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Impact of vaccine platform and BCG vaccination on antibody responses to COVID-19 vaccination

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Multiple factors, including vaccine platform and prior vaccinations, influence vaccine responses. We compared antibody responses to CoronaVac (Sinovac) and ChAdOx1-S (AstraZeneca-Oxford) vaccination in 874 healthcare workers in Brazil. As participants were randomised to BCG vaccination or placebo in the preceding 0–6 months as part of the BCG vaccination to reduce the impact of COVID-19 in healthcare workers (BRACE) trial, we also investigated the influence of recent BCG vaccination on antibody responses to these COVID-19 vaccines. Twenty-eight days after the second dose of each vaccine, ChAdOx1-S induced a stronger anti-spike IgG response than CoronaVac vaccination. Recent BCG vaccination did not impact IgG antibody responses to ChAdOx1-S or CoronaVac.

KEYWORDS

vaccine, COVID-19, antibody, ChAdOx1-S, CoronaVac, BCG

1 Introduction

SARS-CoV-2, the causative pathogen of COVID-19, spreads through the respiratory tract and contact, posing a significant challenge to the healthcare system due to the potential transmission *via* aerosol and droplets (1). The risk of infection is particularly high for healthcare workers, particularly those in urgent care settings, where infection control measures and personal protective equipment may be insufficient (2).

To combat the COVID-19 pandemic, vaccines based on different platforms were developed in parallel. Initially, these included replication deficient adenovirus, mRNA and whole-inactivated virus-based vaccines, with protein sub-unit vaccines being available later in the pandemic. Prior to the COVID-19 pandemic, mRNA and adenovirus-based vaccines had limited use in human and animal models and therefore differences in immune responses to these, and vaccines based on more established platforms (e.g. whole-inactivated, protein subunit) are under investigation.

Due to global demand and limited supply, access to vaccines was limited. Given the increased SARS-CoV-2 exposure in healthcare settings, healthcare workers (along with the elderly) were prioritized for access to COVID-19 vaccinations. In Brazil, the predominant COVID-19 vaccines used for primary vaccination have been ChAdOx1-S (AstraZeneca-Oxford, AZD1222), BNT162b2 (Pfizer-BioNTech), and CoronaVac (Sinovac). ChAdOx1-S, a replication deficient adenovirus-based vaccine, and BNT162b2, an mRNA-based vaccine, induce both humoral and T cell responses to the SARS-CoV-2 spike protein and receptor binding domain (RBD) (3–5). In contrast, CoronaVac, which contains a whole inactivated virus (CN2 strain), primarily provokes a humoral response (6, 7). The primary series schedule for these COVID-19 vaccines is two doses (1, 8), with different intervals depending on the vaccine (9). All three vaccines protect against COVID-19, however reported efficacy is variable with 91–95% reported for BNT162b2 (10, 11), 70–81% for ChAdOx1-S (6, 10, 12) and 50–67% for CoronaVac (6, 10, 11).

Several factors influence the magnitude and persistence of immune responses to COVID-19 vaccines including prior SARS-CoV-2 infection, age and sex (13–16). The immunomodulatory effects of the Bacillus Calmette-Guérin (BCG) vaccine include altering *in vitro* immune responses to SARS-CoV-2 and antibody response to unrelated vaccines, therefore BCG vaccination may also influence immune responses to COVID-19 vaccines (2, 17–23). This study aimed to compare ChAdOx1-S (AstraZeneca-Oxford) and CoronaVac (Sinovac) antibody responses and evaluate the impact of BCG vaccination on the vaccine responses.

2 Methods

2.1 Participants and sample collection

Participants were recruited as part of the ‘BRACE COVID-19-specific’ (BCOS) sub-study of the *BCG vaccination to reduce the impact of COVID-19 in healthcare workers* (BRACE) trial (24)

(human research ethics committee approvals: Royal Children’s Hospital (RCH) approval no. 62586;

Brazilian National Commission of Ethics in Research (CONEP): approval no. 4.572.593). BRACE trial participants in Brazil (recruited from BRACE trial sites in Mato Grosso do Sul, Rio de Janeiro and Amazonas) were eligible for inclusion if: (i) they consented to be contacted for future ethically approved projects; (ii) had a post-vaccination serum sample taken 28 (\pm 2 days) after the first or second dose of a COVID-19 vaccine.

2.2 Vaccination and randomisation

Participants received COVID-19 vaccinations through the Brazilian healthcare system and as per guidelines from the Brazilian Ministry of Health, no treatment was provided for symptoms resulting from COVID-19 vaccination.

Prior to consent to this sub-study and COVID-19 vaccination, participants had been randomised at a 1:1 ratio to receive a 0.1 ml intradermal injection with BCG-Denmark (AJ Vaccines – Batch: 1198019D/119053A) or placebo (0.9% saline solution) as part of the BRACE trial. Eighteen participants consented to this sub-study received BNT162b2, and due to this low number were excluded from statistical analyses.

2.3 Sample collection

Serum samples were collected between 19th March 2021 and 10th September 2021. Peripheral blood was collected in serum tubes (BD Vacutainer SS II Advanced, United Kingdom, Cat #456010) at study visits 28 (\pm 2) days following the first (ChAdOx1-S, BNT162b2) or second (ChAdOx1-S, BNT162b2, CoronaVac) COVID-19 vaccine dose. The recommended 21-day window between the first and second dose of CoronaVac precluded assessment of antibody responses 28 days after the first dose of CoronaVac. Peripheral blood collected in serum tubes was centrifuged and serum samples were stored at -80°C prior to testing.

For plasma samples, peripheral blood was collected into lithium heparin tubes (Greiner BioOne – Austria, Cat #455084), at baseline, 3 months or 6-months after randomisation as part of the BRACE trial. Peripheral blood was centrifuged, and plasma samples were stored at -80°C . The participant’s most recent plasma sample available prior to COVID-19 vaccination was used in this study.

2.4 Antibody measurement

Plasma and serum samples were tested for antibodies against the spike receptor-binding domain of SARS-CoV-2 at Unidade de Apoio ao Diagnóstico do COVID-19 (UNADIG-RJ) using the SARS-CoV-2 IgG (Abbott, United States of America) chemiluminescent microparticle immunoassays (CMIA). Seroconversion was defined as a positive anti-SARS-CoV-2 spike antibody result in the post-vaccination sample following a negative anti-SARS-CoV-2 spike antibody result in the pre-vaccination sample. A positive anti-SARS-

CoV-2 spike antibody result was defined as per the manufacturer's defined cut-off. Researchers involved in sample processing, selection and testing were blinded to the participants randomisation group and were unaware of which COVID-19 vaccine they had received.

2.5 Interferon gamma release assay

Peripheral blood collected on the day of randomisation in the BRACE trial was used for IGRA to identify *Mycobacterium tuberculosis* infection. The QuantiFERON-TB Gold Plus (QFT-Plus) assay (Qiagen, Hilden, Germany) was done as per manufacturers instructions. Results of IGRA testing were assessed according to the manufacturer's criteria, using Qiagen software version 2.71.2.

2.6 Statistical analysis

For seroconversion analysis, 850 (99.3%) participants had a pre-vaccination plasma sample available for analysis. Differences in proportion were determined using the z-test for independent proportions.

For assessment of antibody concentrations, values below the lower limit of detection/extrapolation were assigned a value of half of the lowest detected/extrapolated value, values above the upper limit of detection/extrapolation were assigned a value of 1.5 times the highest detected/extrapolated value. Differences between COVID-19 vaccines were assessed using linear regression of log-transformed antibody data adjusted for region (Mato Grosso do Sul/Rio de Janeiro/Amazonas), age (<40 years; 40 to 59 years; ≥60 years), sex (male/female), presence of cardiovascular disease, diabetes, chronic respiratory disease, workplace COVID-19 direct contact at baseline (yes/no), SARS-CoV-2 PCR result at baseline (detected/not detected/not performed). Differences between randomisation groups were assessed using linear regression of log-transformed antibody data. Sensitivity analysis for differences between BCG- and placebo-vaccinated participants was done adjusting for time between most recent COVID-19 vaccination and blood collection (continuous), age (<40 years; 40 to 59 years; ≥60 years), sex (male/female) and region (Mato Grosso do Sul; Rio de Janeiro; Amazonas). Data analysis was done using Stata version 17.0 (StataCorp LLC, USA)

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files.

3 Results

Of participants who consented to this sub-study, 874 completed COVID-19 vaccination courses before 9th August 2021 and provided post-vaccination blood samples (Figure 1). Blood samples were taken a median of 28 (IQR 27-29) days after the first (ChAdOx1-S, BNT162b2) and 28 (IQR 27-30) days after the second COVID-19 (ChAdOx1-S, BNT162b2 or CoronaVac) vaccination. The

demographics of participants are detailed in [Supplementary Tables 1, 2](#). The mean time between randomisation in the BRACE trial and the first and second doses of SARS-CoV-2 vaccines was 80 (standard deviation, SD, 25) days and 145 (SD 26) days, respectively ([Supplementary Table 2](#)). Only 4% of individuals in the BCG arm and 2% in the placebo arm had another vaccination between randomisation and the first dose of a COVID-19 vaccine. For both randomisation arms, 18% of participants received another vaccination between randomisation and the second dose of a COVID-19 vaccine. SARS-CoV-2 infection at the time of randomisation (as determined by respiratory swab SARS-CoV-2 PCR test) and *M. tuberculosis* infection (determined by IGRA), was similar between randomisation arms and between ChAdOx1-S and CoronaVac recipients ([Supplementary Tables 1, 2](#)).

3.1 Level of anti-spike IgG between COVID-19 vaccines

The concentration of anti-spike IgG antibodies induced by a single dose of ChAdOx1-S was similar to that induced by two doses of CoronaVac (adjusted geometric mean ratio (aGRM) 0.99, 95% CI 0.76-1.30) ([Table 1](#)). Anti-spike IgG antibody levels were more than two times greater following two doses of ChAdOx1-S than two doses of CoronaVac (aGMR 2.58, 95% CI 2.19-3.03).

3.2 Impact of recent BCG vaccination on level of anti-spike IgG

Prior to receiving COVID-19 vaccinations, participants were randomised to receive vaccination with BCG-Denmark (n=435) or saline placebo (n=439) as part of the BRACE trial ([24](#)). Amongst ChAdOx1-S and CoronaVac recipients, there was no difference in the anti-spike IgG seroconversion rate or geometric mean anti-spike IgG responses between BCG-vaccinated and placebo-vaccinated participants ([Table 2](#); [Supplementary Table 3, 4](#)). Adjustment for time between the most recent COVID-19 vaccination and post-vaccination blood collection, age, sex, and region did not impact these findings ([Supplementary Table 4](#)).

4 Discussion

4.1 Effect of COVID-19 vaccine platform

Our finding that ChAdOx1-S induced higher anti-spike IgG levels compared to CoronaVac may be explained by the fact that this vaccine is an attenuated virus vector, which activates a broader range of T cells and induces a stronger cytokine production ([3](#)), ultimately associated with a higher efficacy ([13](#)).

COVID-19 vaccines are a vital tool in protecting against the disease and particularly severe forms. Randomised control trials (RCTs) of ChAdOx1-S have reported an overall protective efficacy of 81% ([25](#)) against symptomatic COVID-19, with lower protection associated with COVID-19 caused by the variant of concerns ([26, 27](#)) Previous studies

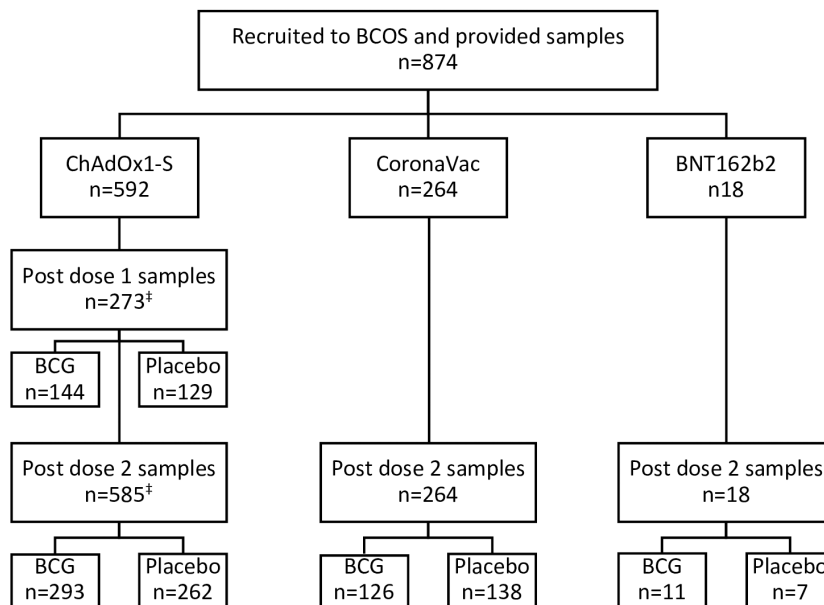


FIGURE 1 Participant flow chart. Participants in the BRACE COVID-19-Specific vaccine (BCOS) sub-study were a subset of participants from the BRACE trial. BRACE trial participants in Brazil who received (or were due to receive) two doses of COVID-19-specific vaccines prior to August 9th 2021 and provided at least 1 BCOS-post-vaccination serum sample were eligible for inclusion. Participants were randomised to BCG or placebo vaccination in the BRACE trial a mean of 80 (standard deviation 41 days) prior to their first COVID-19 vaccination. †266 participants were included for dose 1 and dose 2 of ChAdOx1-S.

have shown that levels of neutralizing antibodies correlate with vaccine efficacy (28).

Phase III RCTs of CoronaVac have reported protective efficacy varying between 51% in Brazil (29) and 84% in Turkey (30). More recently, a national cohort study on over 10 million persons in Chile reported an efficacy of 65.9% (31). The difference we observed in the anti-spike IgG responses is consistent with the observed higher protective efficacy of ChAdOx1-S against symptomatic COVID-19 than CoronaVac, and the observed magnitude is consistent with reported differences in neutralizing antibodies between these vaccines (32).

Used in an emergency, CoronaVac was approved for vaccination in healthcare workers due to direct contact with COVID-19 patients,

leading to higher SARS-CoV-2 exposure than the general population. In a recent cohort study of healthcare workers in Brazil, seropositivity for anti-spike IgG was 99.8% 30 days after the second dose of CoronaVac, and decreased to 97.9% six months after the second doses was 97.0-100% (33). Antibody positivity as well as antibody titre were higher in females compared to males, with this sex effect maintained across a range of ages.

Prior SARS-CoV-2 infection is associated with stronger responses to COVID-19 vaccines (14, 15, 34). In our study, workplace and household exposure, as well as study region were evenly distributed between the BCG and placebo groups but were unbalanced between participants with samples 28 days after the second dose of ChAdOx1-S and CoronaVac. Due to differences in

TABLE 1 Comparison of anti-spike IgG antibody concentration following ChAdOx1-S or CoronaVac vaccination among healthcare workers in Brazil.

	ChAdOx1-S (n=592)	CoronaVac (n=264)	aGMR* (95% CI)	P-value*
	1 st dose	2 nd dose		
No. of available samples	273	264	0.99 (0.76, 1.30)	0.97
Geometric mean (95% CI)	970.4 (789.6, 1192.6)	972.3 (869.4, 1087.4)		
	2 nd dose	2 nd dose		
No. of available samples	585	264	2.58 (2.19, 3.03)	<0.001
Geometric mean (95% CI)	2439.3 (2225.4, 2673.9)	972.30 (869.4, 1087.4)		

*Adjusted for region, age, sex, presence of cardiovascular disease, diabetes, chronic respiratory disease, workplace COVID-19 direct contact at baseline, SARS-CoV-2 PCR result at baseline 95% CI, 95% confidence interval; aGMR, adjusted geometric mean ratio; Ig, immunoglobulin; No., number

TABLE 2 Comparison of anti-spike IgG antibody concentration following ChAdOx1-S or CoronaVac vaccination among healthcare workers in Brazil by BCG randomisation group.

	Placebo (N=439)	BCG (N=435)	GMR (95% CI)	P-value
1st dose of ChAdOx1-S				
No. of available samples	129	144	1.01 (0.67, 1.52)	0.97
Geometric mean (95% CI)	965.8 (718.9, 1297.4)	974.5 (730.1, 1300.8)		
2nd dose of ChAdOx1-S				
No. of available samples	292	293	0.92 (0.77, 1.11)	0.38
Geometric mean (95% CI)	2540.8 (2254.3, 2863.8)	2342.2 (2037.7, 2692.2)		
2nd dose of CoronaVac				
No. of available samples	138	126	1.03 (0.83, 1.30)	0.76
Geometric mean (95% CI)	956.5 (819.7, 1116.2)	989.9 (841.1, 1165.1)		

95% CI, 95% confidence interval; GMR, geometric mean ratio; Ig, immunoglobulin; No., number.

SARS-CoV-2 prevalence across the regions over time, the risk of SARS-CoV-2 infections among participants will have varied between regions (35, 36). Higher workplace patient- and household-COVID-19 contact for participants who received CoronaVac, might also have influenced our findings.

4.2 Effect of recent BCG vaccination

Due to its beneficial off-target effects in protecting against unrelated infections (20, 37–39), BCG vaccination has been investigated for its potential to protect against COVID-19. Ecological studies have reported variable effects of BCG with some studies showing that countries with BCG vaccination programs had fewer COVID-19 cases, while others found effect of BCG vaccination (19). RCTs of BCG vaccination to protect against COVID-19 are ongoing, however thus far results have also been variable (40). One such study, the Dutch BCG-Corona study, found that BCG-Denmark vaccination did not reduce COVID-19 incidence, duration or severity in healthcare workers (2). This study did however report a transient effect of BCG vaccination on antibody responses to SARS-CoV-2. In the first three months after randomisation, participants in the BCG-vaccinated group had higher rates of seroconversion and anti-spike antibody titres compared to the placebo group, although this effect was not maintained at later time points (2). It remains unclear whether this was a direct effect of BCG vaccination on humoral responses to SARS-CoV-2 or an indirect effect such as being the result of differences in SARS-CoV-2 clearance and viral load.

Our findings, that recent BCG vaccination did not alter IgG responses to CoronaVac or ChAdOx1-S, are consistent with a study that reported the lack of an increase in B cell responses to SARS-CoV-2

in vitro in BCG-vaccinated participants compared to placebo controls (22). Similarly, a Polish phase III RCT also found that mycobacterial exposure (positive tuberculin skin test or those BCG-Moreau vaccination) did not affect anti-spike IgG responses to BNT162b2 compared to the placebo (41). BCG-induced trained immunity occurs rapidly and persists for at least 1 year following neonatal BCG vaccination (42–45), therefore it is unlikely that the lack of an observed effect of BCG is due to waning of its immunomodulatory effects in the months between randomisation and COVID-19 vaccinations. Although BCG vaccination did not impact antibody responses in this study, prior BCG vaccination may alter innate or T cell responses to COVID-19 vaccines and may be a viable adjuvant for novel COVID-19 vaccines (20, 37, 46, 47).

Prior mycobacterial exposure among placebo-vaccinated participants may have reduced the observed effects of recent BCG vaccination in our study. In animal models, exposure to environmental mycobacteria, such as *Mycobacterium avium*, can reduce BCG-induced protective immunity against TB (48, 49). However, these effects have been shown to be variable, depending on factors such as mycobacterium species and route of exposure (50). In human trials, stringent tuberculin testing and younger age at BCG vaccination, both of which reduce the likelihood of prior *M. tuberculosis* and environmental mycobacterial exposure, are also associated with greater BCG efficacy (51). The effects of environmental mycobacterial exposure on the off-target effects of BCG vaccination are unknown.

Being based in a high-tuberculosis (TB) prevalence setting, most study participants had received prior BCG vaccination, over 95% of which was more than 5 years prior, likely as part of routine infant BCG vaccination programmes. Infants whose mothers had also been BCG-vaccinated in the past have been reported to have stronger clinical and immunological off-target effects of BCG vaccination (44, 52, 53). BCG revaccination later in life may

provide limited additional effect (54–56). Therefore, the lack of an observed boosting effect of recent BCG vaccination may be attributable, at least in part, to prior mycobacterial exposure.

4.3 Limitations

The study's limitations include that participants were not randomised to the different COVID-19 vaccines, and there were some differences in baseline demographics between the groups. However, factors previously associated with antibody responses to COVID-19 vaccines, such as sex, age and comorbidities (13–16), were evenly distributed between the groups and we tried to account for the impact of any differences in demographics in the analysis. As the study was done in a high COVID-19 prevalence setting and pre-vaccination samples were taken up to six months before the post-COVID-19 vaccination samples, seroconversion and vaccine-induced antibody titres may have been overestimated due to COVID-19 infections between pre and post-vaccination sample collections. This may be due to measurement of SARS-CoV-2-induced antibody responses directly, as well as the increased antibody responses to COVID-19 vaccine among previously SARS-CoV-2-infected participants (14, 15, 34). Other factors that can influence antibody responses to COVID-19 vaccines, including participant health, self-administration of antipyretics, immune-stimulants and immune suppressants by participants was not recorded and therefore their potential impact on the effects of BCG or COVID-19 vaccine-induced antibody responses could not be determined (57, 58). As a sub-study of an RCT in healthcare workers, the participants included were predominantly females under 60 years of age. As sex and age are factors that influence vaccine responses (13–16), it is plausible that antibody titres in response to ChAdOx1-S and CoronaVac, and the effects of BCG may be different among different subgroups.

4.4 Conclusions

In conclusion, in healthcare workers in Brazil, vaccination with the adenovirus-based ChAdOx1-S vaccine induced greater anti-spike IgG responses than whole-inactivated virus CoronaVac vaccine, and recent BCG vaccination had no effect on anti-spike antibody vaccine responses.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Royal Children's Hospital (RCH) (no. 62586) and Brazilian National Research Ethics Committee (no. CAAE 34101620.5.1001.0021). The patients/participants provided their written informed consent to participate in this study.

Author contributions

NM, NC, and JC conceived and designed the project. NM and NC acquired funding. NM, JC, MP, MD, TJ, PS, RO, MG, and BJ executed the study. CM, NM, NC, and LP developed the statistical analysis plan. CM completed the statistical analysis. NM, NC, CM, JC, MP contributed to interpretation of the data. MS, NM, and NC contributed to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2023.1172851/full#supplementary-material>

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