Use of BCG Vaccine as a Preventive Measure for COVID-19 in Health Care Workers

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RESEARCHERS:

Fernanda Carvalho of Q. Mello; MD, MSc, PhD; CPF 916357567 15

Afrânio Lineu Kritski; MD, MSc, PhD; CPF 299576569 53

José Roberto Lapa e Silva; MD, MSc, PhD; CPF 127025017 53

Elisangela Costa da Silva; MSc. PhD; CPF 016548077 70

Rafael Galliez; MD, MSc. PhD; CPF 082542837 88

Maria Armanda Monteiro da Silva Vieira; MD, MSc, PhD; CPF 707915627 00

Ana Paula Santos; MD, MSc, PhD; CPF 047498367 61

Antonio Guilherme Fonseca Pacheco; MD, MSc, PhD; CPF 012999877 02

Martha Maria Oliveira; MSc, PhD; CPF 042874657 84

Guilherme Loureiro Werneck; MD, MSc, PhD, CPF 770037137 72

Rogério Lopes Rufino Alves; MD, MSc, PhD; CPF 821459627 00

Luís Cristóvão de Moraes Sobrino Porto, MD, MSc, PhD; CPF: 429488357 04

Hanna Silva Condelo, MSc; CPF: 135268117 09

Walter Costa; MD; CPF 547574877 20

Robson de Souza Leo; MSc, PhD; CPF 034374517 82

Luciana Silva Rodrigues; MSc, PhD; CPF 075691427 21

Janaina Aparecida of Medeiros Leung; MSc; CPF 160954918 00

Fabio Silva Aguiar; MD; MD, MSc, PhD; CPF 077792197 98

Paulo Fernando Guimarães Morando Marzocchi Tierno; MD; CPF 293080448 33

Roberto de Andrade Medronho; MD, MSc, PhD; CPF 508401427 49

Terezinha Marta Pereira Pinto Castineiras; MD, MSc, PhD; CPF 982013527 34

PARTICIPATING INSTITUTIONS:

1. Hospital Complex: Clementino Fraga Filho University Hospital - Institute of Chest Diseases, Federal University of Rio de Janeiro - Rio de Janeiro/RJ
2. Pedro Ernesto University Hospital - Piquet Carneiro Polyclinic, State University of Rio de Janeiro - Rio de Janeiro/RJ
3. Dr. Francisco Moran Municipal Hospital - Barueri/SP

SUBSCRIPTION PAGE (RESEARCHER - SPONSOR)

I inform you that this Protocol has been revised and approved.

We will supervise and coordinate the clinical trial, conducted in 2 centers in Brazil as described and ensure compliance with Good Clinical Practices (BPC) and Good Laboratory Practices (BPL), the principles described in the Helsinki Declaration and the applicable regulatory requirements.

Name: Fernanda Carvalho de Queiroz Mello

Institution: Institute of Chest Diseases - Federal University of Rio de Janeiro

Signature: _____

day/month/year

STATEMENT BY THE PRINCIPAL INVESTIGATOR

By signing this page, I agree to conduct the study in accordance with all current Brazilian regulations regarding applicable clinical research and international guidelines, as stated in the protocol and other information provided to me.

I will ensure that the requirements for obtaining review and approval from the Research Ethics Committee (CEP) are met. I will promptly report to cep any and all changes in research activities covered by this protocol.

I certify that the individuals involved with this study have completed training in Good Clinical Practices (BPC) in the last 3 years and, if applicable, training in the protection of human beings.

I understand that all information obtained during the conduct of the study regarding the health status of individuals will be considered confidential. No individual name or personally identifiable information may be disclosed. All subject data will be anonymized and identified by numbers assigned in all Case Report Forms and laboratory samples.

I will maintain the confidentiality of this protocol and all other related investigative materials. The information obtained from the study protocol cannot be disclosed or discussed with third parties without the express consent of the researcher – Dr. Fernanda Carvalho de Queiroz Mello.

Full Name of the Principal Investigator:

Fernanda Carvalho de Queiroz Mello

Signature: _____

Signature date: _____

day / month / year

SUMMARY:

The disease promoted by coronavirus (COVID-19) is caused by SARS-Cov2, the first cases identified in December 2019 in China after exposure to the animal market in wuhan city, China (reviewed by Adhikari et al, 2020). From the first case to the present day, the COVID-19 epidemic has been identified in 185 countries, with the notification of 2,666,154 cases and 186,144 deaths (Source: COVID Visualizer, 23Apr2020). In Brazil, more than 45,757 cases and 2,906 confirmed deaths by COVID-19 have been confirmed (Source: COVID Visualizer, 23Abr2020). In our country, to date, testing for COVID-19 occurs only in severe cases and few centers offer the service to health professionals, a population at high risk of infection. BCG is a vaccine produced from a live attenuated strain derived from a *Mycobacterium bovis* isolate and is widely used worldwide as a tuberculosis (TB) vaccine, but there are studies demonstrating non-specific immunotherapeutic mechanisms of this vaccine that signal a possible relationship with the lowest morbidity and mortality associated with COVID-19 infections worldwide. The present study aims to analyze the role of BCG in the prevention of SARS-CoV-2 infection and also in the occurrence of severe forms of COVID-19 in addition to evaluating the immune response mediated by this vaccine in voluntary health professionals.

INTRODUCTION:

The disease promoted by coronavirus (COVID-19) is caused by SARS-Cov2, the first cases identified in December 2019 in China after exposure to the animal market in wuhan city, China (reviewed by Adhikari et al, 2020).

From the first case to the present day, the COVID-19 epidemic has been identified in 185 countries, with the notification of 2,666,154 cases and 186,144 deaths (Source: COVID Visualizer, 23Apr2020). In Brazil, more than 45,757 cases and 2,906 confirmed deaths by COVID-19 have been confirmed (Source: COVID Visualizer, 23Abr2020). In our country, to date, testing for COVID-19 occurs only in severe cases and few centers offer the service to health professionals, a population at high risk of infection. Thus, the data in Brazil present a high of underreporting. According to the UFRJ Technical Note, it is estimated that the cases reported in Brazil represent approximately only 11% of the cases (Kritski *et al*, 2020).

It is known that the impact of the disease differs between countries. These differences are attributed to the different cultural norms, mitigation efforts, available health infrastructure and socio-economic level. Recently, Miller *et al* (2020) proposed, through an ecological study, that countries that continued with the use of *vaccination with Bacillus Calmette Guérin* (BCG) after birth have a lower occurrence of COVID-19.

BCG is a vaccine produced from a live attenuated strain derived *from an isolate of Mycobacterium bovis* and is widely used worldwide as a tuberculosis (TB) vaccine, and countries such as Portugal, Japan and China adopt a universal BCG vaccination policy in newborns. Other countries such as Italy, the Netherlands, Spain, France, Switzerland and the United States of America have discontinued or have not adopted universal vaccination policies due to the low risk, when compared to countries with high prevalence of the disease, of developing *Mycobacterium tuberculosis* (MTB) infections, and also due to the variable efficacy in the prevention of tuberculosis in adults (Miller *et al*, 2020).

Interestingly, shortly after its introduction in the 1920s, epidemiological studies showed that the BCG vaccine reduced infant mortality regardless of its effect on TB (reviewed by Shan *et al*, 2009). In several observational studies in West Africa, there was a 50% reduction in the overall mortality of children vaccinated with BCG, an effect too large to be explained by the protection against tuberculosis only (Garly *et al*, 2003; Roth *et al*, 2005). More recently, these findings have been validated in controlled trials (Aaby *et al*,

2011). Therefore, the reduction in mortality in infants by BCG seems to be due to the induction of protection against unrelated infectious agents. These beneficial effects have been called "hemorologist" or "non-specific" effects.

There are two basic mechanisms on the effect of BCG vaccine on herterologist responses:

1) BCG induces recteral lymphocytic responses, resulting in more efficient immune responses in relation to secondary responses not related to infectious agents (Goodridge *et al*, 2016). For example, mice are protected by BCG vaccination against vaccinia virus infection by increasing the production of IFN-γ by CD4+ T cells (Mathurin *et al*, 2009). The recurrent lymphocytic responses may also involve the activation of CD4+ and CD8+ memory T cells that are specific to non-target antigens, thus modulating TH1 and TH17 responses to secondary non-mycobacterial infections. In healthy volunteer humans, BCG vaccination increased non-specific responses of TH1 and TH17 subpopulations for at least 1 year after vaccination (Kleinnijenhuis *et al*, 2014). In addition, in patients with recurrent respiratory papillomatosis, which is caused by human papillomavirus in 90% of cases, adjuvant therapy with BCG restored the efficiency of antiviral T-cell response by stimulating th1 cytokine production and TREG cell induction •

2) Less than a decade ago, only B and T lymphocytes were considered capable of constructing memory responses. However, in recent studies it has been shown that the functional program of innate immune cells is altered in certain infections or vaccinations, resulting in increased immunity when cells encounter a secondary stimulus (Netea *et al*, 2016). The induction of a non-specific memory in innate immune cells is known as "*trained immunity*" and is mediated by epigenetic and metabolic remodeling (Netea *et al*, 2016). Evidence suggests that the key mechanism by which BCG induces its non-specific effects is probably through the induction of immune memory in innate immune cells, especially in *natural killer* cells (NK), monocytes and macrophages, and not by means of adaptive immune mechanisms based on T cells and B cells.

In mice with severe combined immunodeficiency, the BCG vaccine provided protection against a non-mycobacterial secondary challenge, confirming the importance of innate

immune cells in mediating this effect (Arts *et al*, 2018). In healthy human volunteers, BCG vaccination improved the production of pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α in peripheral blood mononuclear cells (PBMC) for up to 3 months after *vaccination on in vitro stimulation with unrelated pathogens* (Arts *et al* , 2018). This response was associated with an increase in CD11b, TLR4 and CD14 activation markers and epigenetic reprogramming of human monocytes at gene-promoting sites for pro-inflammatory cytokines. In a randomized BCG study, volunteer humans were vaccinated with the YFV vaccine, a yellow fever attenuated virus vaccine. In this study, a significant reduction in viremia was observed after 1 month or placebo in volunteers vaccinated with BCG when compared to volunteers who received placebo. And, this effect was correlated with epigenetic mutations induced by BCG in monocytes (Arts *et al*, 2018). BCG induced higher production of IL-1β during viral infections, rather than the production of IFN-γ by lymphocytes and NK cells. The protective role of IL-1β is related to recent findings suggesting a crucial role of this interleukin in antiviral immunity (Rathinam *et al*, 2010).

Finally, several authors have discussed the role of BCG in inducing the IFN-I pathway in circulating monocytes, inducing the production of IFN-α and -β, cytokines known to be important in the control of viral replication (Moorlag *et al*, 2019). Modulation of a $_{TH1}$ response, with monocyte/macrophage activation, may help reduce the severe responses associated with COVID-19, as several authors point out that inflammatory responses associated with severe forms of Severe Acute Respiratory Syndrome (ARDS) are associated with neutrophil-mediated responses, $_{TH17}$ profiles, pro-inflammatory cytokines such as IL-6, in an event called a cytokine storm (reviewed by Jiang *et al*, 2019).

To build an antiviral response, the ininate immune system recognizes molecular structures that are produced by virus invasion, called pathogen-associated molecular patterns (PAMPs). For RNA viruses such as coronavirus, it is known that the PAMPs found in virus replication in the form of viral genomic ssRNA or double-taped RNA are recognized by the endosomal receptors of RNA TLR8 and TLR7 in ssRNA and by the cytosolic RNA sensor, RIG (retinid inducible gene)/MDA5 (gene 5 associated with melanoma differentiation) (Li *et al*, 2020; by Wit *et al*, 2016; Channappanavar *et al*, 2017). This recognition leads to the activation of several signaling routes. Transcription

factors such as nuclear factor κB (NF-κB), activating protein 1 (AP-1), interferon response factor 3 and 7 (IRF3 and IRF7). NF-κB and AP-1 stimulate the expression of genes encoding various molecules necessary for inflammatory responses, including inflammatory cytokines (e.g., TNF-α and IL-1) and chemokines (CCL2 and CXCL8). IRF3 and IRF7 promote the production of type I interferon (IFN-α and IFN-β), important for innate antiviral immune responses capable of suppressing early-stage replication and viral dissemination (Deng *et al*, 2019; Yang *et al*, 2015). This process can cause complications such as exhaustion, weakness and cough in patients (Kindler *et al*, 2016). The important point in the current pandemic scenario by COVID19 is that, for SARS-CoV-2, the response to ifn virus type I infection is suppressed (reviewed by Rokni *et al*, 2020), because hospitalized patients with severe COVID-19 have high levels of pro-inflammatory cytokines, including IL-2, IL-7, G-CSF, IP-10, MCP-1, MIP-1α, and TNF-α. This phenomenon known as "cytokine storm" or cytokine release syndrome becomes an important factor in the pathogenesis of COVID-19 (Wongi et al, 2004; Conti *et al*, 2020). New strategies should be researched to analyze and improve the individual's response against SARS-CoV2 infection.

Thus, it is understood that non-specific immunotherapeutic mechanisms of bcg vaccine in the light of current epidemiological data, indicate a possible relationship between the existence of universal BCG vaccine policies and the lower morbidity and mortality associated with COVID-19 infections worldwide.

JUSTIFICATION:

Considering the above, the present project aims to study the effect of BCG vaccine on health professionals active in reference hospitals for the care of COVID-19.

This project presents an innovative proposal, addressing a BCG vaccine with known efficacy against severe TB in childhood and reliable for the use of adjuvant therapy in patients with malignant bladder neoplasia.

BCG is an easily accessible vaccine, widely used by SUS in newborn children, thus facilitating its incorporation into a new preventive proposal for COVID-19. In addition, the selection of health professionals will be based on diagnostic screening and confirmatory tests of COVID-19.

It is intended to analyze the role of BCG in the prevention of infection and also in the occurrence of severe forms of COVID-19 in addition to evaluating the immune response mediated by this vaccine in voluntary health professionals.

GOALS:

In the period of 6 months, to analyze the effect of BCG as a protective factor for COVID-19 in research volunteers, aiming at greater efficiency in the prevention of subjects at high risk of infection and evolution to severe forms of the disease, as well as to evaluate the specific and non-specific immune responses to SARS-CoV-2 in vaccinated and unvaccinated volunteers who evolved with COVID-19 or not.

Primary Objectives:

1. Assess the impact of BCG vaccine administration on the cumulative incidence of SARS-CoV-2 infection
2. Assess the impact of BCG vaccine administration on the cumulative incidence of severe forms of COVID-19
3. Evaluate the bcg vaccine-mediated immune response in volunteers

Secondary Objectives:

In groups receiving BCG or placebo, infected and not infected with SARS-CoV-2:

- Epidemiological:

1. Estimate whether BCG vaccination compared to placebo prolongs the time to the first respiratory disease proven by SARS-CoV-2;
2. Estimate whether BCG vaccination compared with placebo reduces the proportion of symptoms associated with COVID-19 (fever or at least one sign/symptom of respiratory disease);
3. Estimate whether BCG vaccination compared to placebo reduces absenteeism (missing days) in volunteers who are health professionals;
4. Analyze the safety of BCG vaccination in adult volunteers;

- Laboratories:

5. Describe the inflammatory profile by dosing neutrophils, platelets, cit-H3, alpha 1 antitrypsin (α1AT), galectin-2, ferritin, transferrin-R, glycated hemoglobin (HbA1c),

C-reactive protein (CRP), D-dummer, LDH, albumin and hemosedimentation velocity (ESR);

6. Describe the profile of inflammatory cytokines identified in the BCG and placebo groups, comparing to the protective events to SARS-CoV-2;
7. Describe the epigenetic alterations associated with monocytes and neutrophils related to the protection of COVID-19;
8. Characterize the cell population (T, B lymphocytes, neutrophils and monocytes) in peripheral blood of the study groups (BCG and placebo);
9. Relate the profile of immune response associated with mild and severe forms observed in the study groups (BCG and placebo);
10. Analyze the serum concentration of antibodies and relate it to the immune response;
11. Identify possible antibodies that may confer immunity to new COVID-19 infections;
12. Perform transcriptomic analysis of neutrophilic, lymphocytic and monocytic responses in the study groups (BCG and placebo) involved in the modulation of immune responses in the study groups;
13. Identify predictive biomarkers of infection and/or illness by SARS-CoV-2;
14. Develop a predictive model of infection and/or illness by SARS-CoV-2;
15. Identify socio-demographic factors, life habits, associated diseases and previous vaccinations associated with immune response and the risk of COVID-19 infection.

With a view to the approval of specific vaccines against COVID-19 for emergency use by ANVISA in January 2021 and incorporated by the National Immunization Program of the Brazilian Ministry of Health, the objectives for the subgroup of individuals who receive the intervention of this study with BCG or placebo and who are also immunized with specific vaccines against COVID-19 will be carried out in the part, taking into account in the future analysis, the time between the intervention of the study and vaccination against COVID-19, the vaccine used and the performance of the second dose of the vaccine used, aiming to identify a possible adjuvant effect of bcg vaccine as a stimulant

of cellular immunity, which also plays a role in immunopathogeny against SARS-CoV-2, including action prior to humoral response.

Furthermore, in cases of participants immunized with specific vaccines against SARS-CoV-2, the identification of asymptomatic infection by the virus, diagnosed through the conversion of serologies to COVID-19 in the moments M3 and M6 after the intervention, will not be evaluated, considering the bias caused by the use of vaccines against viral disease. In these cases, in order to characterize the diagnosis of COVID-19, there will be a need for a report (self-reported) of characteristic signs/symptoms associated with detection of SARS CoV-2 by RT-PCR.

METHODOLOGY:

Study Definitions:

The definitions of the Brazilian Ministry of Health (Brazil, 2020) will be used:

Flu syndrome (GS): Individual with acute respiratory condition, characterized by at least two (2) of the following signs and symptoms: fever (even if reported), chills, sore throat, headache, cough, runny nose, olfactory disorders or taste disorders.

Severe Acute Respiratory Syndrome (SARS): Individual with **GS sit** : dyspnea/respiratory distress OR persistent pressure in the chest OR O2 saturation less than 95% in room air OR bluish coloration of the lips or face.

Confirmed cases of COVID-19:

- By clinical criterion: Case of GSs or SRAG with clinical confirmation associated with anosmia (olfactory dysfunction) OR ageusia (gustatory dysfunction) acute without other previous cause.

- **Por clinical - epidemiological criterion:** Case of GSs or SRAG with a history of close or household contact, in the 14 days prior to the appearance of signs and symptoms with case confirmed for COVID-19.

- **By clinical criterion - imaging:** Case of GSS or SRAG or death from RagS that could not be confirmed by laboratory criterion And that presents at least one (1) of the following tomographic changes:

- Peripheral, bilateral **, horizontal matte glass opacity** , with or without consolidation or visible intralobular lines ("paving"), **OR**

- **Multifocal frosted glass** opacity of rounded morphology with or without consolidation or visible intralobular lines ("paving"),**OR**

- Reverse halo sign or other findings of organizing pneumonia (later observed in the disease).

- By laboratory criterion: Case of SG or SRAG with test of:

- Molecular biology: detectable result for SARS-CoV-2 performed by the RT-PCR method in real time.

- **Immunological:** reactive result for IgM, IgA and/or IgG performed by the following methods: Enzyme-Linked Immunosorbent Assay (ELISA); Immunochromatography (rapid test) for antibody detection; Electrochemiluminescence Immunoassay (ECLIA);

- Antigen Research: reagent result for SARS-CoV-2 by immunochromatography method for antigen detection**.**

- **By laboratory criterion in asymptomatic individual:** Asymptomatic individual with test results:

- Molecular Biology: detectable result for SARS-CoV-2 performed by the RT-PCR method in real time;

- Immunological: reagent result for IgM and/or IgA performed by the following methods: ELISA or Immunochromatography (rapid test) for antibody detection.

- Health Professional: For the purposes of this study, in addition to the definition of a health professional established in Resolution 218 - 97 - Regulation of health professions, the Brazilian Classification of Occupations will be used, and in an expanded way will be included undergraduate students in the area of health sciences who attend the hospital and are in direct contact with patients. Administrative technical professionals who circulate or perform activities in sectors within the hospitals participating in the study and who have direct contact with patients may also be included in the study, such as receptionists, maqueiros, security guards, among others.

Study Design:

The project proposes to monitor research participants for 6 months and thus conduct a controlled clinical trial, phase II-B, which compares the occurrence of SARS-CoV-2 infection as well as the time to infection among health professionals receiving bcg vaccine (experimental group) compared to those receiving placebo (control group). Coupled with the clinical trial, participants will also be analyzed in an observational study of two cohorts of volunteers, vaccinated with BCG and not vaccinated. In this part of the

study, participants will be analyzed in relation to the inflammatory profile, considering biomarkers predicting infection and evolution to severe form of COVID-19.

Study site:

The possible participants of this research will be recruited in two hospitals in the city of Rio de Janeiro (Hospital Complex Institute of Chest Diseases - Clementino Fraga Filho University Hospital of UFRJ and Pedro Ernesto University Hospital Complex - Piquet Carneiro Polyclinic of UERJ) and a hospital in the city of Barueri in SP (Hospital Municipal Dr. Francisco Moran - Barueri/SP).

Eligibility criteria:

Health professionals (according to the definitions of this study), who are in the exercise of their activities in the units of the study or not, of both sexes over the age of 18 years and who agree to participate in the study by signing the Informed Consent Form (TCLE).

***Inclusion criteria**:*

1. Individuals aged 18 ≥, male or female, not infected with SARS-CoV-2
2. Agreement to participate in the study by signing the TCLE
3. Not being pregnant in case of women able to become pregnant
4. Have not received a specific vaccine against COVID-19 or, if vaccinated against SARS-CoV-2, have received the complete immunization schedule only with vaccines approved by ANVISA and implemented by the National Immunization Program, (including the second dose) within a minimum of 15 days prior to the date of inclusion in the study
5. If the participant have not received a specific vaccine against COVID-19 approved by ANVISA, be aware and agree to be able to receive them only 15 days after the intervention proposed in this study

***Exclusion criteria**:*

1. Professionals with a history of sars-cov-2 confirmed infection through RT-PCR or who have already presented clinical and molecular diagnosis of COVID-19 prior to the study

2. Individuals who have not underwent confirmatory tests for COVID-19
3. Breastfeeding
4. Individuals who report having primary or acquired immunodeficiency
5. Individuals who report being affected by malignant neoplasms
6. Patients reporting treatment with high-dose corticosteroids (equivalent to the prednisone dose of 20 mg/day or more) for more than two weeks
7. Patients who report being on other immunosuppressive therapies (antineoplastic chemotherapy, radiotherapy, among others)
8. Individuals who report being diagnosed with autoimmune diseases
9. Dermatological conditions at the vaccine site or generalized
10. Individuals who report being treated for active tuberculosis
11. Individuals who report a history of previous treated tuberculosis
12. Individuals with febrile symptoms (body temperature ≥ 37.5ºC in the last 48h)
13. Participation in other clinical prevention trials for COVID-19 (vaccines already approved by ANVISA for use by the National Immunization Program are not included in this item)
14. Report of vaccination with live microorganism administered in the month prior to randomization
15. Require that, if another vaccination with live microorganism is required, it is administered in the month following randomisation (If the other live vaccine can be administered on the same day, this exclusion criterion does not apply)
16. Known anaphylactic reaction to any ingredient in bcg vaccine
17. Adverse reaction prior to BCG vaccine [significant local reaction (abscess) or suputive lymphadenitis]
18. BCG vaccine administered in the last year

Intervention Plan:

Intervention:

Randomly eligible participants who have provided informed written consent will be recruited. Participants will then be allocated to one of the study regimens in a ratio of 1:1.

1. **Intervention:** BCG vaccine, single dose
2. **Control regimen**: placebo, single dose.

In the BCG clinical trial, participation in the study will last 6 months: during the first 4 months, or for the duration of the recruitment period, participants will receive the randomly designated BCG regimen or only control group. After the recruitment period, all study participants should have received BCG or placebo, and both groups will be monitored through clinical and laboratory examination for 6 months of follow-up. Biosafety care to combat COVID-19 adopted in the participating Health Units will also be monitored during the study period.

Comparator:

The natural evolution of SARS-CoV-2 infection in health professionals will be used to evaluate the efficacy of bcg use as preventive therapy, considering the use of specific vaccines against COVID-19, the time of positivity of COVID-19 tests and the evolution to severe forms of COVID-19.

Specific vaccines against SARS-CoV-2:

- Coronavac® (Sinovac® / Butantan Institute):

The vaccine developed by the Sinovac laboratory® in partnership with the Butantan Institute is a vaccine containing the inactivated SARS-CoV-2 virus. Seroconversion studies of the Sinovac/Butantan vaccine showed results of >92% in participants who took both doses of the vaccine within 14 days and > 97% in participants who took both doses of the vaccine within 28 days.

The efficacy of this vaccine was demonstrated in a regimen containing 2 doses with an interval of 2 weeks. For the prevention of symptomatic cases of COVID-19 that required outpatient or hospital care, efficacy was 77.96%. There were no serious cases in vaccinated subjects, compared to 7 severe cases in the placebo group.

Considering the absence of co-administration studies, covid-19 vaccines are currently not recommended simultaneously with other vaccines. Thus, a minimum interval of 14

days is recommended between COVID-19 vaccines and the different vaccines of the National Vaccination Calendar.

- Covishield® (AstraZeneca® / Fiocruz):

The vaccine developed by the AstraZeneca/University of Oxford laboratory and produced in partnership with Fiocruz is a vaccine containing 5 × 1010 viral particles (pv) of the replication-deficient chimpanzee recombinant adenovirus vector (ChAdOx1), which expresses the Glycoprotein SARS-CoV-2 Spike (S). Produced in human embryonic brain cells (HEK) 293 genetically modified. After vaccination, in participants who were seronegative at the beginning of the study, seroconversion was demonstrated in ≥ 98% of participants at 28 days after the first dose and > 99% at 28 days after the second. For prevention of illness by COVID-19 the vaccine demonstrated efficacy of 73% 22 days after the first dose (in a period of at least 3 months) and with strong indication of increased immune response when the second dose is provided within 3 months, which provides an increase of about 7.5 times in levels of humoral response (antibody production). Exploratory analyses showed that increased immunogenicity was associated with a longer dose interval and efficacy is currently more certainly demonstrated for intervals of 8 to 12 weeks. It is notepoint that there were no severe cases or deaths 21 days or more after vaccination, and 10 hospitalizations for severe COVID-19 were observed in the placebo group, including 1 death.

Allocation and randomization:

The randomization process will be carried out centrally and managed by the team of the Ribeirão Preto School of Medicine of USP (FMRP-USP), under the coordination of Professor Domingos Alves. The process will be performed using the Microsoft Excel random number generation function®. The result will be composed of a randomization table with size appropriate to the N expected for the study (752 participants) and with a ratio of 1:1, which will be integrated to REDCap prior to the beginning of electronic data collection. The creation and validation of the table will be the responsibility of the data manager/project coordinator.

By including a participant in the study, REDCap will consult the existing randomization table and excel® sequentially to allocated it to one of the groups defined in the study. It is noteworthy that REDCap does not, on its own, perform any form of randomization, which will be based exclusively on the said table in Excel®.

Study visits:

Social networks and electronic media will be used to disseminate the study through folders and calls about the research.

Health professionals from other institutions besides those participating in the study may participate in the research. In this case, they will contact the participating centers, through telephone contact or email, informing their interest. All follow-up of these participants will be carried out by the center in which the subject was recruited.

The first evaluation (*Screening* or recruitment) of the study can be initiated in person or through telephone contact, email or electronic media. If the eligibility criteria are met, the individual will be invited to attend the study center for the signing of the Informed Consent and collection of exams that will confirm his or her eligibility or not.

Over four other face-to-face visits [M0 or inclusion, M1 (10 days after the intervention), M3 (90 days after the intervention) and M6 (180 days after the intervention)] the *status* of Coronavirus infection will be monitored by real-time PCR, in the case of symptomatic, and coronavirus serology every 3 months after vaccination with BCG or placebo (chart below). The tests will be performed with the purpose of having the diagnosis of the infection either through the real-time polymerase chain reaction (RT-PCR) technique and/or through the qualitative identification of IgM+IgA and/or IgG antibodies in the detection of infections that occurred asymptomatically and that may have been attenuated by the intervention with the BCG vaccine. For individuals receiving specific immunization against SARS CoV 2, serology will not be used for diagnosis of asymptomatic infection.

At the time of recruitment hiv serology and BHCG dosage will also be collected for *women with status* in order to meet the eligibility criteria. The time between the examinations collected in the recruitment and the effective inclusion in the study should be up to 10 calendar days. If the return occurs between the third and tenth day, and the

participant presents symptoms compatible with COVID-19 upon return, the participant should recollect the RT-PCR.

The gamma interferon release assay (IGRA) will preferably be performed at the inclusion visit or M0 for the purpose of identifying *patients previously infected with Mycobacterium tuberculosis* and detecting gamma interferon (IFN) levels, as well as its role in preventing viral infection, despite knowledge about alpha and beta INFs in immunopathogeny against the virus. In case of non-conformities that make the quality in the performance of this test, in view of being an examination that requires technical details crucial for its performance, the participant will be invited to perform a recollection. In case of participants with previous IGRA reagent and who prove through the exam report will not be submitted to the new collection for this purpose. The other tests will be useful in evaluating the inflammatory response of individuals vaccinated and not vaccinated with BCG and those who became ill or not by COVID-19.

If RT-PCR tests for nasoarcheal swab coronavirus, coronavirus serology and/or HIV serology performed during recruitment, generate inconclusive or indeterminate results, participants will be invited to a new collection and confirmation of the result. Participants will only be included with certain negative or non-reagent results for the three tests, with the exception of cases that have received vaccines against COVID-19, when serology will not be used as a determinant for inclusion or not. In case of participants who present reagent results in hiv serology, they will be referred to the Infectious Diseases service of the study centers for evaluation and follow-up.

In addition to face-to-face visits, participants will receive a telephone contact or a text message by phone via SMS or receive email, the *latter two with a link* for the participant to access and answer the questionnaire about signs and symptoms of COVID as well as sending a photo with the aspect of the injection site. This will occur weekly until M3, and then monthly seeking information on fever and/or respiratory symptoms and the site of vaccine application. The participant will be asked to send a photo of the vaccine application location in each of these contacts. If the participant signals any respiratory manifestation or fever, they will be instructed to seek the Worker Health Divisions of their respective work units or the private or public health services, according to their availability, for the nasoarcheal swab to perform RT-PCR for coronavirus, as well as for medical care directed to the condition. In case of adverse events to the vaccine or

placebo, patients will be evaluated in person at the study centers where they were recruited.

If any participant presents the diagnosis of OVID-19 they will be invited to attend the study for a face-to-face visit once they are asymptomatic and 14 days after the onset of symptoms. In this face-to-face visit, the participant will answer questions from a questionnaire about signs of symptoms of the disease and will be asked to present laboratory or radiological tests performed during COVID-19 infection.

Individuals who show interest in participating in the research should not have been previously vaccinated with any specific vaccine against COVID-19 or if vaccinated, only those immunized with both doses of vaccines, and the second dose should be applied within a minimum of 15 days.

Participants already included and submitted to the intervention, must wait up to 15 days after the intervention with BCG/Placebo to receive specific vaccines against COVID-19 already approved by ANVISA for use by the National Immunization Program, according to the guidelines of the Brazilian Ministry of Health.

In the case of recruited individuals who are eligible for inclusion and intervention, but who give up receiving the investigational product of the study, they should withdraw consent to participate in the ProBCG study.

Participants who present some test results collected during the study outside the normal range will be evaluated by the study physician. It should be indicated in the participant's folder that this result has been verified and whether or not there is clinical significance. If any, the participant should be referred for specialist evaluation at the hospital where his follow-up is being carried out.

Visits	Evaluations	Exams	Procedures
***Screening* or Recruitment**	Face-to-face assessment of eligibility criteria, application of the TCLE	NASOarchyeal swab RT-PCR, anti-SARS-Cov-2 antibody levels, BHCG dosage and HIV serology	Invitation to participate in the study, application of the TCLE, collection of clinical samples (nasopharine swab and blood).
Inclusion or M0 **0 day of preventive treatment (ToT) (BCG or placebo)**	Face-to-face assessment with confirmation of eligibility, clinical inclusion visit, collection of exams and dose of BCG or placebo	IGRA; Complete blood count, lipidogram, albumin, HbGlic, LDH, PCR, ESR, D-D-Dmer; and biomarkers*.	Nursing and medical consultation. Collection of clinical sample (blood). Application of preventive treatment.
S1 - S12 **7-90 days of preventive TTO (±3 days)**	Telephone and/or SMS contact, and/or weekly email and sending photo of the injection site	No exams	Fever questionnaires and/or respiratory signs and symptoms and photo submission of the injection site
M1 **10 days of preventive TTO (±5 days)**	Face-to-face visit to the laboratory for blood collection for biomarkers	Anti-Anti-SARS-Cov-2 antibody levels; Complete blood count, lipidogram, albumin, HbGlic, LDH, PCR, ESR, D-D-Dmer; and biomarkers*.	Collection of clinical sample (blood).
M3 **90 days of preventive TTO (±14 days)**	Face-to-face clinical visit for follow-up and blood collection for serology	Anti-Anti-SARS-Cov-2 antibody levels	Nursing and/or medical consultation. Collection of clinical sample (blood).
M4/M5 **120/150 days of preventive Tom (±14 days)**	Telephone contact and/or SMS, and/or weekly email	No exams	Fever questionnaires and/or respiratory signs and symptoms
M6 **180 days of preventive TTO (±14 days)**	Face-to-face clinical visit to close the study and for blood collection for serology	Anti-Antibody levels anti-SARS-Cov-2.	Nursing consultation. Collection of clinical sample (blood).
Fever and/or respiratory signs and symptoms	Face-to-face visit to the Worker's Health sector of its study center to collect exams	RT-PCR	Clinical sample collection (swab and blood).
	Face-to-face clinical visit to the study center since asymptomatic and 14 – 30 days after the onset of symptoms	Anti-Anti-SARS-Cov-2 antibody levels; Complete blood count, lipidogram, albumin, HbGlic, LDH, PCR, VHS, D-Dallin and biomarkers*.	Nursing or medical consultation. Copy of the tests performed during COVID-19 infection. Collection of clinical sample (blood).

Subtitles: M0: Moment zero; S1-S12: Weeks 1 to 12; M1: Moment 1; M3: Month 3; M4: Month 4, M5: Month 5; M6: Month 6; *Clinical Analyses and Biomarkers: Will be performed only in hucff-idt ufrj, HUPE-PPC UERJ centers.

Storage of specimens during study visits:

The nasopharine swab will be *obtained for the realization of the real time – polymerase chain reation* (RT-PCR) for COVID-19. The collection will be performed using the technique described in the Manual of the Ministry of Health. The collection should be performed with the rubbing of the swab in the posterior region of the nasal caress trying to obtain some of the mucosal cells. The swab will be stored in 15ml tubes containing DMEM + Fetal Bovine Serum 10% + L-Glutamine Solution–Penicillin–Streptomycin.

After collection, the specimen will be packed in a refrigerator of specimens from the research center with temperature control between 2 - 8 °C.

Blood collection will comply with the guidelines of the Ministry of Health in the Manual Techniques for Blood Collection (2001) for the performance of blood count, lipidogram, albumin, LDH, ESR, C-reactive protein, D-dummer and dosage of biomarkers described in the study protocol. The collected material will be distributed in 11 blood tubes [1 SST tube, 2 EDTA tubes – purple cap (4.5ml/each), 1 serum tube - red cap (4 ml/each), 1 gel activator tube - yellow cap (4 ml/each), 2 tubes with soric heparin - green cap (8 ml/each) and 1 Tempus tube – blue cap (3 ml/each). During the *screening or* recruitment visit, blood will be collected for BHCG dosage and HIV serology. In the inclusion visit the material will also be sent for IGRA [4 tubes Kit IGRA (1ml/each)].

After collection, the specimen will be packed in a refrigerator of specimens from the research center with temperature control between 2 - 8 °C.

All specimens will be delivered to their destination laboratories, transported in thermal boxes maintaining the necessary refrigeration and obeying the criteria of storage and maintenance of the viability of the collected material.

Procedure for applying bcg or placebo vaccine:

BCG vaccine:

The application of bcg vaccine as well as its storage and the materials used for application will follow the standards of the Manual of Standards and Procedures for Vaccination of the Ministry of Health (Brazil, 2014). As standardized in this Manual, the

vaccine will be applied intradermally to the lower insertion of the deltoid muscle of the right arm, except contraindications or impossibilities. Other details are described in the standard operating manual (MOP) of the study. After the intervention, participants will be under observation for 20 minutes in the study center waiting room and will be released after the period as long as they do not present adverse events.

Placebo:

A 0.1 ml of 0.9% NaCl saline solution applied intradermally in the lower insertion of the deltoid muscle of the right arm will be used as placebo, except contraindications or impossibilities. Other details are described in the standard operating manual (MOP) of the study. After the intervention, participants will be under observation for 20 minutes in the study center waiting room and will be released after the period as long as they do not present adverse events.

Blinding:

The study participants as well as the researchers, coordinators, nurses, physicians and digitizers of the study will be blind to the allocation in the experimental or control groups.

Only nursing technicians who will apply the vaccine or placebo will be aware of which group the patient will be allocated to. This professional will not follow up on the clinical follow-up or outcome of the study participant.

If the participant has access to the COVID-19 vaccine before 15 days after vaccination with the study product, the "blinding" may be performed at the participant's written request, and if the product applied was placebo, the same may be vaccinated against COVID-19 before the 15 days established by the National Immunization Program. Other details are described in the standard operating manual (MOP) of the study.

Reaction to BCG vaccine and placebo:

The BCG vaccine known to generate a local inflammatory response that lasts about 12 weeks and will be monitored throughout the study. The reactions are described below and will not be considered as adverse events:

- From the first to the second week: reddish macula lasting from 5 mm to 15 mm in diameter.
- From the third to the fourth week: pustule that forms with the softening of the center of the lesion, followed by the appearance of crust.
- From the fourth to the fifth week: ulcer with 4 mm to 10 mm in diameter.
- From the 6th to the 12th week: scar with 4 mm to 7 mm in diameter, found in about 95% of those vaccinated.

Although the response to placebo did not cause any local vaccine reaction, which would question its use, we believe that if we did not use any intervention in the control group, bias could occur, in view of the possible reduction of precautionary measures and care in the use of personal protective equipment (PPE) in the experimental group, believing that they were "protected" by a vaccine, although its effectiveness has not been proven in this situation. On the other hand, those not vaccinated would be more careful in relation to hygiene measures and use of PPE. Using placebo, we believe that we match the usual prevention measures to COVID-19 in the study population at least in the first month of follow-up, a period prior to the onset of the lesions expected by BCG vaccination.

Adverse events:

The definition for an adverse event described in the Manual for Notification of Adverse Events and Safety Monitoring in Clinical Trials (Anvisa, 2016) will be used as a definition for an adverse event: "any adverse medical occurrence in a patient or clinical trial participant to whom a pharmaceutical product has been administered and who does not necessarily have a causal relationship to treatment. As a result, an As can be any unfavorable and unintentional sign, symptom, or disease (including results outside the reference range), associated with the use of a product under investigation, whether related to it or not. A serious AE shall be considered to have one resulting in any adverse experience with medicinal products, biological products or devices occurring at any dose

and resulting in any of the following outcomes: a) death; b) threat to life; (c) persistent or significant disability/disability; d) requires hospitalization or prolongs hospitalization; e) congenital anomaly or birth defect; f) any suspected transmission of infectious agent by means of a drug or; g) clinically significant event."

Adverse events that do not fall within the definition of severe AE (definition above) will be graduated according to ananvisa 2016 manual:

- Light Level: Problem present less than 25% of the time, with an intensity that a person can tolerate and that rarely happened in the last 30 days;
- Moderate Level: A problem that is present less than 50% of the time, with an intensity that interferes with the day-to-day of people and that has occurred occasionally in the last 30 days.
- Sharp Level: Problem that is present in more than 50% of the time, with an intensity that partially alters the day-to-day of the person and that has happened frequently in the last 30 days.
- Complete commitment: It means that a problem that is present in more than 95% of the time, with an intensity that completely alters the day-to-day of the person and that has occurred every day for the last 30 days.
- Unspecified: Means that there is not enough information to specify the intensity.
- Not applicable: It means that it is inappropriate to use a gradation (e.g. menstrual functions).

All EAs will be notified to the local E.R. by the study coordinator at each centre using its own form (Mop Annex 3.3). The EAs should also be reported to the coordination of the proposing institution. The EA notification form as well as proof of sending the notification to the local ZIP Code must be sent by e-mail to the coordinator of the proposing institution no later than 7 days after notification.

In the case of adverse events related to bcg vaccine (ulcer with diameter greater than 1 cm, cold subcutaneous abscess, hot subcutaneous abscess, granuloma, regional lymphadenopathy not suppurated greater than 3 cm, suppurated regional lymphadenopathy, cheloid scar or lupoid reaction), the coordinator of the proposing institution will report to the National Immunization Program of the Ministry of Health.

With regard to placebo, a minimum volume of saline solution applied intradermally will be used. The saline solution itself does not have any immunogenic component and does not cause any adverse events. It is the same solution used for intravenous or oral hydration and for dilution of medicinal products used intravenously. At intradermal application, mild pain may occur and after a few days local redness. Local signs of infection are not expected and the same vaccination standards and protocols established by the National Immunization Program will be used.

Serious adverse events:

Serious adverse events (EAG) should be reported immediately to the coordination of the Proposing Institution through telephone contact (described in mop). From this notification, the EAG must be reported to ANVISA within a maximum of 24 hours by the coordinator of the proposing institution through the agency's electronic portal (Notification Form of Serious Adverse Events in Clinical Trials available on the Electronic Portal of Anvisa > Medicamentos > Clinical Research > Adverse Events > Form for Notification of Serious Adverse Events in Clinical Trials - Notification EC).

All serious adverse events suspected to have been caused by the proposed experimental treatment, including deaths will be reported to CONEP, through notification and within 24 hours.

Participants will receive all care and will be guaranteed assistance.

Both the vaccine and placebo will be applied using disposable materials and patients will have possible adverse events recorded in their own form and notified if they occur (Annex 3.3 of the MOP). In addition, participants will be guaranteed assistance if they present any adverse event.

Adverse events related to Coronavac vaccine®:

Adverse events are classified according to frequency using the following convention: Very common: ≥ 10%; Common: ≥ 1% and <10%; Uncommon: ≥ 0.1% and <1%; Rare: ≥ 0.01% and <0.1%; Very rare: < 0.01% including isolated reports; Unknown: (cannot be estimated from the available data).

Phase I/II clinical studies were conducted in 1,165 adults and elderly aged 18 years or older, and a total of 924 individuals received two doses of basic immunization. Systematic safety follow-up observation was performed within 7 days after each vaccination dose. Adverse events were collected through notifications of participants and regular follow-up of investigators within 8-30 days after each dose. No grade III adverse reactions were found.

The overall incidence of adverse reactions in subjects vaccinated at the target dose was 23.73%, of which 23.24% were mild and 1.94% moderate. The preliminary safety analysis of the phase III clinical study conducted with 5,051 adult (18-59 years) and elderly (over 60 years) clinical study who received the complete regimen with 2 doses of the vaccine showed that the general frequency of occurrence of requested adverse reactions (local and systemic) up to 7 days after administration of the second dose was 50.8% in the group of adults and 36.4% in the elderly group. For unsolicited adverse reactions (local and systemic) up to 7 days after the administration of the second dose, the overall frequency of occurrence was 9.2% in the adult group and 8.1% in the elderly group. The most common reaction observed after the second dose of the vaccine in both groups was pain at the site of administration, which occurred in 40.1% of adults and 27.8% of the elderly. The vast majority of adverse reactions observed were Grade 1/2 and no serious adverse reaction occurred.

- Adverse reactions observed from phase I/II clinical studies in Adults (18-59 years) and the elderly (over 60 years):

- Very common reaction (> 1/10): Location: pain

- Common reaction (> 1/100 and ≤ 1/10): Systemic: fatigue, fever, myalgia, diarrhoea, nausea, headache

- Unusual reaction (> 1/1000 and ≤ 1/100): Systemic: vomiting, lower abdominal pain, bloating, dizziness, cough, loss of appetite, hypersensitivity, high blood pressure; and Site: abnormal staining at the site of administration, swelling, itching, erythema, local hypoesthesia, hardening.

Adverse reactions observed from the phase III clinical study in Adults (18-59 years) up to 7 days after administration of the second dose of the vaccine:

- Very common reaction (> 1/10): Systemic: headache, fatigue; and Location: pain

- Common reaction (> 1/100 and ≤ 1/10): Systemic: nausea, diarrhea, myalgia, chills, loss of appetite, cough, arthralgia, itching, rinorrhoea, nasal congestion; and Location: erythema, swelling, duration, itching

- Unusual reaction (> 1/1000 and ≤ 1/100): Systemic: vomiting, fever, rash, allergic reaction, orohargeal pain, odynophagia, sneezing, astherrries, asthesis, dizziness, abdominal pain, drowsiness, malaise, flushing, pain in the extremities, upper abdominal pain, back pain, vertigo, dyspnea, edema; and Location: hematoma

Adverse reactions observed from the phase III clinical study in the Elderly (over 60 years) up to 7 days after the administration of the second dose of the vaccine:

- Very common reaction (> 1/10): Location: pain

- Common reaction (> 1/100 and ≤ 1/10): Systemic: nausea, diarrhea, headache, fatigue, myalgia, cough, arthralgia, itching, rinorrhoea, odinofagia, nasal congestion; and Location: itching, erythema, local edema, duration

- Unusual reaction (> 1/1000 and ≤ 1/100): Systemic: vomiting, chills, decreased appetite, allergic reaction, asthena, dizziness, ecchymosis, hypothermia, discomfort in the limbs; and Location: hematoma

In addition, there are theoretical risks that vaccinated individuals could develop a more serious disease, but so far there is no report that this has happened to the virus that causes COVID-19. This vaccine was previously tested in animals and they did not present this form of infection more severe.

Adverse events related to Covishield Vaccine®:

The overall safety of the Covishield® vaccine (recombinant) is based on the interim analysis of grouped data from four clinical studies conducted in the UK, Brazil and South Africa. At the time of analysis, 23,745 ≥ 18 years had been randomized and received the vaccine or control. Of these, 12,021 received at least one dose of the recombinant vaccine. Demographic characteristics were generally similar among individuals who received the vaccine and those who received control. Overall, among the individuals who received the recombinant vaccine, 90.3% were between 18 and 64 years old and 9.7% were older than 65 years or more. Most individuals were white (75.5%), 10.1% were black and 3.5% Asian; 55.8% were women and 44.2% were men. The most frequently reported adverse reactions were injection site sensitivity (> 60%); injection site pain, headache, fatigue (> 50%); myalgia, malaise (> 40%); pyrexia, chills (> 30%); and arthralgia, nausea (> 20%). Most adverse reactions were mild to moderate in intensity and usually resolved within a few days after vaccination. Compared to the first dose, adverse reactions reported after the second dose were lighter and less frequently reported.

Adverse reactions were generally lighter and less frequently reported in the elderly (≥ 65 years of age). Analgesic and/or antipyretic medications (e.g., paracetamol-containing products) may be used to provide relief from adverse reactions after vaccination. Adverse drug reactions (ADRs) are organized by MedDRA Organ System Class (SOC). Within each SOC, the preferred terms are organized by decreasing frequency and then by decreasing severity. The frequencies of occurrence of adverse reactions are defined as: very common (≥ 1/10); (≥ 1/100 to < 1/10); unusual (≥ 1/1,000 to < 1/100); rare (≥ 1/10,000 to < 1/1,000); (1< 10,000) and unknown (cannot be estimated with available data).

Risks:

The safety of BCG revaccination among health professionals will be evaluated in order to describe possible injection site reactions and other adverse events.

In addition, we will minimize the occurrence of AS, excluding immunosuppressed individuals or using concomitant medications that impact immune responses.

Local and systemic AEs will be evaluated at relevant times through medical visits, for example in the first 20 minutes after BCG injections and in later visits, either in person or by telephone contact. The EAs will be noted and graduated according to the ANVISA Manual.

Adverse events to the BCG vaccine, more frequent have been abscess and lymphadenopathy, with higher reports for men than for women. The rates of As per 10,000 distributed doses were from 11.6 to 15.4, respectively. Unsuppurated regional lymphadenopathy greater than 3 cm, suppurated regional lymphadenopathy, cheloid scar, or lupoid reaction may occur. The EAs will be monitored and handled throughout the follow-up visits.

The research centers will also be open and available for extra views in case of adverse events. The manifestations will be recorded in the data collection instruments and will be treated and monitored in hospitals linked to the research centers. Similarly, the events will be notified to the National Immunization Program of the Ministry of Health, informing the batch of the vaccine applied as well as the event that occurred. Participants will receive all care and will be guaranteed assistance.

Benefits:

The results of this study may help define a form of prevention of COVID-19 for health professionals and in the future in the population as a whole.

LABORATORY PROCEDURES:

Blood collection:

Conventional examinations will be called those performed by the Clinical Pathology laboratories contracted for the purpose of the study.

These are: blood count, hemosedimentation velocity (ESR), lactate dehydrogenase (LDH), total cholesterol and fractions and triglycerides, D-dummer, C-reactive protein (CRP), albumin, ferritin, iron, glycated hemoglobin. For this purpose, 1 EDTA tube – purple cap and 1 serum tube (red cap) should be delivered for laboratory analysis.

Polymerase chain reaction with real-time reverse transcription:

The presence of SARS-CoV-2 in respiratory samples will be detected by real-time amplification by RT-PCR from the open reading chart SARS-CoV-2 1ab (ORF1ab), nucleocapsid protein (NP) gene fragments using kits provided by IDT USA. The conditions

for amplifications will be 50 °C for 15 minutes, 95 ° C for 3 minutes, followed by 45 cycles of 95 ° C for 15 if 60 ° C for 30 s. When two targets (ORF1ab, NP) test positive by specific real-time RT-PCR, the case will be considered laboratory confirmed. A cycle threshold value (Ct value) less than 37 will be defined as a positive test and a Ct value of 40 or more has been defined as a negative test. An average load, defined as a Ct value of 37 to less than 40, will require confirmation per new test.

Serological test for COVID-19

The Vircell serological test will be® used to define the inclusion or not of health professionals in the study. Vircell reveals® the presence of IgG and IgM/IgA against viral antigens as well as their titration, facilitating the monitoring of absolute antibody values.

Immunoassays

Seros will be obtained from the health professionals enrolled in the study. Two cohorts will be considered: health professionals vaccinated with BCG (n=376) and health professionals not vaccinated with BCG (n=376).

The biomarkers evaluated will be cit-H3, alpha 1 AT, D-dummer, LDH and PCR. The analyzed data will be related to the blood count information, according to the scheme: M0, M1, M3 and M12.

Inflammatory profile and population characterization of neutrophils and T lymphocytes

A panel of surface and intracellular markers more related to exacerbated inflammation will be used, aiming to study the profile of T lymphocytes, monocytes and neutrophils.

Cell profiles will be compared to the occurrence or not of SARS-CoV-2 infection in both cohorts.

These analyses will be performed at the defined times of collection and evaluation of the study groups. The markers to be studied in cell populations will be:

Lymphocyte T: CD3, CD4, CD119, IL-12Rβ2, IFN-γ, TNF-α, IL-4, IL-10, IL-13, IL-21, IL-1RI, IL-6Rα, IL-17A, IL-17F, IL-22, FoxP3, CD25, Galectin-3, IL-35, TGF-β.

Neutrophil: CCL22, CD169, CD68, ZAP70, CD11b, CD11c, LDH, CD64, IL-17.

Monocytes: CD14, CD11c, HLA-DR, CD45, IP-10, MIP-1α, MIF.

Inflammatory and metabolic profiles will be measured by luminex, using serum from patients for the panel: IFN-α2, IFN-γ, IL-10, -12 p40, -12 p70, -13, -15, -1 Ra, -1α, -1β, -2, -4, -6, -8, -23, -17, -18, -22, MIP-1 α, MIP-1β, TNF-α, VEGF-A, Adiponectin, Hepicidine, Insulin, MMP-1, -2, -8, -9, MIF, Glucagon, Greeline, Leptin, CD163s, Galectin-1 and -3, Vitamin D protein, PCR, albumin, citrunilada histona H3, alpha1-anti-trypsin, P-selectin, D-dummer, PSGL-1, tPA, CD40L, PAI-1, Tissue Factor IX, CXCL8 (IL-8).

Transcriptoma

RNA obtained from peripheral blood cells will be used to evaluate the signaling pathways of monocytes and neutrophils in both study groups. RNA sequencing (RNASeq) will be used to evaluate the profiles, according to the manufacturer's instruction (Illumina).

Laboratory procedures to be performed at each research center:

Laboratory procedures will be different according to the research center where the participant is included, as follows:

	Recrutamento	Inclusão ou M0	M1	M3	M6	M9	M12	COVID suspeito ou confirmado	14 – 30 dias após COVID-19 suspeito ou confirmado
HUCFF-IDT UFRJ	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA, análises clínicas, biomarcadores	Sorologia Coronavírus, análises clínicas, biomarcadores	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus, análises clínicas, biomarcadores
HUPE-PPC UERJ	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA, análises clínicas, biomarcadores	Sorologia Coronavírus, análises clínicas, biomarcadores	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus, análises clínicas, biomarcadores
HMFM Barueri SP	RT-PCR Coronavírus swab NF, sorologia Coronavírus, Dosagem BHCG, sorologia anti HIV	IGRA	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	Sorologia Coronavírus	RT-PCR Coronavírus swab NF	Sorologia Coronavírus

DATA COLLECTION AND MONITORING PLAN:

The researchers involved in this protocol will use the forms as source documents (DF). The centers will record the data in the FDs when the data is generated (example: during the participant's visit or during telephone contact). The team will use the REDCap (Electronic Survey Data Capture) system for electronic data capture and data management and its *templates will be called* Case Report Forms (CRF). A copy of the REDCap database will reside on a secure FMRP-USP server that has all physical, technical, and administrative controls. A second copy will be stored on the REDCap server for TB network data. We will use the group of data management researchers from the Faculty of Medicine of Ribeirão Preto-USP. Any professional of the Team of Brazil who is delegated to access the data system, either to access or to do quality control of the data, will have individual access with basic security requirements. The data will be

retrieved from the centers so that no center has access to data from other centers. However, the study's lead investigator will have access to data from all centers. The team responsible for REDCap will manage access to the REDCap system, creating, suspending and exhaling user logins as needed. The centers of Brazil will be responsible for informing the team responsible for data management in advance when a professional is disconnected from the protocol, and then their respective access to the system will be canceled. The list of users who will have access to the protocol REDCap will be reviewed monthly.

As recently, the release of the REDCap software license (https://projectredcap.org/) on behalf of REDE-TB was obtained, it will become part of the REDCap Consortium, a network of all partners that make an instance of the software available. The software was installed in the cloud computing environment of the University of São Paulo. REDCap supports multiple research projects in parallel. Therefore, once installed and configured, it can be used repeatedly for an indefinite period. We will use an intuitive and user-friendly KoBoToolbox (https://www.kobotoolbox.org/) interface as it also allows data collection online and offline, through computers and mobile devices. It can be used together with REDCap. The cloud computing environment of the University of São Paulo, called interNuvem (https://internuvem.usp.br), will be used to create virtual machines to instantiate the technological resources needed to execute the project, such as database servers, REDCap, Web servers, among others. As a way to manage the features of the REDCap and KoboToolbox software suite, the data collection forms, initially defined on paper, will be computerized. Offline data collection can be done through the mobile app (Android, iOS). In this case, the data must be transmitted to the server when internet connectivity is established. The application will be made in the Elixir programming language, which was created on top of erlang, and so it is a functional language. Within the Project there are three main modules are them: Redcap; XLSX Redcap Form Decode and Redcap.Encoder.

The first module is responsible for reading the files used in the translation of the forms provided by KoboToolbox, in XLS format, to Redcap. The main operations involved in this process are migration of the form structure and the collected data. During the first operation, a function is responsible for reading the xls file and uses the XLSX Redcap Form Decode module, to transform each line of the file into an Elixir structure. After

having each row schemated in these structures, it's time to call Redcap.Encoder that will transform this structure received from the previous module and transform into a new data structure by translating each of the rows into its data dictionary equivalents. When all lines are ready the main module uses this structure and "writes" in a data dictionary for Redcap.

In the second operation everything happens in the same Redcap module. In it the data files are read and with the help of a file that I called "data_guide" it reorganizes the data the way Redcap asks for import. It also replaces the headers that are named after the xlsform data for the names that were registered in Redcap. This is done through "data_guide", which is a file that has on one side the column name in xlsform and on the other the name of that information on the Redcap platform. In addition to replacing headers and reorganizing columns, the data also goes through a normalization to be in accordance with what Redcap accepts. For example all "True" and "False" that are put in Ona for multiple selections have to be replaced by 1 and 0 respectively, otherwise Redcap does not accept. After these steps the final structure is written in csv for import.

Specific protocols for electronic quality monitoring and tracking will be performed by a data manager routinely, in order to detect fill errors, inconsistent or missing data, thus ensuring quality control.

An independent study monitor, appointed by the lead researcher, will be responsible for monitoring data quality by standard test operating procedures. Based on the monitoring plan, the field visit and audit will be carried out at different stages. All records of participants, DFs and other source documents of the patients recruited in this study will be made available for analysis by the monitors. A meeting of researchers from each site will take place monthly, via the web-based remote conferencing system, to share the progress of the study and discuss c problems encountered while conducting the test. During the conduct of the research, any critical modifications in the protocol will be informed to the principal investigator, to the study record and, if relevant, to the study participants.

Sample storage plan:

Nasophayngeal swab and peripheral blood specimens collected will be transported to specific laboratories. Specimens may be stored to facilitate the repetition of the test in case of indeterminate results or discordant cases in molecular laboratory tests or not for SARS-CoV-2. Stored clinical specimens will be labeled with a study identifier and not with personal identifiers and will be used only for the purposes of this study.

According to CNS Resolution No. 441 of 2011 (Art. 1) and Ordinance MS No. 2,201 of 2011 (Art. 3), any organized collection of human biological material collected for scientific research purposes, including samples intended for routine examinations in a clinical trial (e.g., blood count and renal function) are considered as constituents of a biorepository or biobank. The biological material will be used as provided for in the research protocol, with no further future analyses different from those foreseen. If there is a proposal for future use of the stored samples, approval in the CEP/CONEP system will be required and a new protocol and TCLE will be submitted so that each participant can choose whether or not to allow the use of the samples for future studies.

Statistical Analysis Plan:

The primary efficacy analysis will compare the cumulative incidence of SARS-CoV-2 infection and the cumulative incidence of severe forms of COVID-19.

These analyses will occur when the last enrolled research subject has completed 30 days and 6 months of preventive treatment and will be performed using the results of COVID-19 diagnostic tests, as well as the data available in participating hospitals and national electronic notification systems of COVID-19. A modified treatment intention analysis (MITT) and protocol (PP) will be performed. The safety and tolerability analysis will consist of all patients who have been randomized and received a dose of the vaccine or placebo. Based on serum immune response concentrations, we will calculate certain immunoinflammatory variables related to the use of BCG versus placebo among subjects infected and not infected by COVID-19 among the categories with COVID-19 vaccine (Coronavac® or Covishield®) and without covid-19 vaccine.

The intervention and control groups will be compared at the time of inclusion in the study in relation to sociodemographic, clinical, radiological and biochemical imaging variables, in order to evaluate the adequacy of the randomization process. Multivariate

analyses using logistic regression or Poisson models with robust variance will be used to evaluate the effect of BCG vaccine on primary and secondary outcomes. Associations will be expressed in the form of risk ratios and respective 95% confidence intervals. Data imputation may be required and the *random forest system will* be used. Statistical software R, version 10.0.

Sample size:

It is estimated that it will take 376 individuals in each of the comparison groups to ensure a statistical power of 80% and a type I error of 5%, considering an incidence of SARS-CoV-2 infection of 20% in BCG vaccinated, 30% in unvaccinated, 15% in covid-19 vaccinated. It is expected a reduction in the joint effect of BCG with the COVID-19 vaccine between 10% and 15% and that the percentage of people vaccinated with BCG in the cohort is 50% and the percentage of people vaccinated for Covid-19 in the cohort is 80%.

We will also use for the two outcomes (below) the sequential design the groups are followed up until there is a group with clear benefits or that both groups do not present difference. This design is used when the results need to be known very quickly. If there is a large difference between the treatments, then this study will be performed in a shorter time when compared to the parallel trial. The main ethical advantage is that if one treatment shows a superiority over the other, the study can already be stopped. However, the interim analyses will be previously planned, in such a way that the calculation of sample size allows the multiplicity of the tests. The meaning of p-values in sequential analyses also changes, because when using sequential analyses, more than one analysis is performed, and the typical definition of a p-value as data "at least as extreme" as observed needs to be reset. One solution is to sort the p-values of a series of sequential tests based on the downtime and at what time the test statistic was in a certain appearance, known as ordering.

OUTCOMES:

Primary Outcome

1. Compare the cumulative incidence of SARS-CoV-2 infection

It is estimated that it will take 376 individuals in each of the comparison groups to ensure a statistical power of 80% for the detection of a difference in effects of 10%, considering that the control group will have an accumulated incidence of SARS-CoV-2 infection of 30% after 6 months of follow-up, for a type I error of 5%.

2. Compare the cumulative incidence of severe forms of COVID-19

We will also use for the two outcomes the sequential design the groups are followed up until there is a group with clear benefits or that both groups do not present difference. This design is used when the results need to be known very quickly. If there is a large difference between the treatments, then this study will be performed in a shorter time when compared to the parallel trial. The main ethical advantage is that if one treatment shows a superiority over the other, the study can already be stopped. However, the

interim analyses will be previously planned, in such a way that the calculation of sample size allows the multiplicity of the tests. The meaning of p-values in sequential analyses also changes, because when using sequential analyses, more than one analysis is performed, and the typical definition of a p-value as data "at least as extreme" as observed needs to be reset. One solution is to sort the p-values of a series of sequential tests based on the downtime and at what time the test statistic was in a certain appearance, known as ordering.

3. Assess the BCG vaccine-mediated immune response in healthcare professionals

SARS-CoV2 infection is estimated to occur in approximately 20% of health professionals vaccinated by BCG (experimental group), in 30% of health professionals not vaccinated by BCG (placebo group) and not vaccinated for COVID-19 and in 15% in COVID-19 vaccinated without BCG vaccine. A reduction of 10% to 15% of the joint effect of bcg vaccine with Covid-19 vaccine is expected.

In this scenario, with the bearing of 376 health professionals in each arm, if we assume that the percentage of covid-19 vaccinated is 80% and equal in each arm, SARS-CoV2 infection is expected to be detected in 15 professionals vaccinated only with BCG, in 23 professionals not vaccinated with BCG and COVID-19 vaccine, 45 professionals vaccinated for COVID-19 without bcg vaccine and 38 professionals vaccinated for COVID-19 and BCG. For each subgroup (vaccinated and not vaccinated with BCG) it will be possible to identify the predictive biomarkers of infection by comparing infected professionals with those who were not infected with SARS-CoV2.

INTERIM ANALYSIS PLAN:

Regarding the effect of BCG vaccine administration on the cumulative incidence of SARS-CoV-2 infection or on severe forms of COVID-19, a single intermediate analysis will be performed when the number of volunteers included has reached half of the total sample scheduled to verify whether the evidence would already be conclusive and would allow the study to be interrupted. For type I error protection, intermediate analysis will use a Z value of 2.78 (Chow & Liu, 2004).

The analyses that will be performed in the intermediate analysis will be restricted to the following outcomes: accumulated incidence of SARS-CoV-2 infection, cumulative incidence of severe forms of COVID-19 and proportion of serious adverse events.

The following rules will be used to indicate early completion of the study after intermediate analysis:

• Significant difference in the effect of BCG judged by comparing the proportions of patients who develop SARS-CoV-2 infection or severe forms of COVID-19 in each arm of the study;

• If the effect of BCG is similar between groups and there is a significant difference in the cumulative proportion of serious adverse events;

The results will be evaluated by the Data and Safety Monitoring Committee, which will express the need to continue the study, based on the vaccine's performance in terms of the incidences of SARS-CoV-2 infection or severe forms of COVID-19 and adverse events.

Independent Data Monitoring and Security Committee:

The Data and Safety Monitoring Committee (CMDS) will be composed of three members not linked to participating institutions, in accordance with the Operational Guidelines for the Establishment and Functioning of Data Monitoring and Safety Committees of the Ministry of Health (2008).

This committee will be responsible for reviewing the protocol and progress of the study in all centers, with real-time meetings by videoconference, as detailed below, and will consist of:

Denise Rossato - Physician - Professor, Faculty of Medicine, Federal University of Rio Grande do Sul

Ronir Raggio - Statistician - Institute of Collective Health Studies of the Federal University of Rio de Janeiro

Silvana Spíndola - Physician - Professor, Faculty of Medicine, Federal University of Minas Gerais

An early safety assessment will be carried out when the first 100 participants (approximately 50 per arm) are included. Security data will be reviewed by a CMDS member or someone designated by a CMDS member. In addition, the Committee will review the progress of the study after the inclusion of every 100 additional participants through real-time meetings by videoconference.

CMDS activity plan:

Time of study	**Activity**	**Form**

Before the start of the study	Protocol and MOP review	Real-time video conferencing scheduling
During the study	Early safety assessment after inclusion of the first 100 participants	Real-time video conferencing scheduling
	Evaluation of study progression per 100 participants included	Real-time video conferencing scheduling
	Adverse event analysis	Real-time video conferencing scheduling every 2 weeks
	Analysis of serious adverse events	Extraordinary meeting via real-time videoconferencing
	Intermediate analysis after the inclusion of 500 participants	Real-time video conferencing scheduling
At the close of the study	Evaluation of the conduct of the study and its results	Real-time video conferencing scheduling

Plan for monitoring and analysis of adverse events:

The definition for an adverse event described in the Manual for Notification of Adverse Events and Safety Monitoring in Clinical Trials (Anvisa, 2016) will be used as a definition for an adverse event: "any adverse medical occurrence in a patient or clinical trial participant to whom a pharmaceutical product has been administered and who does not necessarily have a causal relationship to treatment. As a result, an As can be any unfavorable and unintentional sign, symptom, or disease (including results outside the reference range), associated with the use of a product under investigation, whether related to it or not. A serious AE shall be considered to have one resulting in any adverse experience with medicinal products, biological products or devices occurring at any dose and resulting in any of the following outcomes: a) death; b) threat to life; (c) persistent or significant disability/disability; d) requires hospitalization or prolongs hospitalization; e) congenital anomaly or birth defect; f) any suspected transmission of infectious agent by means of a drug or; g) clinically significant event."

All EAs will be notified to the local EC by the study coordinator at each centre using their own form. EAs shall also be reported to the coordination of the proposing institution no later than 7 days after notification of the EA.

In the case of adverse events related to bcg vaccine (ulcer with diameter greater than 1 cm, cold subcutaneous abscess, hot subcutaneous abscess, granuloma, regional lymphadenopathy not suppurated greater than 3 cm, suppurated regional lymphadenopathy, cheloid scar or lupoid reaction), the coordination of the proposing institution should be notified to the National Immunization Program of the Ministry of Health.

Serious adverse events (EAG) should be reported immediately to the coordination of the Proposing Institution through telephone contact. From this notification, the EAG must be reported to ANVISA within a maximum of 24 hours by the coordinator of the proposing institution through the agency's electronic portal (Notification Form of Serious Adverse Events in Clinical Trials available on the Electronic Portal of Anvisa > Medicamentos > Clinical Research > Adverse Events > Form for Notification of Serious Adverse Events in Clinical Trials - Notification EC).
All serious adverse events suspected to have been caused by the proposed experimental treatment, including deaths will be reported to CONEP, through notification and within 24 hours.
There will be a review of The EA data every 2 weeks. If adverse events are unexpected or not previously described, case review will be requested through a meeting of the data monitoring and security committee, which should take place in real time by videoconference. Serious adverse events will be immediately flagged to the CMDS for extraordinary analysis also by real-time videoconferencing.

Criteria for discontinuation of the study for safety reasons:

No formal rule will be adopted to interrupt the study for safety reasons. The CMDS may recommend stopping the study, based on the review of interval safety results. The study can only be canceled or discontinued after analysis of the reasons for discontinuity by the institution's ZIP Code or CONEP.

Criteria for discontinuation of experimental treatment in the participants:

No formal rule will be adopted to discontinuation of experimental treatment in the study participants. The CMDS may recommend this study interruption, based on the review of interval results as described above. Nevertheless, this decision can only be effected after evaluation and approval by the institution's ZIP Code or CONEP.

QUALITY MANAGEMENT:

All studies will be conducted in accordance with the ICH-GCP Guidelines (ICH-GCP E6 R2), as far as possible in the research environment, as well as all national legal and regulatory requirements (as applicable).

BUDGET:

Cost	**Value**
Legal entity	R$84,000.00
Bags	R$160,800.00
Inputs	R$297,188.57
Other	R$43,571.43
Capital	R$14.440,00
Total	R$600,000.00

SCHEDULE:

The schedule presented below is a schedule, and the study should be started only after approval of the CEP.

Phase	**Beginning**	**End**
Submission of amendment to CEP/CONEP	01/08/2021	31/08/2021
Recruitment/Inclusion/Intervention	30/10/2020	31/12/2021
Follow-up of patients	30/10/2020	31/06/2022
Data analysis	31/06/2022	31/09/2022
Reporting/Results	31/09/2022	31/12/2022

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