid-March.⁽³⁾ By June 2020, almost 9 million cases and 470 thousand deaths have been reported worldwide.⁽⁴⁾ In Brazil, the first reported cases were in late February, and by June, the country accumulates more than one million cases and 50,000 deaths.

The development of pneumonia that progresses to a systemic inflammatory response syndrome (SIRS) and multiorgan failure indicates that pro-inflammatory cytokines are a significant contributor to morbidity and mortality in SARS-CoV-2 infection.^(5,6) The rapid rise in cytokines reminiscent of that seen in chimeric antigen receptor therapy (CAR-T) is proposed as an essential element in the rapid clinical deterioration.⁽⁷⁻⁹⁾ The COVID-19 SIRS is usually observed in the second week of infection, where a slew of pro-inflammatory signals are triggered, leading to respiratory compromise and multiorgan failure. After SARS-CoV-2 initially binds to the angiotensin-converting enzyme 2 (ACE2) receptor, several cell types are infected thereafter from the respiratory, gastrointestinal, neurologic, endothelial, and reticuloendothelial systems giving rise to a wide clinical presentation ranging from oligosymptomatic to multiorgan failure and death.⁽¹⁰⁻¹⁵⁾ Clinical and laboratory risk factors which associate with worse outcomes are being recognized as the pandemic unfolds globally.⁽¹⁶⁻¹⁸⁾

Macrophage infection and activation, initially in the lungs and then systemic, is emerging as an essential source of pro-inflammatory cytokines such as IFN γ , IL-6, IL-12, TNF, IL-1RA, and CXCL10 amongst others.⁽⁵⁾ Other systems, such as the coagulation cascade, is activated by the crescent inflammatory response leading to thrombotic and haemorrhagic events both in the lung and systemically.^(19,20) This multisystem havoc triggered by SARS-CoV-2 is now recognized as a critical element to its pathogenesis which has guided therapeutic strategies that could block its more lethal consequences. For example, an old drug, etoposide, and several more targeted approaches have emerged as a consequence of macrophage activation, including anti-cytokine, anti-chemokine, anti-complement therapies, Janus Kinase (JAK) and Bruton Tyrosine Kinase (BTK) inhibitors.^(8,21) It is likely that, if active, the benefit of these therapies will be incremental and a combination strategy with antivirals, anticoagulants, or possibly convalescent plasma provide for a therapeutic approach.⁽²²⁻²⁴⁾

Tocilizumab is an interleukin (IL)-6 inhibitor approved for rheumatoid arthritis, giant cell arteritis, and cytokine release syndrome during CAR-T.^(25,26) Preliminary data show that tocilizumab and other IL-6 blockers may have a role in severe SIRS cases, but its role in COVID-19 has not been confirmed and its use should be considered experimental.^(27,28) Given the mode of action, it is plausible that tocilizumab is active in patients with an already established SIRS (that could worsen) as opposed to early on in the infection cycle with no demonstrable inflammatory activity. Thus, to test this hypothesis, we devised a trial where tocilizumab be tested to avert the more lethal consequences of SIRS related to COVID-19. To investigate the effect of blocking an ongoing inflammatory response, we designed a randomized controlled trial that compares the efficacy of tocilizumab plus standard of care compared to standard of care alone in patients with moderate to severe SARS-CoV-2 infection who require supplemental oxygen and have increased markers of systemic inflammation. The requirement of having elevated systemic inflammatory markers, in our hypothesis, better select those who are more likely to benefit from this strategy.

METHODS

Study design

TOCIBRAS was developed according to SPIRIT guidelines (**Appendix 1**) and registered in clinicaltrials.gov as NCT04403685. TOCIBRAS trial is an open-label, parallel-group, superiority, multicentre, randomized controlled trial with the primary objective to show that tocilizumab added to standard of care is superior to standard of care alone in moderate to severe cases of COVID-19 (**Figure 1**).

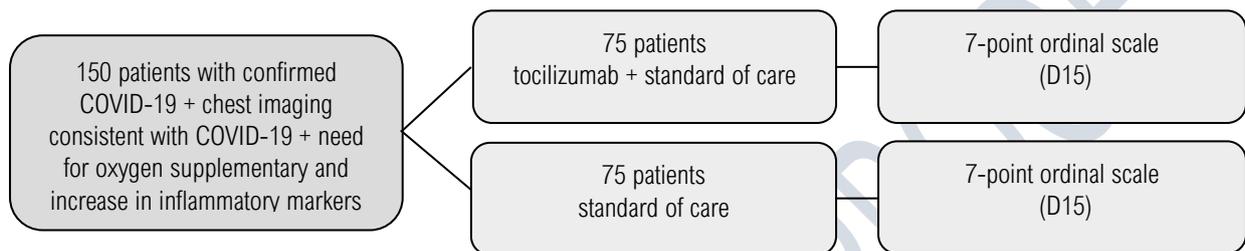


Figure 1 - Schedule of enrollment, interventions, and assessments.

Coalition COVID-19 Brazil is conducting this study, a collaborative research network comprised of the following hospitals: *BP - A Beneficência Portuguesa de São Paulo, HCor-Hospital do Coração, Hospital Israelita Albert Einstein, Hospital Sírio-Libanês, Hospital Alemão Oswaldo Cruz, Hospital Moinhos de Vento*, Brazilian Clinical Research Institute (BCRI), and the Brazilian Research in Intensive Care Network (BRICNet) (**Appendix 2**).

Eligibility criteria

Inclusion criteria

1. Confirmed diagnosis of SARS-CoV-2 infection
2. Computed tomography (or chest X-ray) of the chest consistent with COVID-19
3. More than three days of symptoms related to COVID-19
4. 18 years of age or older
5. Need for oxygen supplementation to maintain $SpO_2 > 93\%$ OR need for mechanical ventilation less than 24 hours before the randomization
6. Two or more of the following inflammatory tests:
 - D-dimer $> 1,000\text{ng/mL}$
 - C reactive protein (CRP) $> 5\text{mg/dL}$
 - Ferritin $> 300\text{mg/dL}$
 - Lactate dehydrogenase (LDH) $>$ upper limit of normal

Exclusion criteria

1. Need for mechanical ventilation for 24 hours or more before the randomization
2. Hypersensitivity to tocilizumab
3. Patients without therapeutic perspective or in palliative care
4. Active non-controlled infections (other than COVID-19)
5. Neutrophil count $< 0.5 \times 10^9/L$
6. Platelet count $< 50 \times 10^9/L$
7. Liver disease, cirrhosis or elevated AST or ALT above 5 times the upper limit of normal
8. Renal disease with estimate glomerular filtration below $30\text{mL}/\text{min}/1.72 \text{ m}^2$ (MDRD or CKD-EPI scores)
9. Breastfeeding women
10. Pregnancy
11. Other clinical conditions that contraindicate tocilizumab, according to the attending physician

Outcomes

Primary objective

To assess the effect of tocilizumab plus standard of care compared to standard of care alone on the clinical status on day 15, as measured by a 7-point ordinal scale, in adults hospitalized with moderate to severe COVID-19. The 7-point ordinal scale applied in this study is as follows:

1. Patient not hospitalized, with no limitation in activities
2. Patient not hospitalized, with limitation in activities
3. Patient in the hospital, without supplemental oxygen
4. Patient in the hospital, with supplemental oxygen
5. Patient in the hospital on non-invasive positive pressure ventilation (NIPPV) or high flow nasal cannula
6. Patient on mechanical ventilation
7. Death

Secondary objectives

To assess the treatment effect with tocilizumab plus standard of care compared to standard of care, in adults hospitalized with COVID-19, on the following outcomes:

1. All-cause mortality from randomization to day 28.
2. Hospital mortality
3. Degree of organ dysfunction assessed by the Sequential Organ Failure Assessment (SOFA) score at day 8 and day 15 after randomization
4. Clinical status at days 8 and 29 after randomization, using the 7-level ordinal Scale

5. Ventilator free days within 29 days
6. Time until oxygen support independence within 29 days
7. Length of hospitalization
8. Incidence of secondary infections
9. Occurrence of thromboembolic events (stroke, myocardial infarction, deep vein thrombosis, pulmonary thromboembolism)
10. Incidence of adverse events

Exploratory secondary objectives

1. To assess the association of inflammatory markers and cytokines with clinical outcomes
2. To assess the kinetics of haemostatic parameters, inflammatory markers, cytokines, peripheral blood flow cytometry, complete blood count, renal and liver function tests
3. To assess viral clearance of SARS-CoV2 at D8

The following exploratory tests will be conducted and correlated with clinical outcomes:

- Biomarker measurement of D-dimer, CRP, LDH, ferritin, IL-6, TNF α , IL2 receptor (CD25), and IL-10
- Peripheral blood flow cytometry for T (CD4+, CD8+, double-negative T cells subpopulations), B (transitional, naïve, non-class switched memory cells, class switched memory cells and plasma cells) and NK (CD16+/CD56-, CD16+/CD56+, CD16-/CD56++) lymphocytes, monocytes (classical, intermediate and non-classical), plasmacytoid and myeloid dendritic cells, eosinophils, basophils and neutrophils.
- Coagulation studies PT/PTT, fibrinogen, vWF, ristocetin cofactor and factor 8.

Details of the methodology for all these tests is provided in **appendix 3**.

Interventions

The concomitant use of hydroxychloroquine, azithromycin, corticosteroids, or other therapies are allowed in this trial as standard of care if local institutional guidelines/protocols include these agents as part of the standard management in COVID-19 patients. Antibiotics are permitted at any time during the study per the discretion of the treating physician. The standard of care treatment for COVID-19 is not yet defined thus investigators can apply what is considered their standard approach for these patients per local policies.

Eligible patients after giving written informed consent will be randomized to receive tocilizumab plus standard of care (n = 75) or standard of care alone (n = 75). In the experimental arm, tocilizumab will be administered as a single intravenous infusion at 8 mg/kg dose. The maximum dose is 800 mg. To allow for a homogenous dose rounding between centres, the following weight-based scale will be applied:

- < 50kg - 8mg/kg/dose
- from 50 to 56kg - 400mg/dose
- from 57 to 68kg - 500mg/dose
- from 69 to 81kg - 600mg/dose
- from 82 to 93kg - 700mg/dose
- \geq 94kg to 800mg/dose (max dose)

There is no dose adjustment for renal or hepatic impairment. Infusion-related reactions will be treated with antihistamines and corticosteroids if needed and reported as adverse events. All infusions will be administered in hospitalized patients in a regular ward or intensive care unit.

Randomization

Participants will be randomly assigned to either experimental (tocilizumab) or the control (standard of care) group with a 1:1 allocation considering blocks (2, 4, 6, and 8) with random variation applying age (< 60 and \geq 60 years) and gender as strata, as per a computer-generated randomization schedule. The random sequence will be generated by a statistician not involved with patient care using an algorithm in Software R 3.6.3.⁽²⁹⁾

Allocation concealment is ensured by a web-accessed system (REDCap) which displays the random treatment assignment only after the participant is properly registered into the trial system and all eligibility criteria are met.^(30,31)

Blinding

Patients, investigators, and caregivers will not be blinded to assigned treatment. Clinical outcome assessors and statisticians performing the analysis are not involved with the patient care teams and are independent of the treating sites.

Data collection, management, analysis

Study data will be collected and managed using REDCap electronic data capture tools hosted at *BP - A Beneficência Portuguesa de São Paulo*.^(30,31) REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Data will be entered by the local study personnel who have a unique centre designated access, which is not transferable. A limited number of designated staff have access to the trial in REDCap and are given proper delegation from their pertinent interaction with the platform and entry. The data will be kept on the institutional server, with the security requirements recommended by the REDCap

Consortium.⁽³¹⁾ This system's functionalities include patient registration, concealment of randomization 24 hours per day, data entry, data cleaning, data export for statistical analysis, and event adjudication. Clinically relevant laboratory studies will be done in real-time, while those in the exploratory list will be analysed later from frozen serum or plasma. Data from the primary and secondary endpoints will be included in REDCap in the appropriate time points. The independent Data Monitoring Committee (DMC) will have full access to the data of the study.

The follow-up data for this trial are collected until day 29. A one-year follow-up study of hospitalized patients enrolled in all Coalition trials will continue follow-up of patients enrolled in this study who accepts to participate. Patients who die within this period will be censored at the time of death, and those still hospitalized will be followed on study until discharged before enrolling in the long-term study. Contacts by telephone and other means of communication will be used to ensure the highest retention during follow-up. We anticipate a high retention rate up to D29, given that most will still be in recovery from the recent episode of viral pneumonia. Adherence will be monitored by reconciling protocol specified data collection and the data entry into REDCap.

Statistical analysis

Sample size

Considering an ordinal outcome with seven stages with probabilities 30%, 20%, 8%, 8%, 4%, 15%, 15%, respectively for stages 1 to 7, under the model of proportional odds ratios for the accumulated probabilities for the outcome levels, a sample of 75 cases per arm (150 cases) has 80% power to detect an odds ratio of 0.44, with a 5% significance level.

Statistical methods

The main analysis will follow the intention-to-treat principle. The primary endpoint will be assessed by ordinal logistic regression assuming proportional odds ratios adjusted for stratification variables (age and gender). Logistic regression models will assess binary cumulated outcomes. If odds proportionality does not hold in the final analysis, we intent to switch primary outcome to a binomial endpoint collapsing categories 1 to 5 and 6 to 7 (alive versus dead or on mechanical ventilation). Secondary outcomes will be evaluated by generalized linear regression using appropriate distributions. All models will be adjusted for age and results will be presented with their 95% confidence intervals effect measures. Subgroup analyses will be presented in forest plot evaluated with interaction terms of group and the following variables: age (< or > 60 years), sex, types of comorbidities (cardiovascular, pulmonary, hepatic, renal, obesity, high blood pressure, cancer, diabetes), and altered study entry inflammatory markers. Analyses will be performed with R software.⁽²⁹⁾

Monitoring

An interim analysis will be performed when 50% of the planned accrual is reached (n = 75). The interim analysis will be performed by an independent DMC who will analyse efficacy and safety data, rate of recruitment, adherence to the protocol, data quality, and follow-up loss. These data will be provided by the study coordinating site to the committee via a report. The Lan DeMets method and O'Brien Fleming thresholds will be applied as a pre-defined criterion for study interruption. If the committee recommends continued accrual, the study will go onto completion (n = 150).

Adverse events

Adverse events (AE) are defined as any untoward medical occurrence, including an exacerbation of a pre-existing condition, in a subject during the course of the clinical trial. The event does not necessarily have to have a causal relationship with the treatment.

A serious adverse event is defined as any adverse event that results in death, offers immediate risk to life, results in persistent or significant disability, requires or prolongs the patient's hospitalization, or if it is a major medical event that based on proper medical judgment could threaten the patient's life or could require medical or surgical intervention to prevent one of the other results listed above. Adverse events classified as serious will be notified within 24 hours to the coordinating site.

The AEs will be graded according to the most recent version of the Common Terminology Criteria for Adverse Events (CTCAE). Monthly monitoring will evaluate the integrity of the screening, inclusion/criteria, and data reporting from the sites for adequacy and consistency. Every 15 days, a statistical methodology will be applied to look at inconsistency and data errors.

Given the biologic characteristics of tocilizumab, the following AEs will be of specific interest and will be captured:

- Secondary infections
- Anaemia
- Liver function test abnormalities
- Diverticulitis
- Herpes zoster
- Headache
- Haemorrhage
- Thromboembolic events
- Serious infusion-related toxicities

Ethics and dissemination

The TOCIBRAS study was approved by local and central (national) ethical committees following current national and international guidelines/directives. The National Committee in Research and Ethics

(CONEP) is the entity in Brazil regulating ethical standards for clinical research. TOCIBRAS was developed and will be conducted under the normative set forth by CONEP, which adopts international ethical standards in clinical research. Each participating centre had the study approved at their institutional review boards before initiating protocol enrolment. A first amendment was submitted to both central and local ethical committees, which address clarifications that were left unclear by the original version of the protocol. This amendment did not change the primary or secondary points, study design, statistics, safety/efficacy monitoring, informed consent, exclusion criteria, or interim analysis. The inclusion criteria were left unchanged, with the only exception being to limit intubation time up to 24 hours before protocol enrolment, given that the benefit of an anti-inflammatory strategy, including tocilizumab, is likely before a fully established SIRS.

Delegated investigators will obtain informed consent in this study. The trial only includes adult subjects; thus, a minor assent is not included. Once the informed consent is signed, it is safeguarded by the centre's research personnel. When a written informed consent cannot be provided due to clinical status or other logistical impediments, verifiable audio will be permitted temporarily as a means of study entry. The process of audio recording is being applied per CONEP guidelines in Brazil for COVID-19 trials, given the inability at times to obtain written informed consent in real-time given the pace of the pandemic and significant restrictions in visitations and companion stays in the ward or intensive care units treating for COVID-19 patients. Once the clinical circumstances allow, written informed consent will be signed by the patient or next of kin and will be added to the patient's records. The physician will discuss the risks, benefits, and caveats in participating in the trial and answer all questions. This conversation will be documented in the medical record by the delegated physician, which will reflect the patient's knowledge about the study and consent.

TOCIBRAS is an independent investigator-initiated trial funded by the COALITION COVID-19 Brazil. The exploratory laboratory analysis will be conducted and funded by Fleury Laboratory in São Paulo. A donation from Instituto Votorantim was kindly provided for the purchase of tocilizumab for this study.

The data derived from this trial will be published regardless of the results. The dataset will be analysed independently by statisticians not involved with the teams involved with data entry and patient care in the participating centres. The publication policy will follow that of the sites member of the Coalition. The publication venues will include medical meetings/conferences and submission to peer review journals. Depending on the results, preliminary results could be disclosed via a press release if it is determined that it is in the public's best interest. The investigators in TOCIBRAS will write the manuscript, which will be approved by all authors before any submissions.

CONCLUSION

The result of this trial will shed light on the potential role of an interleukin blocking strategy in patients with moderate to severe COVID-19. Apart from the many other anti-cytokine approaches being investigated in COVID-19, our study selects patients who manifest an ongoing systemic inflammatory response, as evidenced by serum inflammatory biomarkers, which could make this strategy more effective. We hypothesize that tocilizumab could be more active in the earlier stages of the inflammatory response (which tends to occur in the second week of the infection) before it becomes fully established with multiorgan compromise. This approach differs, for example, from antiviral strategies where the more significant benefit is anticipated in the earlier stages of the infection (first week). Therefore the study was designed to detect a difference of tocilizumab plus standard of care versus standard of care in this more select 'window' in the disease course in moderate to severe COVID-19. As with most COVID-19 studies, assumptions had to be made in regards to anticipated treatment differences given the scarcity and inconsistency in the best available data existent at the time of protocol development in regards to outcomes of patients with COVID-19. Nevertheless, we included a robust design and several secondary clinical and laboratory exploratory endpoints, which will allow us to detect if there is an activity of tocilizumab in this patient population. Our work will complement all the anti-inflammatory and other approaches being developed to mitigate the consequences of COVID-19.

Strengths and limitations of this study

- The randomized controlled study design permits a more definitive conclusion regarding the activity of tocilizumab in COVID-19
- The well-defined patient population with a higher propensity to develop inflammatory complications of COVID-19 may define the subset of patients in which a potent anti-inflammatory approach is warranted
- The 7-point ordinal scale is a robust endpoint for COVID-19 trials
- The study is not blinded which presents a weakness of the study design
- The total 'N' is relatively small for a controlled study with more limited statistical power

Authors contributions

DLC Farias, J Prats, P Scheinberg and VC Veiga conceptualized the study, wrote the protocol, recruited patients and drafted the manuscript; AB Cavalcanti, RG Rosa, FR Machado, FG Zampieri, O Berwanger, LCP Azevedo, RD Lopes, A Avezum, L Kawano-Dourado, CG Castro Jr. participated in the protocol development and approved its final version for the COALITION COVID-19 BRAZIL Group, recruited patients, participated in interim discussions and reviewed the manuscript; CZ Oliveira, developed the REDCap database and attended to all data collection related issues; LP Damiani will be performing the statistical analysis; LEC Andrade, AF Sandez, MC Pintão coordinated exploratory sample collection and will be performing the exploratory analysis, participated in the protocol development an interim discussions and reviewed the manuscript.

Funding

This trial was funded by the COALITION COVID-19 Brazil. The exploratory laboratory analysis will be conducted and funded by Fleury Laboratory in São Paulo, Brazil. A donation from Instituto Votorantim has been kindly provided for the purchase of tocilizumab for this study. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

REFERENCES

- Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med.* 2020;382(8):727-33.
- Cohen J, Normile D. New SARS-like virus in China triggers alarm. *Science* 2020;367(6475):234-5.
- Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. *BMJ.* 2020;368:m1036.
- World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. [cited 2020 June 23]. Available from: <https://covid19.who.int/>
- Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol.* 2020;20(6):355-62.
- Zhang Y, Gao Y, Qiao L, Wang W, Chen D. Inflammatory response cells during acute respiratory distress syndrome in patients with coronavirus disease 2019 (COVID-19). *Ann Intern Med.* 2020 Apr 13:L20-0227. Online ahead of print.
- Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* 2020;395(10229):1033-4.
- Vaninov N. In the eye of the COVID-19 cytokine storm. *Nat Rev Immunol.* 2020;20(5):277.
- Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science.* 2020;368(6490):473-4.
- Park MD. Macrophages: a Trojan horse in COVID-19? *Nat Rev Immunol.* 2020;20(6):351.
- Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, et al. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A.* 2020;117(21):11727-34.
- Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* 2020;395(10234):1417-8.
- Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology.* 2020;158(6):1831-3.e3.
- Zubair AS, McAlpine LS, Gardin T, Farhadian S, Kuruvilla DE, Spudich S. Neuropathogenesis and neurologic manifestations of the coronaviruses in the age of coronavirus disease 2019: a review. *JAMA Neurol.* 2020 May 29. Online ahead of print.
- Fu L, Wang B, Yuan T, Chen X, Ao Y, Fitzpatrick T, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: a systematic review and meta-analysis. *J Infect.* 2020;80(6):656-65.
- Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med.* 2020;180(7):1-11.
- Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054-62.
- Esai Selvan M. Risk factors for death from COVID-19. *Nat Rev Immunol.* 2020;20(7):407.
- Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med.* 2020;383(2):120-8.
- von Weyhern CH, Kaufmann I, Neff F, Kremer M. Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *Lancet.* 2020;395(10241):e109.
- Hamizi K, Aouidane S, Belaaloui G. Etoposide-based therapy for severe forms of COVID-19. *Med Hypotheses.* 2020;142:109826. Online ahead of print.

22. Hillen HS, Kokic G, Farnung L, Dienemann C, Tegunov D, Cramer P. Structure of replicating SARS-CoV-2 polymerase. *Nature*. 2020 May 21. Online ahead of print.
23. Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. *JAMA*. 2020 Jun 3:e2010044. Online ahead of print.
24. Connors JM, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*. 2020;135(23):2033-40.
25. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell*. 2020;181(5):1036-45.e9.
26. Scott LJ. Tocilizumab: a review in rheumatoid arthritis. *Drugs*. 2017;77(17):1865-79.
27. Gritti G, Raimondi F, Ripamonti D, Riva I, Landi F, Alborghetti L, et al. IL-6 signalling pathway inactivation with siltuximab in patients with COVID-19 respiratory failure: an observational cohort study. *medRxiv* 2020.04.01.20048561. doi: <https://doi.org/10.1101/2020.04.01.20048561>
28. Antwi-Amoabeng D, Kanji Z, Ford B, Beutler BD, Riddle MS, Siddiqui F. Clinical outcomes in COVID-19 patients treated with tocilizumab: an individual patient data systematic review. *J Med Virol*. 2020 May 21:10.1002/jmv.26038. Online ahead of print.
29. R Foundation for Statistical Computing. The R Project for Statistical Computing 2020. Available from: <https://www.r-project.org/>
30. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-81.
31. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, Duda SN; REDCap Consortium. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208.

Appendix 1 - SPIRIT 2013 checklist

Section/item	Item	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1,2
	2b	All items from the World Health Organization Trial Registration Data Set	1
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1, 11
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	4,11, 16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	16
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	4,6
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	4,5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6,7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	6,7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6,7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5,6

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	5,6
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7,8
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8,9
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	9
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8

Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2,10
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	NA
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	BA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	NA
	31b	Authorship eligibility guidelines and any intended use of professional writers	11
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

Appendix 2 - Steering committee

COALITION COVID-19 Brazil VI Investigators

Viviane Cordeiro Veiga, Phillip Scheinberg, Danielle Leão Cordeiro de Farias, João Prats, Alexandre Biasi Cavalcanti, Flávia Ribeiro Machado, Regis Goulart Rosa, Otávio Berwanger, Luciano César Pontes de Azevedo, Renato Delascio Lopes, Álvaro Avezum, Leticia Kawano-Dourado, Claudio Galvão for the COALITION COVID-19 Brasil VI Investigators

Appendix 3 - Exploratory laboratory testing

This appendix includes the details in the methodology of exploratory laboratory testing as part of the secondary endpoints. As it relates to interleukins testing, the measurement of the cytokines IL-6, TNF α , IL-10, as well as the IL-2 receptor (CD25), will be performed by capture ELISA system. Briefly, the serum samples will be incubated in an appropriate dilution in polystyrene plates pre-coated as monoclonal antibodies against the cytokine of interest for 30 minutes. After washing, there will be incubation with peroxidase-labelled monoclonal antibody for 30 minutes. After a new wash, there will be incubation with 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂). After 10 minutes, the reaction will be stopped by adding 1N H₂SO₄ and each well will be evaluated by spectrophotometry at wavelength 450nm.

As it relates to the flow cytometric studies, all samples will be collected in tubes containing K3 EDTA as anticoagulant. Cells in suspension (2×10^6 cells in 100 μ L per tube) from the peripheral blood samples will be stained with monoclonal antibodies (MAb) directed against cell surface markers using a stain-lyse-and-then-wash, direct immunofluorescence technique. The following panel of 8-color combinations of monoclonal antibodies (MAbs)—fluorescein isothiocyanate (FITC)/phycoerythrin (PE)/peridinin chlorophyll protein (PerCP-Cy5.5)/ PE-cyanine 7 (PE-Cy7)/allophycocyanin (APC)/APC-H7/Brilliant Violet 421 (BV421)/Violet 500 (V500) - will be used in all cases: IgM/CD10/CD20/CD19/IgD/CD38/CD27/CD45, CD57/CD26/CD3/CD25/CD279/CD8/CD4/CD45, CD16/CD123/CD34/CD33/CD56/CD3+CD19+CD14/HLA-DR/CD45 and CD8+Ig(K)/CD56+Ig(L)/CD3/CD19+TCR-gamma-delta/CD5/CD38/CD20+CD4/CD38. A tube containing Ig isotype controls for FITC/PE/PerCPCy5.5/PE-Cy7/APC/APC-H7/BV421/V500 will be performed in all cases. The source of MAbs will be as follows: Ig isotype controls, CD3, CD4, CD8, CD5, CD10, CD14, CD16, CD19, CD20, CD25, CD26, CD33, CD34, CD38, CD45, CD56, CD57, CD123, CD279, TCR-gamma-delta, IgD, Ig(K), Ig(L) are from Becton Dickinson Biosciences (BDB), San Jose, CA, USA; HLA-DR are from Biolegend, San Diego, CA, USA; and IgM from Beckman Coulter, Indianapolis, USA. Data acquisition will be performed immediately after completion of sample staining, using a FACSLyric flow cytometer and the FACSuite software (BDB). For each sample, data from at least 3×10^5 events per tube will be acquired. The Infinicyt software (Cytognos, SL, Salamanca, Spain) was used for the analysis of flow cytometry data. Daily instrument quality control was performed using CS&T beads (BDB) to ensure consistent determination of fluorescence intensity during the study.

In relation to the coagulation the following will be performed. All samples will be collected in tubes containing citrate 3,2% as anticoagulant. Tubes will be centrifuged at 2,200 g and plasma was aliquoted and stored at -80° C. For analysis, samples will be thawed at 37° C for 20 minutes. All assays will be performed on ACL TOP 750 analyser (Instrument Laboratories, Bedford, USA) accordingly to standard protocols. The PT will be performed using Hemosil® RecombiPlasntin 2G, PTT and factor 8 assays will be performed using Hemosil® Synthasil and Hemosil® Factor VIII deficient plasma. Factor VIII assay will be performed using a single-point assay (1/20 dilution in buffer). Fibrinogen will be performed using Hemosil® QFA Thrombin (Bovine) reagent by Clauss method. Von Willebrand assay will be performed using Hemosil® VWF: Ag and ristocetin cofactor assay will be performed using Hemosil® VWF:Rco, all immunoturbidimetric tests. All reagents are from Instrument Laboratories (IL, Bedford, USA).