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Immunogenicity and reactogenicity of an inactivated SARS-CoV-2 vaccine (BBV152) in children aged 2–18 years: interim data from an open-label, non-randomised, age de-escalation phase 2/3 study

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Summary

Background Despite having milder symptoms than adults, children are still susceptible to and can transmit SARS-CoV-2. Vaccination across all age groups is therefore necessary to curtail the pandemic. Among the available COVID-19 vaccine platforms, an inactivated vaccine platform has the advantage of excellent safety profile across all age groups; hence, we conducted an age de-escalation study to assess the safety, reactogenicity, and immunogenicity of an inactivated COVID-19 vaccine, BBV152 (COVAXIN; Bharat Biotech International, Hyderabad, India), in children aged 2–18 years.

Methods In this phase 2/3 open-label, non-randomised, multicentre study done in six hospitals in India, healthy children (male or female) aged 2–18 years were eligible for inclusion into the study. Children who had positive SARS-CoV-2 nucleic acid and serology tests at baseline, or any history of previous SARS-CoV-2 infection, or with known immunosuppressive condition were excluded. Children were sequentially enrolled into one of three groups (>12 to ≤18 years [group 1], >6 to 12 years [group 2], or ≥2 to 6 years [group 3]) and administered with adult formulation of BBV152 as two 0·5 mL intramuscular doses on days 0 and 28. Co-primary endpoints were solicited adverse events for 7 days post-vaccination and neutralising antibody titres on day 56, 28 days after the second dose. Immunogenicity endpoints were compared with Biodefense and Emerging Infections, Research Resources Repository (BEI) reference serum samples and from adults who received two doses of BBV152 in the same schedule in a previously reported phase 2 study. The trial is registered with the Clinical Trials Registry, India (CTRI/2021/05/033752) and ClinicalTrials.gov (NCT04918797).

Findings From May 27, 2021, to July 10, 2021, we enrolled 526 children sequentially into groups 1 (n=176), 2 (n=175), and 3 (n=175). Vaccination was well tolerated, with no differences in reactogenicity between the three age groups, and no serious adverse events, deaths, or withdrawals due to an adverse event. Local reactions mainly consisted of mild injection site pain in 46 (26%) of 176 participants in group 1, 61 (35%) of 175 in group 2, and 39 (22%) of 175 in group 3 after dose 1; and 39 (22%) of 176 in group 1, 43 of 175 (25%) in group 2, and 14 of 175 (8%) in group 3 after dose 2; there were no cases of severe pain and few reports of other local reactions. After dose 1, the most frequent solicited systemic adverse event was mild-to-moderate fever, reported in eight (5%) of 176 participants in group 1, 17 (10%) of 175 in group 2, and 22 (13%) of 175 in group 3. No case of severe fever was reported, and rates of all fever were all 4% or less after dose 2. Geometric mean titres (GMTs) of microneutralisation antibodies at day 56 in groups 1 (138·8 [95% CI 111·0–173·6]), 2 (137·4 [99·1–167·5]), and 3 (197·6 [176·4–221·4]) were similar to titres in vaccinated adults (160·1 [135·8–188·8]) and with BEI reference serum samples (103·3 [50·3–202·1]). Similar results were obtained using the plaque reduction neutralisation test (PRNT), in which 166 (95%) of 175 participants in group 1, 165 (98%) of 168 in group 2, and 169 (98%) of 172 in group 3 seroconverted at day 56. The GMT ratio of PRNT titres in children and adults was 1·76 (95% CI 1·32–2·33), indicating a superior response in children compared with adults.

Interpretation BBV152 was well tolerated in children aged 2–18 years, and induced higher neutralising antibody responses than those observed in adults, in whom the efficacy (ie, the prevention or decrease in the severity of COVID-19 infection) has been demonstrated.

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Introduction

There is an ongoing global inequality in the distribution of effective vaccines against SARS-CoV-2 with some low-income and middle-income countries unable to either afford the most widely used vaccines, or provide the necessary infrastructure to store and distribute the vaccines that require storage at –20°C.[1] To meet this need, a whole-virion adjuvanted inactivated SARS-CoV-2 vaccine

Research in context

Evidence before this study

COVID-19 vaccination efforts to date have focused on the adult and older populations that constituted those with the most serious outcomes; therefore, vaccine studies also focused on these groups. We searched PubMed on April 24, 2022, for research articles using the search terms "SARS-CoV-2", "COVID-19", "vaccine", "paediatric", and "children", with no language or date restrictions. We found several publications on COVID-19 vaccine clinical trials in children and adolescents, mainly with mRNA vaccines, to investigate their safety and reactogenicity and immune responses. Clinical trial results from two other inactivated vaccines reported humoral responses but minimal cell-mediated responses. From our published phase 3 data, we have shown that two doses of BBV152, a Vero cell-based whole-virion inactivated SARS-CoV-2 vaccine, elicits 78% efficacy against RT-PCR-confirmed COVID-19 and 65·2% against PCR-confirmed COVID-19 caused by the delta variant in adults. The low reactogenicity of this vaccine in adults make it an ideal candidate for the extension of vaccination to younger age groups.

Added value of this study

To our knowledge, this study is the first on the immunogenicity and safety of the BBV152 vaccine in children aged 2–18 years. Our preliminary analyses show the vaccine was well tolerated with no serious adverse events, deaths, or withdrawals due to an adverse event reported during the study. This finding is consistent with the larger dataset available from several studies with the vaccine in adults. BBV152 induced immune responses in children that were equivalent or superior to those previously demonstrated in adults in whom the vaccine has demonstrated protective efficacy. This finding suggest that BBV152 might be equally efficacious in protecting children against severe COVID-19. Because BBV152 is an inactivated vaccine, it is unlikely to lead to adverse events that have implied to be associated with paediatric vaccination with mRNA vaccines such as cases of Guillain-Barré syndrome, thromboembolic events, or myocarditis or pericarditis.

Implications of all the available evidence

Our findings with BBV152 are in accordance with humoral immune responses reported for other inactivated SARS-CoV-2 vaccine candidates and support extension of its use from adults to adolescents and children to broaden vaccine-induced immunity against SARS-CoV-2. Inclusion of younger age groups will help to interrupt transmission of the virus and diminish outbreaks. The low reactogenicity might make the vaccine more acceptable in paediatric populations than the more reactogenic mRNA vaccines, and as BBV152 can be stored at 2–8°C, it is compatible with immunisation cold chain requirements for many developing countries, where it will be a valuable tool in the global immunisation effort. Follow-up studies to assess paediatric effectiveness are underway, but our study suggests that similar efficacy might be anticipated in children based on the observation of superior immunogenicity than in adults.

(BBV152 [COVAXIN]; Bharat Biotech International, Hyderabad, India) was developed that can be stored at standard refrigerator temperatures (2–8°C).[2] A 2021 trial involving more than 25 000 adults demonstrated that two doses of BBV152 have 77·8% (95% CI 65·2–86·4) efficacy against RT-PCR-confirmed COVID-19 of any severity.[3] A smaller study, in 3732 Indian health-care workers in conditions of high infectious pressure during the second wave of COVID-19 (probably of the B.1.617.2 [delta] variant), found 46–57% efficacy against symptomatic PCR-confirmed SARS-CoV-2 infection depending on the vaccination interval.[4]

Paediatric COVID-19 infections have not been associated with the high rates of morbidity and mortality observed in older adults, and paediatric deaths are rare.[5,6] Children have been shown to be equally at risk of infection as adults although such infections tend to be asymptomatic, and might be a source of the ongoing infections in more susceptible populations.[7] Moreover, the increasing occurrence of variants of concern[8] has been associated with an increasing proportion of infections in children.[9] This increase has led several countries, including the US Centers for Disease Control and Prevention (CDC) and European Medicines Agency (EMA), to extend licensure of current adult mRNA vaccines to include children older than 5 years,[10,11] making the development of effective vaccines or the establishment of current vaccines as being suitable for use in children a medical priority, both to protect this population and to increase herd immunity. To meet this need, we assessed the safety and immunogenicity of BBV152 in an open-label, age de-escalation study in three cohorts of children aged between 2 years and 18 years.

Methods

Study design and participants

This was a phase 2/3 open-label, non-randomised, multicentre study done across six hospitals in India. Eligible participants were healthy children (male or female), aged 2–18 years. The trial was conducted in compliance with all International Council for Harmonisation Good Clinical Practice guidelines. The trial was approved by the National Regulatory Authority (Central Drugs Standard Control Organization, India) and also by the respective hospital ethics committees (Pranam Hospital, Hyderabad; All India Institute of Medical Sciences [AIIMS], Patna; Prakhar Hospital, Kanpur; Cheluvambha Hospital, Mysore; Meditrina Institute of Medical Sciences, Nagpur and AIIMS, New Delhi; appendix p 2). The protocol is registered with the

See Online for appendix

Clinical Trials Registry (India), CTRI/2021/05/033752, and ClinicalTrials.gov, NCT04918797. A data safety monitoring board comprising independent medical and vaccine experts was also convened to assess the safety of the vaccinations throughout the study and to approve the age de-escalation.

Inclusion criteria included general good health in the investigator's opinion, ability to fulfil the study criteria, residence in the study area for its duration, and no participation in any other clinical trial. The main exclusion criteria were any history of previous COVID-19 vaccination, SARS-CoV-2 infection confirmed by RT-PCR or ELISA at screening, a temperature (>38·0°C) or symptoms of an acute illness within 3 days of a vaccination, known sensitivity to any vaccine component, receipt of any other vaccine within 4 weeks of the study or any know immunosuppressive condition or treatment likely to interfere with the immune response. Children were excluded from receiving a second dose, if they displayed any anaphylactic reaction to the first vaccination or had a virologically confirmed SARS-CoV-2 infection between doses.

Parents or legal guardians of all participants supplied written informed and audio–visual consent; verbal assent was also obtained from children aged 7–12 years, and written consent from children older than 12 years to 18 years.

Procedures

The vaccine antigen is a β-propiolactone-inactivated whole virion of SARS-CoV-2 vaccine strain NIV-2020–770 (GISAID; GenBank accession number EPI_ISL_420545), isolated from an Italian tourist taken ill while making a visit to New Delhi, India, and sequenced at the Indian Council of Medical Research National Institute of Virology (Pune, India).[12] Each 0·5 mL dose of BBV152 contains 6 μg antigen with a toll-like receptor 7/8 agonist molecule (imidazoquinoline; IMDG) adsorbed to alum (Algel-IMDG). The vaccine was supplied in glass vials, stored at 2–8°C, for administration by intramuscular injection in the upper deltoid of the non-dominant arm (usually the left arm) in a two-dose regimen on days 0 and 28.

Age de-escalation was done by recruiting three age groups in sequence, beginning with the oldest group: group 1, older than 12 years to 18 years. A small number of participants were initially recruited to group 1 and received their first vaccination. The data safety monitoring board then assessed 7 days of safety and reactogenicity data before giving approval to recruit and vaccinate the remaining participants in that age group. Only after completion of the group 1 vaccination was the data safety monitoring board approval process initiated with children older than 6 years to 12 years (group 2), again in a small initial cohort before recruiting all participants, and then this was repeated with the youngest children in group 3, aged at least 2 years to 6 years.

All children were screened for SARS-CoV-2 infection by RT-PCR (appendix pp 11–12) and antibody detection assay (ELISA/CLIA; appendix pp 13–15) on day 0, when eligible participants had a general physical examination and assessment for potential SARS-CoV-2 infection. They were then vaccinated, and after administration of each dose, the participant was monitored for 2 h for immediate reactions. Parents or guardians were trained on how to complete study diaries for 7 days in which solicited local reactions (pain, redness, swelling, stiffness, and tenderness at the injection site) and systemic adverse events (body pain, fatigue, headache, loss of appetite, nausea, vomiting, pain in extremities, arthralgia, weakness, and fever), which were graded by the parents for severity as mild, moderate, or severe.

Active surveillance of reactogenicity and possible SARS-CoV-2 infection was also done during these 7 days by telephone contact with the parents or guardians who were also instructed to record any occurrence of a serious adverse event or adverse event of special interest and to report the event immediately to the investigator. Blood was drawn on days 0, 28 (before dosing), and 56 (4 weeks, post second dose), to assess immune responses.

Serum samples were separated immediately after the blood collection and stored at –20°C for measurement of immune responses (appendix pp 15–17). SARS-CoV-2 virus neutralising antibodies were measured using micro-neutralisation (MNT) and plaque reduction neutralisation (PRNT) tests, with titres expressed as reciprocal of the serum sample dilution achieving 50% neutralisation (MNT_{50} or $PRNT_{50}$). Because there is no established correlate of protection against COVID-19, vaccine-induced responses were compared with a panel of 18 internationally recognised reference serum samples (Biodefense and Emerging Infections, Research Resources Repository [BEI], NIAID, NIH). IgG antibody titres against SARS-CoV-2 S-protein, receptor-binding domain (RBD), and nucleocapsid protein (N-protein) were measured by ELISA and expressed as the reciprocal of the highest serum sample dilution showing binding to antigen.

Outcomes

The co-primary immunogenicity objective was measured as the geometric mean titres (GMTs) and seroconversion rates of neutralising antibodies elicited by vaccination in each age group. As there was no internal control these values in children were compared with the same measurements done in an adult population from a previously reported phase 2 study with a similar design. This interim report presents data obtained up to 4 weeks after the second dose, but the study is ongoing to collect data up to 6 months after the last vaccination, which will be reported separately.

Post-hoc analysis using subset of samples (15 from each age group) was performed to evaluate the capacity of vaccine-induced antibodies to block the interaction between human ACE-2 receptor and the RBD from the

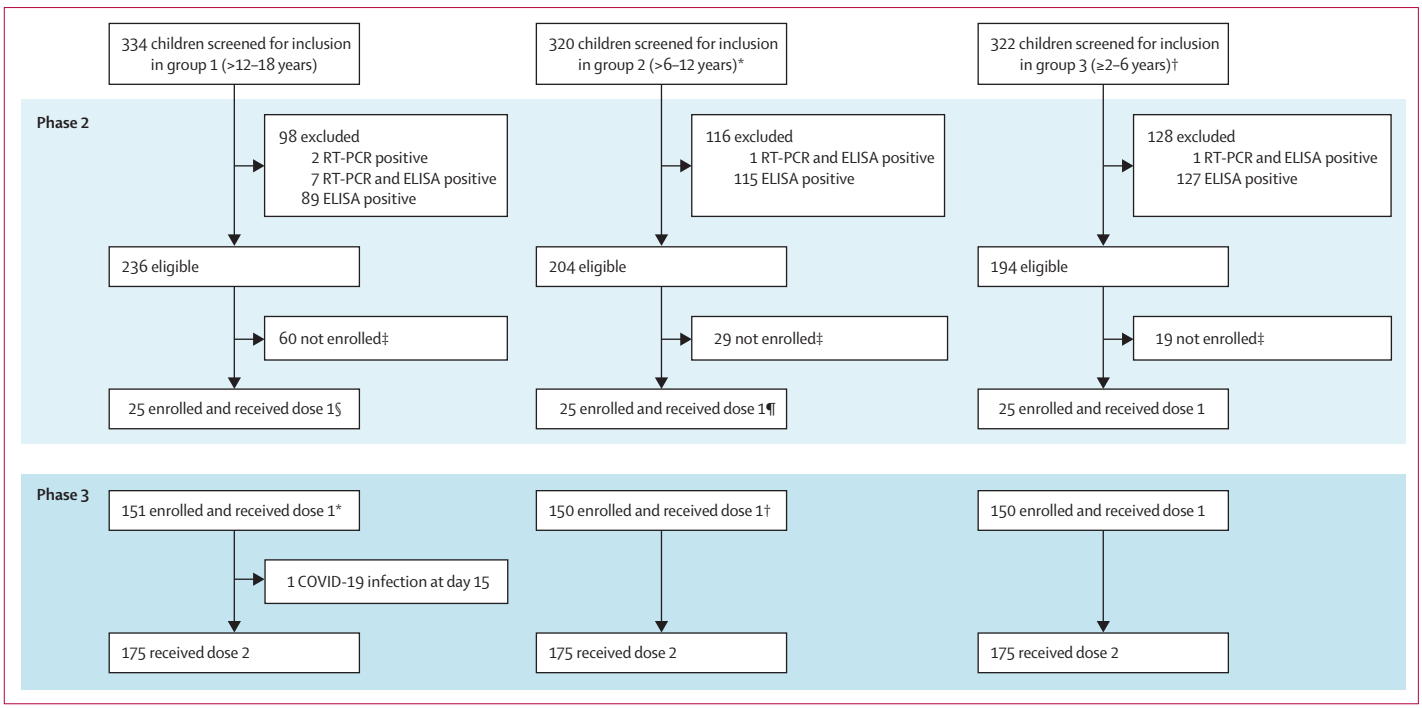


Figure 1: **Study flow chart**
Chart shows the sequential enrolment of the three age groups initially in phase 2, then in phase 3, following approval by the data safety monitoring board. *After completion of participant recruitment to group 1, the study progressed to the recruitment of participants for group 2. †After completion of participant recruitment to group 2, the study progressed to the recruitment of participants for group 3. ‡More potential participants were screened than were required for the protocol numbers (the sample size had been achieved); hence, these participants were not enrolled. §After completion of 7 days post first dose for 25 participants in group 1, safety data of these participants were reviewed by data safety monitoring board, and based on their recommendation, the study progressed by enrolling an additional 151 participants to group 1. ¶After completion of 7 days post first dose for 25 participants in group 2, safety data of these participants were reviewed by data safety monitoring board, and based on their recommendation, the study progressed to enrolling an additional 150 participants to group 2.

	Group 1, >12–18 years (n=176)	Group 2, >6 to 12 years (n=175)	Group 3, ≥2 to 6 years (n=175)
Age, years	14·52 (1·59)	9·01 (1·67)	3·72 (1·10)
Sex			
Female	84 (48%)	72 (41%)	68 (39%)
Male	92 (52%)	103 (59%)	107 (61%)
Weight, kg	55·5 (13·7)	34·3 (9·8)	16·4 (3·7)
Body-mass index, kg/m²	21·1 (3·9)	17·6 (2·8)	15·4 (2·6)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics**

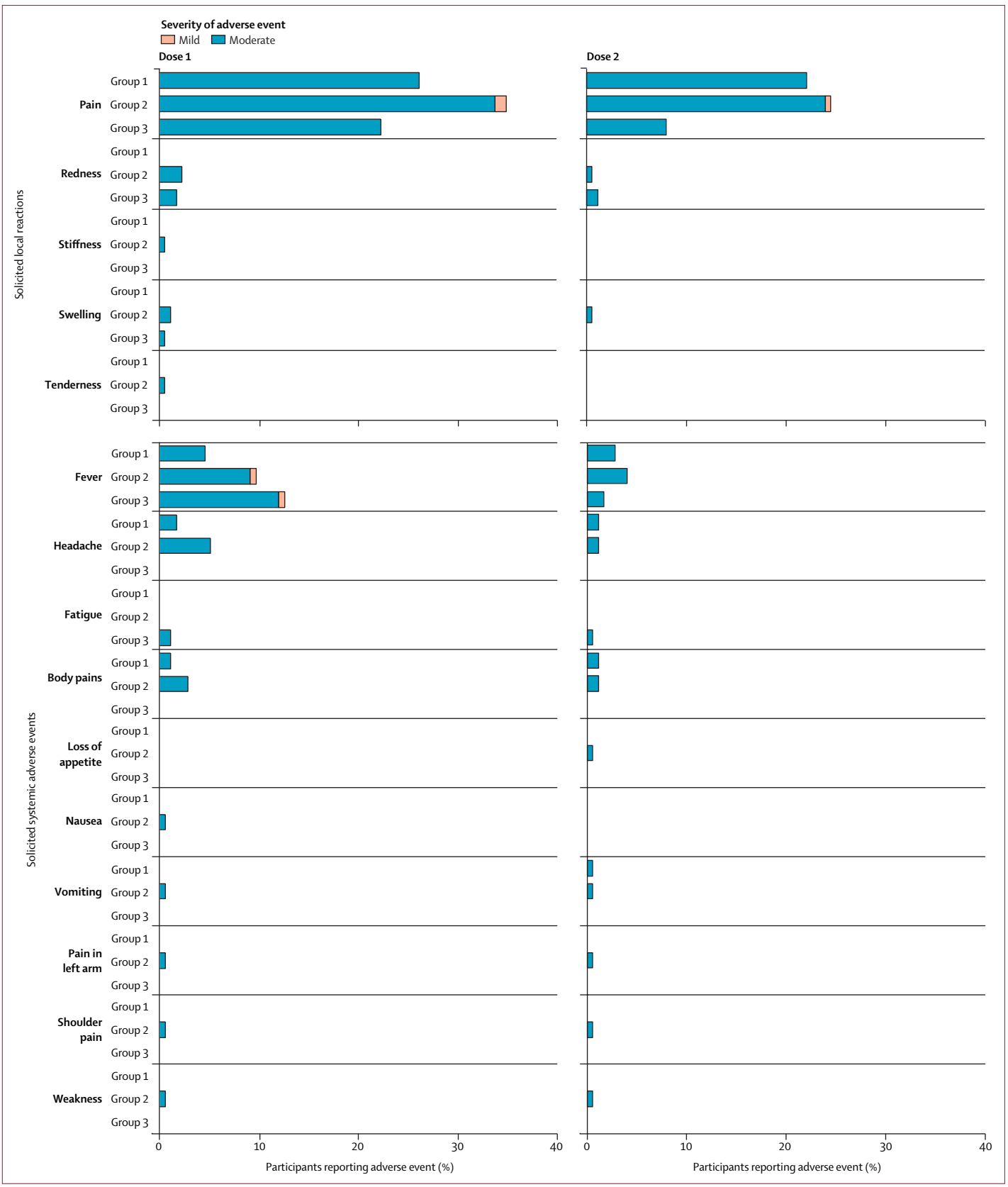
wild-type SARS-CoV-2 strain (D614G), and the delta (B.1.617.2) and omicron (B.1.1.529) variants with a surrogate virus neutralisation test (sVNT).[14,15] Similarly, another post-hoc analysis of randomly selected serum samples (n=24) was done to measure IgG1:IgG4 ratios as an indicator of the ratio of the Th1/Th2 responses.[13,16]

The outcomes for the primary safety and reactogenicity objectives were the assessments of the occurrence of solicited adverse events and their severity within 7 days after the administration of each dose, and unsolicited adverse events, serious adverse events, and adverse event of special interest throughout the study.

Statistical analysis

The sample size was intended to allow separate comparisons of GMTs at day 56 between each of the three age groups, and against titres observed in adults in an earlier phase 2 study.[13] Per-protocol non-inferiority analyses were based on those who received two doses of vaccine. Paediatric age groups were considered non-inferior to the adult group if the 95% CI for GMT ratio (paediatric group GMT/adult GMT) had a lower limit of at least 0·5 (non-inferiority margin). Based on the phase 2 trial data, we assumed the SD of $\log_{10}$ (titre) to be 0·5. We conservatively assumed a true GMT ratio of 0·8 for each paediatric group, such that the true underlying GMT is lower in the paediatric subgroup. Using a t-test for non-inferiority on the difference in means of $\log_{10}$ (titre) with assumed true difference of $\log_{10}(0·8)$ of –0·096910 and non-inferiority margin of $\log_{10}(0·5)$ of –0·30103. The power to show non-inferiority for a single paediatric age subgroup is approximately 0·95615 (PASS 2020, NCSS, Kaysville, Utah, USA). The

Figure 2: **Solicited reactogenicity**
Occurrence of solicited reaction is shown by severity in the three age groups in the 7 days after the first and second doses of BBV152 in groups 1 (>12–18 years), 2 (>6–12 years), and 3 (≥2–6 years).


Severity of adverse event
Mild
Moderate
Dose 1
Dose 2
Solicited local reactions
Solicited systemic adverse events
Pain
Redness
Stiffness
Swelling
Tenderness
Fever
Headache
Fatigue
Body pains
Loss of appetite
Nausea
Vomiting
Pain in left arm
Shoulder pain
Weakness
Group 1
Group 2
Group 3
0
10
20
30
40
Participants reporting adverse event (%)

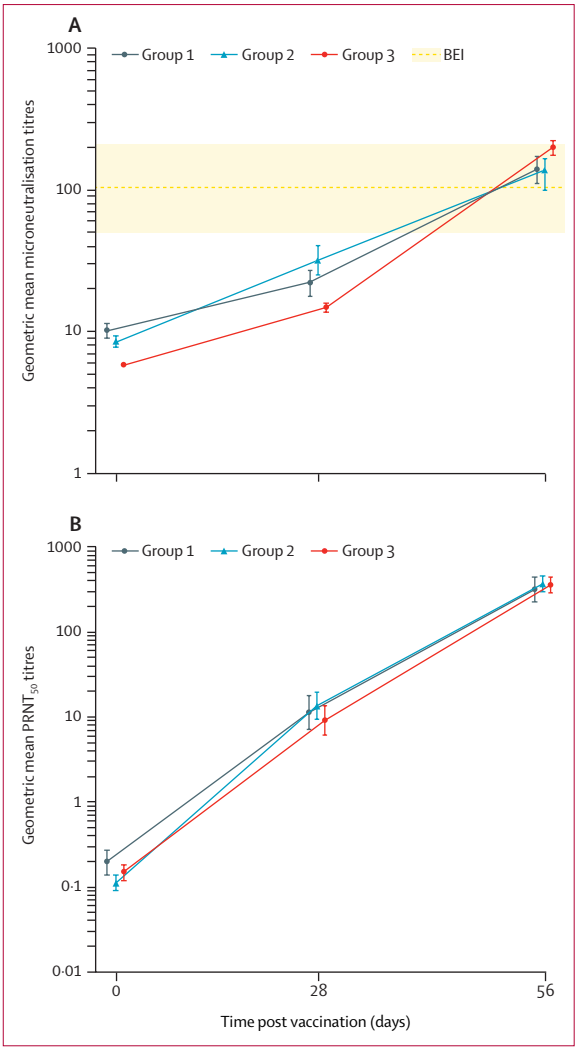


Figure 3: **Geometric mean titres of SARS-CoV-2 neutralising antibodies**
Levels of antibodies were measured by MNT_{50}, (A) or $PRNT_{50}$ (B) at baseline (day 0) and days 28 and 56 following two doses of BBV152 in group 1 (>12–18 years), group 2 (>6–12 years), and group 3 (≥2–6 years). Bars indicate 95% CIs. MNT_{50} GMT for 18 BEI samples are shown as a dotted line in (A) with 95% CI indicated by shading. MNT_{50}=microneutralisation. $PRNT_{50}$=plaque reduction.

probability that the non-inferiority criterion would be met for all three paediatric age subgroups is approximately ($0{\cdot}95615^3$), or approximately 87%. We assumed sample sizes of 150 individuals providing data for each paediatric subgroup and 177 for the adult group, which is the number reported from the phase 2 trial with titre data at day 56. To allow for loss of data due to withdrawals and loss to follow-up, we planned to enrol 175 children into each of the three paediatric age subgroups.

GMTs were calculated for neutralisation antibodies and ELISA IgG antibodies for each age group separately. A two-sided 95% CI for the post-vaccination GMT was calculated from a 95% CI for the mean of log-transformed titre, using a normal approximation for the distribution of log (titre). For each age group, the ratio of GMTs with corresponding 95% CI versus the adult group are presented. The 95% CI for the GMT ratio was calculated from a 95% CI for the difference in means of $\log_{10}$ (titre). Groups were compared using a two-sided two sample t-test on the means of log-transformed titres. The primary comparison was the GMT ratios after the second dose. Secondary post-hoc comparisons were done with earlier reported data obtained 4 weeks after the second dose in initially seronegative adults in a phase 2 study.[13] Seroconversion rates were calculated as group proportions achieving a four-times increase in titre from baseline (day 0) to days 28 or 56 with two-sided 95% CIs. For differences between seroconversion rates, exact 95% CIs were calculated and seroconversion rates compared using two-sided Fisher exact tests, with the p value obtained by doubling the smaller of the one-sided p values. All statistical analyses were done using SAS (version 9.4), R (version 4.1.2), and GraphPad Prism (version 9.0.2 [161]). A p value less than 0·05 was considered significant.

Role of the funding source

The sponsor of the study had no role in data collection, data analysis, data interpretation, or writing the report. A contract research organisation (Sclin Soft Technologies, Hyderabad, India) was responsible for data analysis and generating the report. The unblinded contract research organisation and several authors from Bharat Biotech had full access to the data in the study. Authors from Bharat Biotech had final responsibility for the decision to submit for publication.

Results

From May 26, to July 10, 2021, we screened 976 potential participants sequentially (group 1 n=334; group 2 n=320; group 3 n=322); of the people screened, 342 (group 1 n=98, group 2 n=116, and group 3 n=128) were excluded for seropositivity for SARS-CoV-2 infection according to RT-PCR or ELISA testing (figure 1). Of the 634 eligible children, 526 were enrolled to complete the required study size and these children were vaccinated (group 1 n=176, group 2=175, and group 3 n=175). One child in group 1 did not receive a second dose after being diagnosed with COVID-19 on day 15. There were more males than females in each age group, and this difference was largest in the group 3 (table 1).

There were no serious adverse events, deaths, or withdrawals due to an adverse event during the study, with the exception of one case of COVID-19 in group 1. No cases of Guillain-Barré syndrome, thromboembolic events, myocarditis or pericarditis, or other adverse event of special interest have been reported to date. Vaccination with BBV152 was generally well tolerated, with mild-to-moderate reactogenicity profiles (figure 2). There were no differences in these profiles between

	Group 1	Group 2	Group 3	Adults[13]
MNT_{50}				
Day 0				
n	175	175	175	NA
GMT	10·1 (8·9–11·5)	8·5 (7·8–9·3)	5·8 (5·7–5·9)	NA
Day 28				
n	175	157	175	NA
GMT	21·9 (17·8–27·1)	31·8 (24·9–40·6)	14·8 (13·7–16·0)	NA
Seroconverted*	44 (25%; 19·3–32·1)	70 (45%; 37·0–52·4)	26 (15%; 10·3–20·9)	NA
Day 56				
n	175	157	173	177
GMT	138·8 (111·0–173·6)	137·4 (99·1–167·5)	197·6 (176·4–221·4)	160·1 (135·8–188·8)
GMTR (group *vs* adults)	0·87 (0·66–1·14)	0·86 (0·64–1·16)	1·23 (1·01–1·51)	0·98 (0·80–1·19)†
Seroconverted*	158 (90%; 84·9–94·2)	141 (90%; 84·0–94·1)	173 (100%; 97·9–100)	171 (97%; 92·8–98·7)
p value *vs* adult	<0·05	<0·05	<0·05	NA
$PRNT_{50}$				
Day 0				
n	175	175	175	NA
GMT	0·20 (0·14–0·27)	0·11 (0·09–0·14)	0·15 (0·12–0·18)	NA
Day 28				
n	175	175	175	NA
GMT	11·2 (7·0–17·9)	13·5 (9·3–19·6)	9·1 (6·1–13·5)	NA
Seroconverted*	111 (63%; 56·1–70·2)	143 (82%; 75·3–86·8)	126 (72%; 64·3–77·6)	NA
Day 56				
n	175	168	172	177
GMT	317·4 (224·4–449·2)	366·9 (297·0–453·3)	358·6 (287·2–447·8)	197·0 (155·6–249·4)
GMTR (group *vs* adults)	1·61 (1·05–2·43)	1·86 (1·35–2·55)	1·82 (1·31–2·51)	1·76 (1·32–2·33)†
Seroconverted*	166 (95%, 90·5–97·6)	165 (98%; 94·9–99·6)	169 (98%; 95·0–99·6)	174 (98%; 95·1–99·6)
p value *vs* adult	ns	ns	ns	NA

MNT_{50}=microneutralisation test. NA=not applicable. GMT=geometric mean titres. GMTR=geometric mean titre ratio. $PRNT_{50}$=plaque reduction neutralisation test. ns=non-significant (p value >0·1). *Seroconversion defined a four-times increase in titre over baseline at day 0. GMTR for neutralising antibodies measured by MNT or PRNT between paediatric groups 1, 2, and 3 in this study and adults in phase 2 study.[14] Thus, a paediatric age subgroup was considered non-inferior to the adult group if the 95% CI for GMTR (GMT in a paediatric subgroup/GMT in adults) had a lower limit of at least 0·5. The PRNT GMTR (GMT in a paediatric subgroup/GMT in adults) had a lower limit of at least 1, indicating a superior response in children when compared with adults. †All children versus adults.

Table 2: **Neutralising antibody titres Data are n, GMT (95% CI), GMTR (95% CI), or n (%; 95% CI). GMTs, GMTRs, and seroconversion rates for SARS-CoV-2 neutralising antibodies measured by MNT_{50} or plaque $PRNT_{50}$ with values from a phase 2 adult study**

groups 1 and 2, which both had higher reactogenicity than group 3. Local reactions mainly consisted of mild injection site pain, reported by less than 35% of any group after the first dose (46 of 176 in group 1, 61 of 175 in group 2, and 39 of 175 in group 3), and less than 25% after the second dose (39 of 176 in group 1, 43 of 175 in group 2, and 14 of 175 in group 3); there were no cases of severe pain. There were few reports of other local reactions. Systemic adverse events were less frequent, especially after the second dose. After dose 1, the most frequent systemic adverse event was mild-to-moderate fever, reported in eight (5%) of 176 group 1 participants, 17 (10%) of 175 group 2 participants, and 22 (13%) of 175 of children in group 3. No case of severe fever was reported and rates of all fever were all 4% or less after dose 2.

Unsolicited adverse events were also infrequent after vaccination, reported for three (2%) of 176 children in group 1, eight (5%) of 175 children in group 2, and four (2%) of 175 children in group 3. All unsolicited adverse events, which consisted of individual reports of typical childhood complaints likely to occur independent of vaccination (appendix p 3), were described as mild and resolved without sequelae.

Immune responses measured as MNT antibody titres were similar in all three age groups. Vaccine-induced MNT responses in all groups (at day 56) were similar to the GMT of 103·3 (95% CI 50·3–202·1) from 18 BEI reference serum sample run in the same assay (figure 3). On day 56, the GMT ratio comparing all children with adults was 0·98 (95% CI 0·80–1·19; table 2). 4 weeks after the second dose, all (173 [100%] of 173) group 3 participants seroconverted, with a GMT of 197·6 (95% CI 176·4–221·4). 158 (90%) of 175 participants in group 1 and 141 (90%) of 157 participants in group 2 seroconverted, with GMTs of 138·8 (95% CI 111·0–173·6; group 1) and 137·4

(99·1–167·5; group 2). When assessed by PRNT, there was no difference between groups in terms of GMTs or seroconversion rates after two doses (figure 3; table 2). On day 56, the GMT ratio comparing children with adults was 1·76 (95% CI 1·32–2·33). Seroconversion 4 weeks after the second vaccination was high (95–98%) in all three groups, and similar to the phase 2 study in adults. Neutralising GMTs measured by PRNT were higher in all three groups of children (317·4 in group 1, 366·9 in group 2, and 358·6 in group 3) than those previously reported in adults (197·0 [95% CI 155·6–249·4]; table 2).

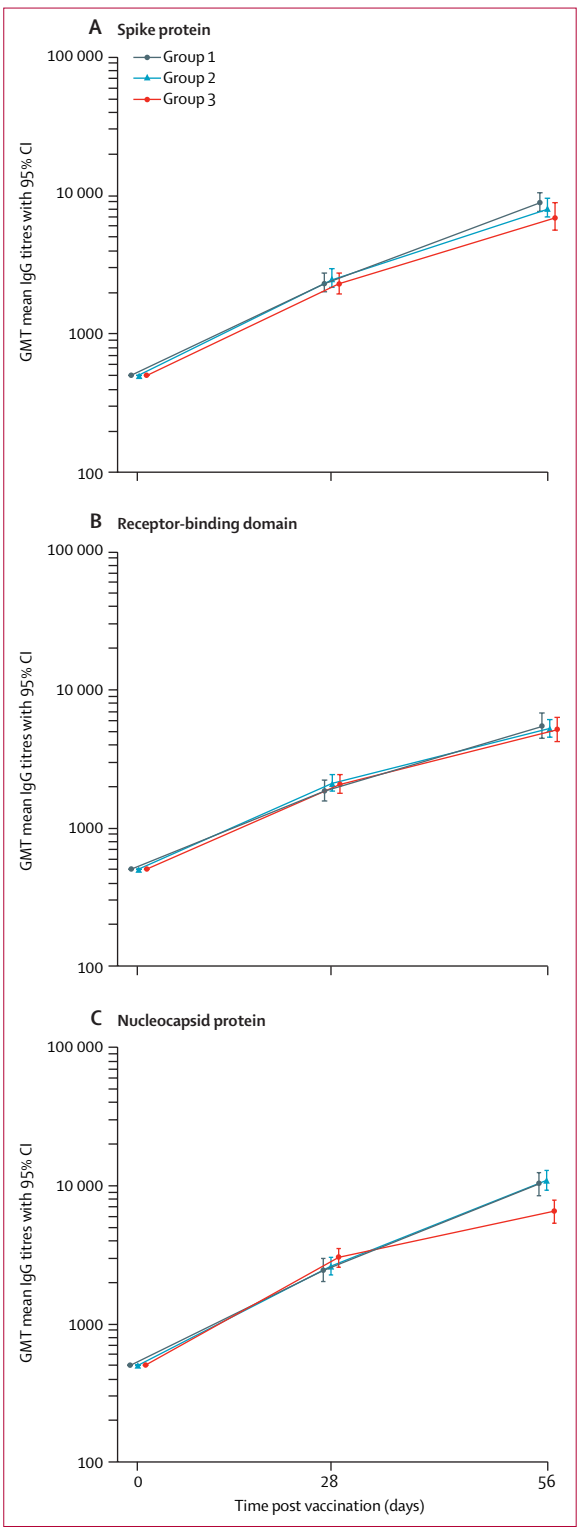


Figure 4: **GMT of IgG antibodies**
IgG antibodies were measured by ELISA against SARS-CoV-2 S-protein (A), receptor-binding domain (B), or nucleocapsid protein (C) at baseline (day 0) and days 28 and 56 after two doses of BBV152 in group 1 (>12–18 years), group 2 (>6–12 years), and group 3 (≥2–6 years). Bars indicate 95% CIs. Groups were measured at the same timepoints, but are separated laterally for clarity. GMT=geometric mean titre.

In an exploratory investigation using the sVNT assay we found that vaccine-induced antibodies were able to block the interaction between human ACE2 and RBD of both D614G and delta variants with low activity against the omicron variant. On day 56, 15 (100%) of 15 participants across all three age groups) showed neutralisation against D614G across all age groups, with GMTs of inhibition ranging from 63·6% to 94·4% at 1:10 serum dilution. Neutralisation was age dependent against the delta variant, observed in 12 (80%) of 15 participants in group 1, and 15 (100%) of 15 participants in groups 2 and 3, with GMTs of inhibition of 49·4% in group 1, 84·2% in group 2, and 91·1% in group 3. When tested with neat sera (without dilution) against the omicron variant, four (27%) of 15 participants from groups 2 and 3 demonstrated neutralisation with 1·2–4·7% inhibition, whereas none of the participants in group 1 demonstrated any neutralisation of the omicron variant. Vaccine-induced neutralisation efficiency at day 56 was statistically significant ($p<0·0001$) compared with neutralisation efficacy at day 0 across all age groups against D614G, and the delta and omicron variants, except in group 1 against omicron (appendix pp 8–9).

Binding IgG antibody responses against the three main antigenic SARS-CoV-2 protein components, S-protein, RBD, and N-protein, are illustrated in figure 4 (appendix pp 4–6); all three age groups responded in a similar manner with similar magnitude against the three proteins except for a lower GMT at day 56 for N-protein in group 3. Isotyping ratios (IgG1/IgG4) at day 56 were above 1 for all vaccinated groups, indicative of a Th1 bias (appendix p 10).

Discussion

We previously demonstrated that two doses of BBV152 are effective in preventing COVID-19 due to SARS-CoV-2 infection in adults,[2,3] and now show that the vaccine is equally well tolerated and immunogenic in seronegative children aged 2–18 years. No adverse events of special interest have been reported to date; however, a supplementary surveillance study is ongoing, which will provide more information on rarer adverse events. Neutralising antibody responses measured by MNT or PRNT after two doses in a cohort of children aged 2–18 years are non-inferior to those observed in adults. The GMT ratio of neutralising antibodies measured by PRNT in children versus adults had a lower limit of 1 or more, indicating a superior response in children. Although neutralising titres are not a correlate of protection, the fact that this vaccination schedule has been

shown to protect against infection in adults thus suggests BBV152 might also be efficacious in the paediatric cohort. Furthermore, preliminary assessments show the induced antibodies were able to block the interaction between human ACE2 and RBD from both the wild-type (D614G) and delta variant, whereas the effectiveness against omicron was low. These results suggest the importance of a third dose, to enhance effectiveness against omicron, which has been shown (in a preprint) in adults with BBV152[17] and other COVID-19 vaccines.[18] Further studies with a third dose of BBV152 in children will be required to confirm any protection against such infections.

A Th1-dominant response has been suggested to be preferable for COVID-19 vaccines;[19] we also demonstrated that the immune response towards BBV152 was skewed towards a Th1 response with IgG1/IgG4 ratios above 1.[13,16] An earlier study in adults vaccinated with BBV152 reported cross-reactive T-cell responses to several SARS-CoV-2 variants (alpha, beta, and delta).[20]

The pattern of baseline neutralising antibody GMTs suggests an age-dependent increase (appendix p 7), possibly due to the older children interacting with a greater number of other children socially and thus more likely to be exposed to COVID-19. This study was conducted during the second wave of COVID-19 in India from March to July, 2021, which peaked on May 3.[21] The only case of symptomatic COVID-19 detected in the study was in the oldest age group; however, visits to assess severity or duration of disease were not scheduled, and routine SARS-CoV-2 nucleic acid testing was not conducted, so asymptomatic or mild symptomatic cases of COVID-19 might have been missed.

Schoolchildren were not prioritised for global vaccination programmes generally and thus remained vulnerable to SARS-CoV-2 infection.[22] With the new emerging variants,[23] the number of cases and hospitalisation in children started to increase compared with earlier in the pandemic. Hence, preventing SARS-CoV-2 infection across all age groups, including children, by vaccination will continue to be critical to curtail the pandemic. Vaccination of younger age groups with licensed adult COVID-19 vaccines[10,11] would not only reduce the severity, hospitalisations, and long-term complications of COVID-19, but might also reduce household transmission and severity in vulnerable individuals, including immunocompromised or comorbid individuals.[24] The pandemic necessitated regulatory body approval of the use of available safe and effective COVID-19 vaccines in children as a precautionary measure.[11]

Globally, inactivated vaccines and mRNA vaccines form the majority of COVID-19 vaccines approved for administration to children younger than 18 years. An advantage of this study is the demonstration of acceptable tolerability and significant immunogenicity of BBV152 in children as young as 2 years, supported by a safety profile similar to that in adults. Inactivated COVID-19 vaccines have been shown to be well tolerated and immunogenic in phase 1/2 trials in Chinese children aged 3–17 years.[25,26] An advantage of whole-virion vaccines such as BBV152 is that multiple epitopes are present, as illustrated by the marked responses against S-protein, RBD, and N-protein in this study. Furthermore, after a booster (third) dose BBV152 showed (in a preprint) enhanced in vitro neutralisation against variants of concern, including omicron in adults,[17,27] despite inducing only low or moderate neutralising antibody titres compared with mRNA vaccines. Vikkurthi and colleagues, also show (in a preprint) that, ex vivo, BBV152-generated T cells (Tfh) assist in B-cell production of antibodies against SARS-CoV-2 variants of concern.[20]

Our study was limited by the ethical considerations of doing a study in children, and it was for these reasons that our study was small, open label, and without a placebo group. However, the low rates of reported reactogenicity, except for injection site pain, suggest that a control group would not have revealed any medically significant tolerability issues. Transient mild-to-moderate injection site pain is the main adverse event reported by adults not only with BBV152,[2,13] but also with other COVID-19 vaccines.[28] The small size of the study means that we cannot draw firm conclusions about vaccine safety in children as rare events, eg myocarditis, will only be detected in larger surveillance studies, which are ongoing. Finally, we have assessed only the immunogenicity of BBV152, not clinical efficacy, in children.

In conclusion, the inactivated SARS-CoV-2 vaccine BBV152, was well tolerated and immunogenic in children aged 2–18 years, with neutralising antibody responses at least similar to those observed in adults in whom the vaccine has been proven to be efficacious against symptomatic and asymptomatic COVID-19. This, combined with their ability to be stored stably at warmer (fridge) temperatures than the mRNA[29,30] and vector-based vaccines that have been widely used in high-income countries, make BBV152 an attractive alternative to those vaccines for wider global use. The vaccine has been approved for use by the Indian government in children aged 15–18 years.[31] We await further safety data from the large surveillance study in children aged 2 years and older.

Contributors

All authors read and approved the final version of the manuscript and met the criteria for authorship set forth by the International Committee for Medical Editors. KMV, SRe, BG, SPrasad, RE, and KE reviewed the manuscript. KMV and SRe were responsible for overall project coordination. SRe led clinical operations and helped with protocol design. HJ was responsible for neutralisation assays. BG was responsible for Th1 biased responses and sVNT data. WB was involved with the study design and statistical analysis plan. All principal investigators (NM, VNT, CS, VK, SPrasan, and SRa) were involved in the scientific review of this paper. KMV, SRe, and RE had full access and verified the masked data in the study and can vouch for its accuracy and completeness. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

This work was funded by Bharat Biotech International. KMV, SRe, BG, HJ, and SPrasad, are employees of Bharat Biotech, with no stock options

or incentives. Co-author, KE, is the Chairman and Managing Director of Bharat Biotech and owns equity in the company. RE and WB are independent clinical development and statistical consultants, respectively (who received fees from Bharat Biotech). NM, VNT, CS, VK, SPrasan, and SRa were principal investigators representing the study sites.

Data sharing

Individual participant (de-identified) data will be made available when the trial is complete upon direct request to the corresponding author with an appropriate research proposal. Once such a proposal is approved, data will be shared through a secure online platform.

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