Online Supplement 1

This supplement contains the following items:

1. Final study protocol

2. Summary of changes in the study protocol

CLINICAL TRIAL PROTOCOL

Suspension of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers and adverse outcomes in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – The BRACE CORONA Trial

PROTOCOL NUMBER 001/2020

Proponent: Instituto D'Or de Pesquisa e Ensino (IDOR)

Partner Institution: Brazilian Clinical Research Institute (BCRI)

SYNOPSIS OF PROTOCOL NUMBER 001/2020

BRACE CORONA Trial

Study title	Suspension of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers and adverse outcomes in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – The BRACE CORONA Trial
Proponent's Name	Instituto D'Or de Pesquisa e Ensino (IDOR)
Partner Institutions	Brazilian Clinical Research Institute (BCRI)
Responsible Researchers	André Feldman Andréa Silvestre de Sousa Ariane Macedo Guilherme Arruda Olga Ferreira de Souza Pedro Gabriel Melo de Barros e Silva Renata Moll Bernardes Renato D. Lopes (Principal investigator)
Study Design and Methods	Patients treated in emergency departments of the D'Or São Luiz network hospitals with a diagnosis of COVID-19. Randomization 1:1: Group 1 will continue to use angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs); group 2 will discontinue the use of ACEI and ARBs.
Primary Objective	To assess the impact of the discontinuation of ACEI and ARB therapy on days alive and out of the hospital in patients hospitalized with COVID-19.
Inclusion Criteria	• Hospitalized patients with confirmed COVID-19 diagnosis, using ACEI or ARBs; • Age ≥18 years; • Using no more than 3 antihypertensive drugs; • Ability of patient (or legal representative) to provide informed consent
Exclusion Criteria	• Patients hospitalized for decompensated congestive heart failure in the last 12 months; • Use of sacubitril/valsartan; • Use of mechanical ventilation, and/or hemodynamic instability in the first 24 hours or until confirmation of COVID-19 diagnosis; • Acute renal failure; • Shock; • Pregnancy.
Sample size	Approximately 700 patients
Participating sites	D'Or São Luiz Network Hospitals and field hospitals managed by D'Or São Luiz Network
Follow-up	30 days after randomization

List of Acronyms and Abbreviations:

TIA: transient ischemic attack

COVID-19: coronavirus disease 2019

CVA: cerebrovascular accident

ARB: angiotensin receptor blocker

CVD: cardiovascular disease

AE: adverse event

SAE: serious adverse event

ACE-2: angiotensin-converting enzyme 2

ICH: International Council for Harmonisation

ACEI: angiotensin-converting enzyme inhibitors

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Table of Contents

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus disease 2019 (COVID-19) pandemic, was first detected in Wuhan, China in December 2019. Due to the rapid spread of the virus to more than 100 countries, COVID-19 was declared a pandemic by the World Health Organization on March 11, 2020 (WHO, 2020).

Clinical presentation of COVID-19 is variable and most patients are asymptomatic or present with mild symptoms commonly seen with other viral infections such as cough, fever, dyspnea, myalgia, fatigue, and diarrhea. Abnormal laboratory values such as lymphocytopenia in patients with COVID-19 are not unique and are commonly found with other viruses. The most severe cases of COVID-19 may present as pneumonia and acute respiratory distress syndrome and may evolve to multiple organ failure and death (Chen et al., 2020; Huang et al., 2020; Wang et al., 2020). Children may be less susceptible to severe forms of the disease, possibly due to superior immunity and the absence of comorbidities. A difference in the maturation of the viral receptors also may be associated with the lower risk in children (Wang et al., 2020; Wu et al., 2020).

Data now show that the infection has a direct impact on the cardiovascular system (Lippi et al., 2020). Those with pre-existing cardiovascular disease (CVD) may be more susceptible to COVID-19. Elderly patients or those previous CVD have a higher risk of developing severe forms of the disease and have higher mortality (Ruan et al., 2020).

In patients with COVID-19 the incidence of cardiovascular symptoms is high due to the systemic inflammatory response and immune system disorders during the progression of the disease (Zheng et al., 2020). The infection is associated with myocardial ischemia, myocarditis, arrhythmias, and thromboembolic events. Studies suggest the involvement of the angiotensin-converting enzyme 2 (ACE-2) in the physiopathology of the coronavirus infection. This enzyme is a membrane-bound aminopeptidase that has a vital role in the cardiovascular and immune systems and is related to the development of hypertension and diabetes mellitus. SARS-CoV-2, like other coronaviruses, uses ACE-2 as a functional receptor to enter the cells (Turner et al., 2004). The presence of ACE-2 in alveolar cells makes the lungs a preferred entry site for the virus. ACE-2 also plays a role in pulmonary protection, so the binding of the virus to the receptor may interfere with this protective effect thereby contributing to the virus' pathogenicity.

Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs) are widely used in patients with cardiovascular diseases such as arterial hypertension, coronary disease, and congestive heart failure.

Although some experimental studies in animal models report an association between the use of ACEI and ARB and an increase in ACE-2 levels, there is currently no definitive evidence regarding the association between the use of these drugs and an increased risk of SARS-CoV-2 infection. The Brazilian, American, and European Cardiology Societies (SBC, ACC, AHA, and ESC) recommend the continued use of these drugs in patients with heart failure, hypertension, and ischemic heart disease. If a patient with cardiovascular disease is diagnosed with COVID-19, decisions regarding treatment must be made on a case-by-case basis weighing the risk of stopping these drugs versus the potential risk of complications of COVID-19. Urgent new studies are needed, properly delineated and conducted, so these recommendations can be updated with a higher degree of certainty about the safety of continuing or discontinuing these drugs in the presence of COVID-19.

Treatments investigated for COVID-19 may have adverse cardiovascular effects. Preliminary studies suggest the use of chloroquine or hydroxychloroquine, drugs that may inhibit in vitro SAR-CoV-2 infection (Liu et al., 2020), may cause hypotension, vasodilation, suppression of myocardial function, and cardiac arrhythmias. Patients with COVID-19 with previous cardiovascular disease or cardiac decompensation, particularly those taking hydroxychloroquine, should be monitored using echocardiography or electrocardiography and biomarkers such as troponin and D-dimer (Lippi et al., 2020).

The independent effect of chronic treatments like ACEI and ARBs on outcomes in patients with COVID-19 is unknown. In addition, there is conflicting observational evidence about the potential impact of ACEIs/ARBs on patients with COVID-19 (Gheblawi et al., 2020; Soler et al., 2008; Patel et al., 2020). On one hand, ACEI and ARB use could increase ACE2 receptor expression and thus enhance viral binding and viral entry leading to worse outcomes in patients with COVID-19. On the other hand, diminishing production of angiotensin II with an ACEI or ARB enhances the generation of angiotensin (1–7), which attenuates inflammation and fibrosis and therefore could attenuate lung injury. Given the frequent use of these agents worldwide, randomized clinical trial evidence is urgently needed to guide the management of patients with COVID-19.

Objective

Primary Objective

To assess the impact of the discontinuation of ACEI and ARB therapy on days alive and out of the hospital in patients hospitalized with COVID-19.

Methodology

Study Design

Patients treated in the emergency departments of the hospitals in the D'Or São Luiz network with a suspected diagnosis of COVID-19 will be included in an observational registry. Data will be collected for the purposes of epidemiological surveillance, healthcare management, and to learn more about COVID-19 in clinical practice. Patients with a confirmed diagnosis of COVID-19 who are using ACEI and/or ARBs will be invited to participate in the proposed trial. After meeting eligibility criteria and providing informed consent, patients be randomized in a 1:1 ratio to either continue ACEI/ARB therapy (Group 1) or discontinue ACEI/ARB (Group 2) for 30 days.

The use of other anti-hypertensive drugs will be determined by the clinical team caring for the patient. A sampling of 500 patients will be made, stratified by drug that the patient is taking at randomization (ACEI or ARB). All decisions about procedures and treatments during hospitalization will be made by the medical team according to current guidelines and in the best interest of the patient. The only study intervention will be the continuation or discontinuation or ACEI and/or ARB therapy.

The study team will recommend substitutions for ACEI/ARB therapy, if deemed necessary, following recommendations from the Brazilian Guideline of Arterial Hypertension. The participant will be assisted by the medical team during the follow-up of the proposed study and, if necessary, the need for reintroduction of the drug previously used (ACEI or ARB) will be assessed.

Thirty days after randomization the patient will be reassessed, or in case of discharge, telephone contact will be made to evaluate the status of the patient. The outcomes reported by the investigators from each hospital will be verified by the IDOR research team.

Randomization Process

Randomization will be done in 1:1 ratio and will be made using an electronic web-based system.

Risks and Benefits

Available information indicates that an increase in ACE-2 expression may increase the virus binding; however, there are also studies that indicate that an increase in ACE-2 expression may be protective. Therefore, there may be a balance between risk and benefit (equipoise) with the continuation or discontinuation of ACEI/ARB in patients with COVID-19. Observational data have not been clear about the potential impact of ACEIs/ARBs on patients with COVID-19. The lack of clear guidance on how to manage these agents in patients with COVID-19 has generated confusion among physicians leading to

heterogeneous clinical practices around the world. The risk of routinely stopping ACEI/ARB in COVID-19 is not known. This risk might be particularly high given that patients with cardiovascular diseases (hypertension, heart failure) are at high risk for developing severe COVID-19. Therefore, between the balance of risk and benefit of discontinuing ACEI/ARBs, the patients eligible for the study will be those with less dependency on ACEI/ARBs, such as hypertensive patients who have other treatment alternatives (use of <4 anti-hypertensive drugs) and heart failure patients with no decompensation in the last 12 months. In patients with heart failure, there will be the option to use treatment alternatives, such as hydralazine and nitrate, which are commonly used in place of ACEI/ARB, especially during hospitalizations. During the course of the study, a security committee coordinated by BCRI will assess efficacy and safety outcomes.

Finally, in the context of a global pandemic, the information generated by this trial could benefit a great number of individuals. The availability of data on best practices in patients using ACEI/ARBs will inform clinical practice in the treatment of this high-risk population with cardiovascular comorbidities infected with COVID-19.

Eligibility

Inclusion Criteria

- Hospitalized patients with confirmed COVID-19 diagnosis, using ACEI or ARBs;
- Age ≥18 years;
- Maximum use of 3 anti-hypertensive drugs;
- Patient (or legal representative) able to give informed consent in accordance with ICH Good Clinical Practice guidelines and local legislation and/or regulations.

Exclusion Criteria

- Patients hospitalized for decompensated congestive heart failure in the last 12 months;
- Use of sacubitril/valsartan;
- Use of mechanical ventilation and/or hemodynamic instability in the first 24 hours or until the moment of confirmation of the COVID-19 diagnosis;
- Acute renal failure;
- Shock;
- Pregnancy.

Study Procedures

Procedures	Screening Visit	Randomization Visit	30-Day Visit or Telephone Call
Confirmation of Inclusion/Exclusion criteria	x		
Informed consent form signature	x		
Clinical assessment	x	x	(x)*
Demographic data/Medical history	x		
COVID-19 diagnosis confirmation		x	
Severity assessment and patient eligibility		x	
Outcome assessment			x
Verification of adverse events/Serious adverse events			x

*If patient is still hospitalized

Outcomes of Interest

Primary Outcomes

Days alive and out of the hospital will be separately calculated for each included patient and will be calculated from the day of randomization until the 30th day post-randomization. Days alive and out of the hospital represents the follow-up time (30 days) subtracted from the days in the hospital and/or the days between death and completion of follow-up.

Secondary Outcomes

Cardiovascular and non-cardiovascular outcomes such as progression of COVID-19, mortality (overall and cardiovascular), acute myocardial infarction, CVA/TIA, new heart failure or worsening of pre-existing heart failure, myocarditis, pericarditis, arrhythmias requiring treatment, thromboembolic events, hypertensive crisis, respiratory insufficiency, hemodynamic decompensation, sepsis, renal failure, and biomarkers levels (troponin, B-type natriuretic peptide [BNP], N-terminal pro B-type natriuretic peptide [NT-proBNP], D-dimer, total lymphocytes, CD4, CD8, macrophages, cytokines, in addition to proteomics and metabolomics diagnosed biomarkers) will also be assessed.

The clinical outcomes during 30-day follow-up (death, acute myocardial infarction, CVA, hypertensive crisis, and heart failure) will be assessed and classified by an adjudication committee.

Safety Analysis

Data and Safety Monitoring Committee

The study outcomes (which include efficacy and safety variables) and other serious adverse events not covered by the study outcomes will be reported and monitored continuously by an independent data and safety monitoring committee (DSMC). The DSMC will be managed by the BCRI and will comprise independent investigators who will analyze the data in an unblinded and systematic way with weekly meetings in order to offer greater security to the patients. In addition to the weekly regular analyses, 2 interim formal analyses will be performed (with 150 randomized patients and 250 randomized patients who have completed the study follow-up of 30 days). The DSMC will use a P value <0.001 to determine a statistically significant difference of the outcomes between the groups of these interim analyses (in the final study analysis, a P value of <0.05 will be used to determine a statistically significant difference).

Severe Adverse Events

A severe adverse event (AE) is any adverse event that meets at least one of the following criteria:

- Fatal (AE that causes or leads to death);
- Risk to life (puts the patient at imminent risk of death);
- Requires or extends hospitalization;
- Results in deficiency/incapacity;
- Significant medical event that may require clinical or surgical intervention in order to prevent one of the outcomes listed above.

Statistical Analysis

Sample Size

As there are no studies that allow adequate sample size calculation. Thus, the initial sample size was 500 patients, with possible alteration according to recruitment, event rate, and DSMC interim analysis.

Using data from the ongoing study COALIZAO I [NCT04322123] as a reference (standard deviation of 4 days and average time of 24 days alive and outside the hospital), it will be possible to obtain a power of 90% to detect an average ratio of at least 1.10 in the primary endpoint with a total of 500 patients.

The recruitment of participants occurred faster than expected and in order to be able to carry out the two interim analyzes with a 30-day follow-up of 150 and 250 patients, the recruitment was extended and the sample size increased to approximately 700 patients. Due to a lower number of patients using ACEI in relation to the subgroup of patients using ARBs, we intend, after inclusion of patients in the principal study is completed, to follow with the randomization only of the ACEI patients subgroup until we reach an

estimated sample of 250 patients. The aim is to achieve better statistical power in order to perform a pre-specified subgroup analysis. Patients included will be those treated in the emergency departments of D'Or São Luiz network hospitals with a confirmed diagnosis of COVID-19 who meet the previously described inclusion criteria during a period of 3 months; the recruitment period can be extended depending on rate of recruitment.

Statistical Analysis Plan

Continuous variables will be described as median (25th, 75th percentiles) or mean ± standard deviation according to the distribution. Means will be compared using the t test and medians will be compared using the Kruskal-Wallis test. Categorical variables will be described by absolute and relative frequencies and the proportions will be compared using the Chi-square test. P values will be two-sided and a P value <0.05 will be considered statistically significant. All analyses will be performed using R version 3.6.1.

As the primary outcome will be measured as days alive or out of the hospital, the analysis will be based on mean and median of this outcome as well as mean ratio and difference in means and medians. The analysis of the distribution of days alive and out of the hospital over 30 days will be performed by histograms in the 2 groups (with and without suspension of ACEI / ARB) and the median and 25th and 75th percentiles of the general population and of each group will be calculated. The comparison between the groups will be made using a Quasi-Poisson model. Results will be presented as average ratios between the groups with the respective 95% confidence interval. A sensitivity analysis will also be performed in which patients who have died (even if death occurred after discharge) will be considered as having zero days alive outside the hospital. Interaction tests will be performed for specific subgroups that include sex (male or female); age (> or ≤65 years); symptom days (in tertiles); history of acute myocardial infarction (vs. no); history of stroke (vs. no), history of heart failure (vs. no); history of hypertension (vs. no); use of ACEI vs. use of ARB and ACE or ARB times before inclusion.

Secondary outcomes over 30 days such as mortality and cardiovascular events will be compared using log-binomial models and relative risks will be reported, with the respective 95% confidence intervals.

Ethical and Legal Aspects

This clinical study was designed and should be implemented and reported in accordance with the International Conference on Harmonization Conference (ICH) guidelines for Good Clinical Practice, with applicable local standards, and with the ethical principles outlined in the Declaration of Helsinki. Patients will be invited to participate voluntarily in the study and if they accept, they will sign the free and informed consent term previously approved by the National Research Ethics Commission (CONEP), in

two copies, one of which will be delivered to the participant and the other will be filed by research center staff.

Participant confidentiality

All participants will be identified by a number, maintaining the confidentiality of all their records during the study. Except when required by law, name, personal identification number (e.g., Sistema Único de Saúde [SUS] or health insurance card number, address, telephone number or any other direct personal identifier in the study records will not identify participants.

The participant's identity will remain confidential when the results of the study are published.

References

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., . . . Wei, Y. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet, 395(10223), 507-513.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., . . . Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet, 395(10223), 497-506. doi:10.1016/s0140-6736(20)30183-5

Lippi, G., Lavie, C. J., & Sanchis-Gomar, F. (2020). Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): Evidence from a meta-analysis. Progress in cardiovascular diseases.

Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, Raizada MK, Grant MB, Oudit GY (2020). Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. Circulation Res 126:1456-74.

Soler MJ, Barrios C, Oliva R, Batlle D. (2008). Pharmacologic modulation of ACE2 expression. Curr Hypertens Rep 10:410.

Liu, J., Cao, R., Xu, M., Wang, X., Zhang, H., Hu, H., . . . Wang, M. (2020). Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discovery, 6(1). doi:10.1038/s41421-020-0156-0

Patel AB, Verma A. COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? JAMA. 2020;323:1769-1770.

Ruan, Q., Yang, K., Wang, W., Jiang, L., & Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine, 1-3.

Turner, A. J., Hiscox, J. A., & Hooper, N. M. (2004). ACE2: from vasopeptidase to SARS virus receptor. Trends in pharmacological sciences, 25(6), 291-294.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., . . . Xiong, Y. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. Jama.

WHO. (2020). Coronavirus disease 2019 (COVID-19): situation report, 51.

Wu, Z., & McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. Jama.

Zheng, Y.-Y., Ma, Y.-T., Zhang, J.-Y., & Xie, X. (2020). COVID-19 and the cardiovascular system. Nature Reviews Cardiology, 1-2.

Rio de Janeiro, July 06, 2020.

Investigator: Dr Renato Delascio Lopes

Protocol: "Suspension of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers and adverse outcomes in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – The BRACE CORONA Trial'

AMENDMENT 1 – APPROVED 04/14/2020

JUSTIFICATION: Electronic or oral Informed Consent Form application

AMENDMENT 2 – APPROVED 05/13/2020

JUSTIFICATION:

- Inclusion of another hospital
- Protocol alterations

1. Exclusion criteria: inclusion of sacubitril/valsartan use.

2. Secondary outcomes: Cardiovascular and non-cardiovascular outcomes, such as: progression of COVID-19, mortality (overall and cardiovascular), acute myocardial infarction, cerebrovascular accident (CVA)/transient ischemic attack (TIA), new heart failure (HF) or worsening of pre-existing HF, myocarditis, pericarditis, arrhythmias that require treatment, thromboembolic events, hypertensive crisis, respiratory insufficiency, hemodynamic decompensation, sepsis, renal failure, biomarkers levels (troponin, B-type natriuretic peptide [BNP], N-terminal pro B-type natriuretic peptide [NT-proBNP], D-dimer, total lymphocytes, CD4, CD8, macrophages, cytokines, in addition to proteomics and metabolomics diagnosed biomarkers). The clinical outcomes during 30 days of follow-up (death, acute myocardial infarction, CVA, hypertensive crisis and heart failure) will be assessed and classified by an adjudication committee.

3. Data and Safety Monitoring Committee (DSMC): The study outcomes (which include efficacy and safety variables) and other serious adverse events not included as study outcomes will be reported and monitored continuously by an independent Data and Safety Monitoring Committee (DSMC). The DSMC will be managed by the BCRI and comprises independent investigators who will analyze the data in an unblinded, independent and systematic way with weekly meetings in order to offer greater security to the patients. In addition to the weekly regular analyses, 2 interim formal analyses will be performed (with 150 randomized patients and 250

randomized patients who have completed the study follow-up of 30 days). The DSMCwill use a P value of <0.001 to determine a statistically significant difference of the outcomes between the groups in these interim analyses (in the final study analysis, a P value of <0.05 will be used to determine a statistically significant difference).

4. Size of sample: In assuming a standard deviation of 4 days and an average time of 24 days alive and out of the hospital (based on the ongoing study COALIZAO I [NCT04322123]), the inclusion of 500 patients will have a 90% power to detect an average ratio of at least 1.10 on the primary outcome.

- Informed Consent Form (ICF) alterations

Inclusion of blood collection for cytokine and other biomarkers quantification.

AMENDMENT 3 – APPROVED 06/30/2020

JUSTIFICATION:

- Protocol alterations:

1. Increase of recruitment rate to about 700 patients instead of 500 initially expected patients and presented sample calculation.

2. Due to a lower than initially expected number of patients using ACEI in relation to the subgroup of patients using ARBs, we intend, after completion of the inclusion of patients in the main study, to continue with the randomization only of the ACEI patients subgroup until we reach an estimated sample of 250 patients. The aim is to achieve better statistical power within this group of ACEI and perform a pre-specified subgroup analysis.