

# Live-virus neutralization of the omicron variant in children and adults 14 months after SARS-CoV-2 wild-type infection

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## Funding information

Ministry of Science, Research and the Arts Baden-Württemberg; Dietmar Hopp Stiftung

## Abstract

Data on cross-neutralization of the SARS-CoV-2 omicron variant more than 1 year after SARS-CoV-2 infection are urgently needed, especially in children, to predict the likelihood of reinfection and to guide vaccination strategies. In a prospective observational cohort study, we evaluated live-virus neutralization of the SARS-CoV-2 omicron (BA.1) variant in children compared with adults 14 months after mild or asymptomatic wild-type SARS-CoV-2 infection. We also evaluated immunity to reinfection conferred by previous infection plus COVID-19 mRNA vaccination. We studied 36 adults and 34 children 14 months after acute SARS-CoV-2 infection. While 94% of unvaccinated adults (16/17) and children (32/34) neutralized the delta (B.1.617.2) variant, only 1/17 (5.9%) unvaccinated adults, 0/16 (0%) adolescents and 5/18 (27.8%) children <12 years of age had neutralizing activity against omicron (BA.1). In convalescent adults, one or two doses of mRNA vaccine increased delta and omicron neutralization 32-fold, similar to a third mRNA vaccination in uninfected adults.

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Neutralization of omicron was 8-fold lower than that of delta in both groups. In conclusion, our data indicate that humoral immunity induced by previous SARS-CoV-2 wild-type infection more than 1 year ago is insufficient to neutralize the current immune escape omicron variant.

**KEYWORDS**

children, COVID-19, immune escape, live-virus neutralization, omicron variant, SARS-CoV-2, vaccination

## 1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has infected more than 750 million people worldwide in the past 3 years.<sup>1</sup> Infections in children are frequently asymptomatic or mild.<sup>2-4</sup> Data on long-term vaccine-acquired versus infection-acquired immunity are scarce in this age group. Vaccine efficacy of wild-type vaccines against the immune-evading omicron variant is reduced.<sup>5,6</sup> Therefore, there is a higher reluctance to recommend and administer COVID-19 vaccines to children despite regulatory approval.<sup>7,8</sup>

Due to high SARS-CoV-2 infection rates and low vaccination coverage in children under the age of 12 years, especially in countries with poor vaccine availability, their first SARS-CoV-2 antigen contact is frequently the natural infection rather than the vaccination. Previous studies on the humoral immune response to infection observed that neutralizing antibodies peak at 1 month postinfection and decrease thereafter with a more rapid waning of immunity in the first 2–3 months.<sup>9</sup> Despite humoral waning, immunity was observed in most children with equal<sup>10-13</sup> or higher<sup>14,15</sup> neutralization activity compared to adults several months after mild or asymptomatic SARS-CoV-2 infection. However, data on virus neutralizing activity with a follow-up of more than 1 year are scarce<sup>10,13</sup> and currently missing for the predominant omicron variant. Live-virus neutralization against the omicron variant is of particular interest in this context, because serological assays are designed to detect SARS-CoV-2 wild-type virus and neutralization of previous variants overestimate neutralization of the immune-evading omicron variant.<sup>16</sup>

Therefore, the aim of this study was to investigate live-virus neutralization of the omicron variant in children compared with adults more than 1 year after mostly mild or asymptomatic wild-type SARS-CoV-2 infection to predict their level of humoral protection. Live-virus neutralization was investigated as a surrogate readout for the risk of SARS-CoV-2 (re-)infection, because it has a high predictive value for protection against SARS-CoV-2 infection.<sup>17,18</sup> We also examined immunity to reinfection mediated by prior infection and COVID-19 vaccination.

## 2 | METHODS

### 2.1 | Study design and conduct

This study is part of a multicenter noninterventional, prospective observational cohort study on the seroprevalence and humoral immune response after SARS-CoV-2 wild-type infection in households with at least one RT-PCR confirmed index case and at least one child in the Federal State of Baden-Württemberg, Germany (German Registry for Clinical Studies (DRKS), study ID 00021521). It was initiated by the University Children's Hospitals in Heidelberg, Freiburg, Tübingen and Ulm. Results on household transmissions<sup>19</sup> and humoral immune dynamics<sup>13</sup> including neutralization of the B.1.617.2 (delta) variant in convalescent unvaccinated adults and children have been published previously. Blood samples and data for this substudy were collected at the study site in Heidelberg. 395 households who met the eligibility criteria were invited via the respective local health authorities of the districts Heidelberg/Rhein-Neckar, Mannheim, Karlsruhe, and Neckar-Odenwald; 143 of 395 households (36.2%) voluntarily participated. Households that met all of the following inclusion criteria were eligible for enrollment at T1: (i)  $\geq 1$  household member with a reverse transcriptase polymerase chain reaction (RT-PCR)-proven SARS-CoV-2 infection from a nasopharyngeal or oropharyngeal swab specimen taken  $\geq 4$  weeks before the study visit, (ii)  $\geq 1$  household member  $< 18$  years of age, (iii) residency in the state of Baden-Württemberg, and (iv) all household members were officially released from quarantine. Key exclusion criteria were: (i) missing written consent, and (ii) missing knowledge of the German language. Study participants were investigated at the first time point (T1) from May 11 to June 20, 2020. They were asked to answer a questionnaire on age, sex, on the result of their SARS-CoV-2 RT-PCR test including test date, on COVID-19-associated symptoms and on the requirement for hospitalization.

A subset of children and their parents who were seropositive at T1 were invited for follow-up (T2) from May 20–28, 2021,  $362 \pm 8$  days after T1. Children and their parents or guardians who were seropositive at T1 and had no laboratory-confirmed reinfection since T1 were eligible for follow-up at T2. They answered additional questions on SARS-CoV-2 vaccines since T1 including details on the number, manufacturers, and dates of the respective vaccinations.

Because booster vaccination after SARS-CoV-2 infection was not recommended in children during the study period, we determined the effect of mRNA vaccination on neutralizing live-virus 14 months after wild-type infection in adults. Only vaccinated participants who (i) had received BNT162b2 by BioNTech or mRNA-1273 by Moderna and (ii) were vaccinated 7 to 45 days before blood collection were included. An age-matched and delta neutralization titer-matched healthy control group of mRNA vaccinated adults without prior SARS-CoV-2 infection (negative antinucleocapsid pan-Ig) from a previously published cohort<sup>20</sup> was analyzed for comparison.

The study was designed, analyzed and reported according to the STROBE guidelines (<https://www.strobe-statement.org>).

## 2.2 | Detection of SARS-CoV-2-reactive antibodies

We analyzed samples for the presence of antibodies reactive against Sars-CoV-2 proteins with commercially available immunoassays. The following tests were performed: (i) Elecsys<sup>®</sup> Anti-SARS-CoV-2 test kit detecting pan-Ig against the N protein (09 203 095 190; Roche) run on a Roche Cobas 601 module, (ii) a test kit detecting antibodies reactive against the receptor binding domain (RBD) of the S1 glycoprotein (ADVIA Centaur sCOVG; 11207377; Siemens) run on a Siemens ADVIA Centaur. To convert measured indices to the WHO international standard Binding Antibody Units per milliliter (BAU/mL), we used a factor of 21.8. Sera that tested positive in at least one of these two assays were further subjected to enzyme-linked immunosorbent assay (ELISA) measurements of the S1 domain of the viral spike protein (Euroimmun Anti-SARS-CoV-2-ELISA [IgG] test kit; Euroimmun AG, Lübeck, Germany, EI 2606-9601 G) on a Euroimmun Analyzer I. Sera that were positive in at least two assays were considered positive.

## 2.3 | Neutralization capacity against the SARS-CoV-2 variants B.1.617.2 (delta) and BA.1 (omicron)

The neutralization capacity of sera was determined in titration experiments as described previously.<sup>21,22</sup> The SARS-CoV-2 variants of concern B.1.617.2 (delta) and BA.1 (omicron) were isolated from nasopharyngeal swabs of respective RT-PCR- and sequencing-confirmed SARS-CoV-2-positive patients. B.1.617.2 variant was amplified in VeroE6 cells. Stocks of B.1.1.529 were produced in Calu-3 cells to avoid rapid cell culture adaptation. Virus stocks were validated by genome sequencing and titers were determined by a Median Tissue Culture Infectious Dose (TCID50) assay. To determine neutralization capacity, two-fold serial dilutions of sera were prepared in OptiMEM medium and incubated with  $6 \times 10^4$  TCID50 of SARS-CoV-2 variants B.1.617.2 or BA.1 for 1 h at 37°C. The mixture was then inoculated to VeroE6 cells to detect whether SARS-CoV-2 were neutralized by serum neutralizing antibodies or still active to infect the cells. Virus replication was measured after cells were fixed with 5% formaldehyde at 24 h postinfection using

immunostaining for the viral nucleocapsid with rabbit monoclonal antibody (Sinobiological) and antirabbit HRP-conjugated antibody (Merck). The signal was developed with KPL SureBlue<sup>™</sup> 3,3',5,5'-tetramethylbenzidine peroxidase substrate (Seracare) for 5 min and stopped by the addition of 0.5 M sulfuric acid. Absorbance was measured on a Tecan Sunrise plate reader (Tecan) at 450 nm with a reference wavelength of 620 nm. Values were normalized to those obtained with cells infected in the absence of patient serum (100% infection) and noninfected cells (0% infection = assay background). Inhibition capacity is given as ID<sub>50</sub> (serum dilution that reduces the virus-specific signal by 50%). A neutralization titer of 1:10 represents the cut-off for detection of this neutralization assay.

## 2.4 | Statistical analysis

Analyses were performed using R version 4.1.1 (R Core Team, 2021) and GraphPad Prism Version 9. Results for normally distributed symmetrical variables are presented as mean (standard deviation, SD), results for skewed variables are presented as median (interquartile range, IQR). Results from live-virus neutralization experiments were not normally distributed and skewed towards zero especially for the BA.1 variant. We therefore applied Mann-Whitney U test for comparison of two and Kruskal-Wallis with Conover test (pairwise comparisons) for comparison of more than two independent groups. We used the Wilcoxon matched-pairs signed rank test for comparison of paired groups.

We used linear regression models (least square regression [LS], robust regression [RR] and least trimmed square regression [LTS]) to examine whether commercially available assays detecting anti-SARS-CoV-2 antibodies (anti-S1 IgG, anti-S1-RBD IgG and anti-N pan-Ig) were able to predict neutralization capacity against the B.1.617.2 (delta) variant of concern. Receiver operating characteristic (ROC) curve analysis was performed to compare assays detecting anti-SARS-CoV-2 antibodies and define a cut-off antibody value to predict neutralization of BA.1 (omicron) (ID<sub>50</sub>  $\geq$  1:10) with a sensitivity of at least 80% and highest possible specificity. No a priori hypotheses were tested; therefore, all *p* values are reported as descriptive measures.

## 3 | RESULTS

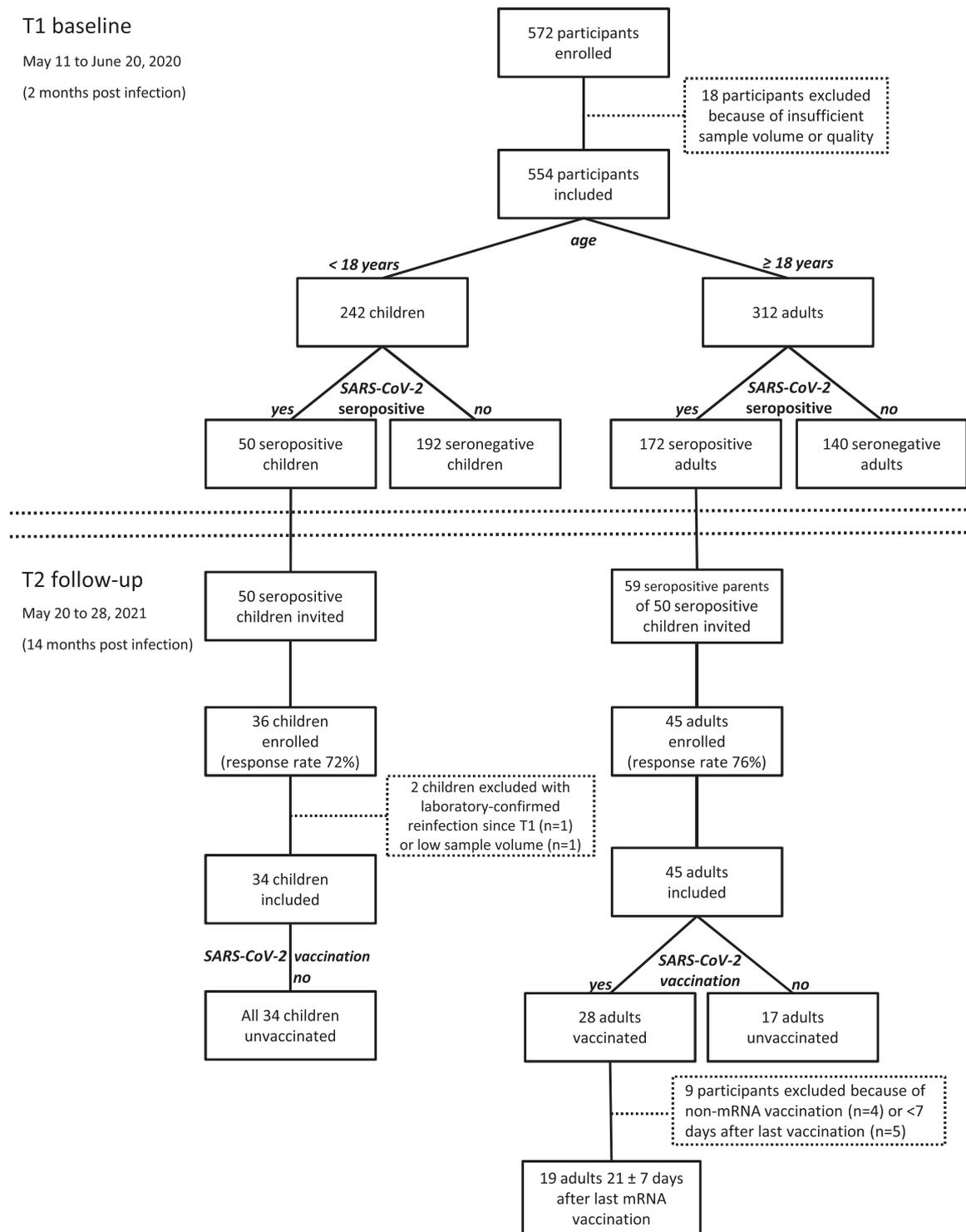
### 3.1 | Participants and characteristics

A total of 554 participants, 312 adults and 242 children, from 143 households were available for analysis 1 to 3 months after acute SARS-CoV-2 infection (Figure 1). A total of 222 participants, 50 children and 172 adults, were classified as seropositive 1 to 3 months after acute SARS-CoV-2 infection. All 50 seropositive children together with their 59 seropositive parents or guardians were invited for a follow-up investigation approximately 1 year after T1. 36 children (response rate, 72%) and 45 parents (response rate, 76%)

## T1 baseline

May 11 to June 20, 2020

(2 months post infection)



**FIGURE 1** Study flow diagram.

responded; one child with a SARS-CoV-2 reinfection and one child with insufficient blood sample volume were excluded (Figure 1).

All 34 children aged  $11.2 \pm 5.0$  years (<12 years,  $n = 18$ ; 12.0–17.9 years,  $n = 16$ ) and 17 of 45 (38%) adults aged  $44.2 \pm 9.9$  years were unvaccinated. 28 of 45 (62%) adults had been vaccinated against SARS-CoV-2; 9 adults were excluded, because they had received a non-mRNA vaccine ( $n = 4$ ) or samples were

collected less than 7 days after the last vaccination ( $n = 5$ ). Nineteen vaccinated adults aged  $44.6 \pm 10.9$  years were included. They were vaccinated once (BNT162b2 by BioNTech [ $n = 9$ ] or RNA-1273 by Moderna [ $n = 4$ ]) or twice (all BNT162b2 by BioNTech [ $n = 6$ ]) 21 ± 7 days before T2 and 13 to 14 months after their respective SARS-CoV-2 wild-type infection (Figure 1). Live-virus neutralization titer ( $ID_{50}$ ) against the SARS-CoV-2 BA.1

**TABLE 1** Demographic characteristics of convalescent study participants 14 months after SARS-CoV-2 wild-type infection.

	All	Children (all unvaccinated)			Adults		
		All	<12 years	12.0–17.9 years	All	unvaccinated	vaccinated
Total no. of participants	70	34	18	16	36	17	19
Age, years mean ± IQR (range),	28.3 ± 18.6 (1–68)	11.2 ± 4.99 (1–17)	7.14 ± 3.24 (1–11)	15.7 ± 1.51 (12–17)	44.4 ± 10.5 (18–68)	44.2 ± 9.89 (26–63)	44.6 ± 10.9 (18–68)
Sex							
Female, no. (%)	35 (50.0)	18 (52.9)	10 (55.6)	8 (50.0)	17 (52.8)	10 (58.8)	7 (36.8)
Male, no. (%)	35 (50.0)	16 (47.1)	8 (44.4)	8 (50.0)	19 (47.2)	7 (41.2)	12 (63.2)
Symptomatic SARS-CoV-2 infection							
No, no. (%)	19 (27.1)	15 (44.1)	9 (50.0)	6 (37.5)	4 (11.1)	1 (5.9)	3 (15.8)
Yes, no. (%)	51 (72.9)	19 (55.9)	9 (50.0)	10 (62.5)	32 (88.9)	16 (94.1)	16 (84.2)
COVID-19 severity							
Outpatient, no. (%)	69 (98.6)	34 (100)	18 (100)	16 (100)	35 (97.2)	16 (94.1)	19 (100)
Hospitalized, no. (%)	1 (1.4)	0 (0)	0 (0)	0 (0)	1 (2.8)	1 (5.9)	0 (0)

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2.

(omicron) variant were not significantly different between vaccinated convalescents who had received one or two vaccine doses (Supporting Information: Figure 1); therefore, we analyzed both groups together. The sex distribution within the adult and child groups was balanced (Table 1). The mean time elapsed since infection in participants with a positive RT-PCR test result was  $423 \pm 15$  days in children and  $425 \pm 13$  days in adults. Fifty-one of 70 participants (72.9%) were symptomatic, only one participant (48-year-old) required hospitalization. Adults were more often symptomatic than children (88.9% vs. 55.9%). 19 vaccinated healthy adults aged  $47.2 \pm 10.9$  years without prior SARS-CoV-2 infection served as controls. They were investigated  $15 \pm 9$  days before and  $22 \pm 6$  days after the third mRNA vaccination, which was 7 to 8 months after the second vaccine dose.

### 3.2 | Neutralization of SARS-CoV-2 B.1.617.2 (delta) and BA.1 (omicron) variants 14 months after wild-type infection

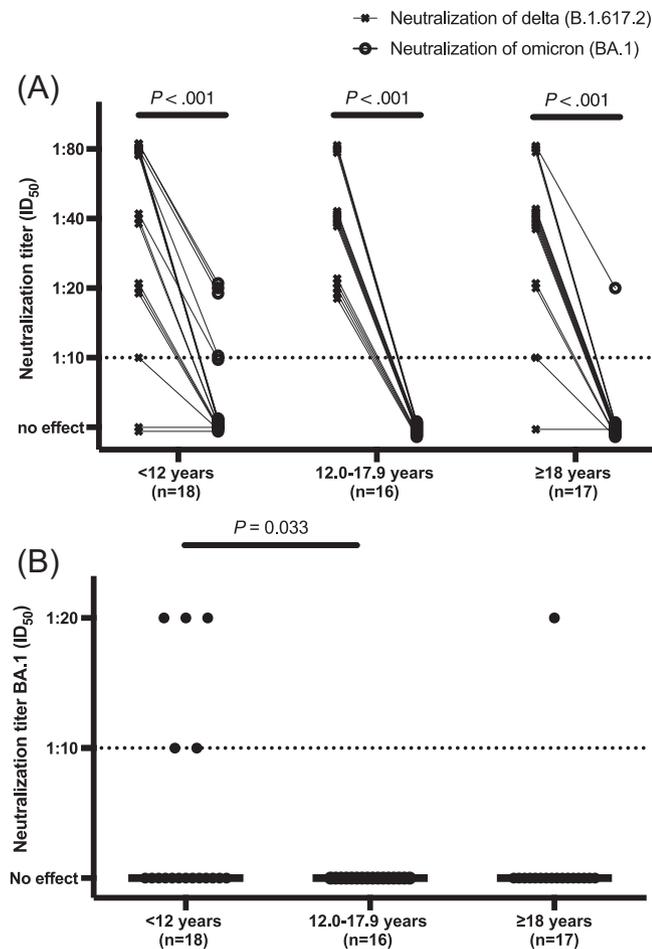
We measured neutralizing antibodies against SARS-CoV-2 variants B.1.617.2 (delta) and BA.1 (omicron) in 51 unvaccinated participants (17 adults, 16 adolescents and 18 children <12 years of age) by a live-virus neutralization assay 14 months after SARS-CoV-2 wild-type infection (Figure 2). In all age groups, there was significant immune escape for omicron compared with delta (Figure 2A; all  $p < 0.001$ ). Only one of 17 (5.9%) adults, none of 16 (0%) adolescents and 5 of 18 (27.8%) children <12 years of age showed any neutralization activity against omicron ( $ID_{50} \geq 1:10$ ), while there was detectable neutralization activity against delta in most adults (16/17; 94%), all adolescents (16/16; 100%) and most children <12 years of age (16/18; 88.9%). A Kruskal–Wallis

rank sum test comparing live-virus neutralizing activity among age groups showed no significant difference for delta ( $p = 0.78$ ) but for omicron ( $p = 0.033$ ). Children <12 years of age had higher neutralization activity against omicron than adolescents ( $p = 0.033$ ; Figure 2B).

Next, we investigated in linear regression models whether commercially available assays detecting anti-SARS-CoV-2 Ig were able to predict live-virus neutralization titer against delta. Anti-S1 and anti-S1-RBD IgG correlated well with neutralizing activity against the delta variant (both  $p < 0.001$ ; Figure 3). Multiplying anti-S1 IgG (ratio) test results with the factor 14 or anti-S1-RBD IgG (BAU/mL) test results with the factor 0.3 gave good estimates of neutralizing activity ( $ID_{50}$ ) against delta ( $R^2 = 0.93$  and  $0.86$ , respectively). Anti-S1 IgG had the highest min–max accuracy of 76% followed by anti-S1-RBD IgG of 63% to 65%. Anti-N pan-Ig was weakest at predicting neutralizing activity against delta ( $R^2 = 0.40$ ;  $p < 0.001$ ) with a min–max accuracy of 36% to 48% (Figure 3). When testing whether the three assays were able to discriminate samples with any ( $ID_{50} \geq 1:10$ ) neutralization activity against the omicron variant, anti-N pan-Ig (ROC-AUC, 0.91; 95% CI, 0.82–1.00,  $p = 0.001$ ) was not worse than anti-S1-RBD IgG (ROC-AUC, 0.88; 95% CI, 0.77–0.99,  $p = 0.003$ ) and anti-S1 IgG (ROC-AUC, 0.80; 95% CI, 0.65–0.95,  $p = 0.02$ ) (Figure 4).

### 3.3 | Effects of vaccination 14 months after wild-type infection on neutralization of SARS-CoV-2 variants B.1.617.2 (delta) and BA.1 (omicron)

All 19 vaccinated convalescent adults had detectable neutralizing activity against B.1.617.2 (delta) and BA.1 (omicron) (Figure 5). The



**FIGURE 2** Live-virus neutralization of SARS-CoV-2 variants B.1.617.2 (delta) (A) and BA.1 (omicron) (A and B) in VeroE6 cells of sera from unvaccinated children ( $n = 34$ ) and unvaccinated adults ( $n = 17$ ) 14 months after wild-type infection. Medians are shown in (B) as black lines.  $p$  values were calculated using a Wilcoxon signed-rank test (A) and Kruskal-Wallis with Conover test (B). SARS-CoV-2, Severe acute respiratory syndrome coronavirus type 2.

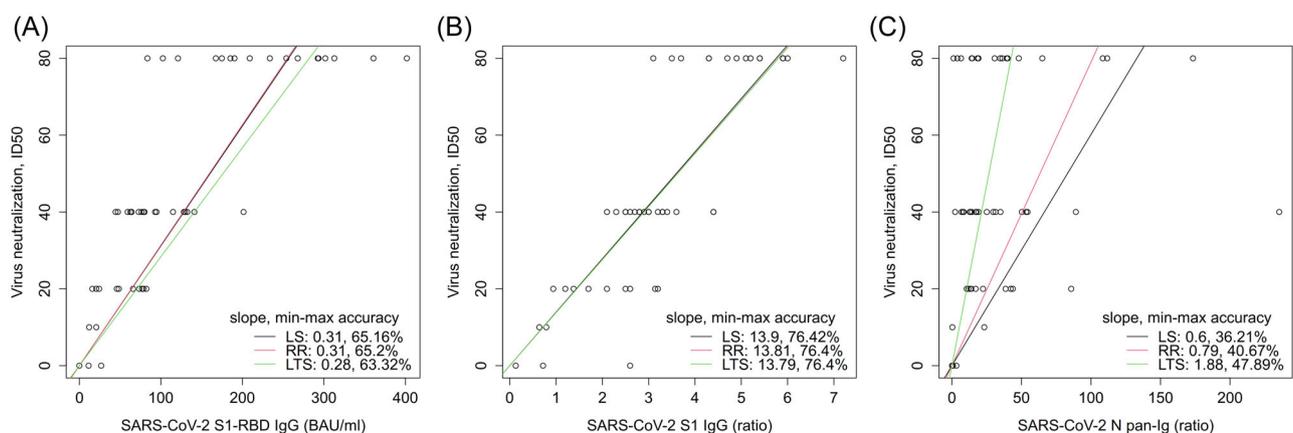
median neutralizing activity (ID<sub>50</sub>) was 32-fold higher against delta (1280 [640–2560] vs. 40 [40],  $p < 0.001$ ; Figure 5B) and at least 32-fold higher against omicron (160 [160–320] vs. no detectable effect,  $p < 0.001$ ; Figure 5C) in the vaccinated compared to unvaccinated convalescent adults 14 months postinfection.

Humoral immune escape of the omicron variant has been reported to be stronger with vaccination alone than with infection plus vaccine-acquired immunity.<sup>23,24</sup> We therefore compared 19 vaccinated convalescents with an age-matched uninfected healthy control group who received three SARS-CoV-2 mRNA vaccine doses (Figure 6A); both groups had similar neutralization activity against delta. The effect of the third vaccination in the uninfected control group (Supporting Information: Figure 2) was comparable to the effect of vaccination 14 months after SARS-CoV-2 wild-type infection (Figure 5B). The median (IQR) of omicron neutralization was similar in the vaccinated convalescents and the control group after the third vaccination (ID<sub>50</sub> of 160 [160–320] vs. 160 [80–320]) and 8-fold lower than delta neutralization in both groups (Figure 6).

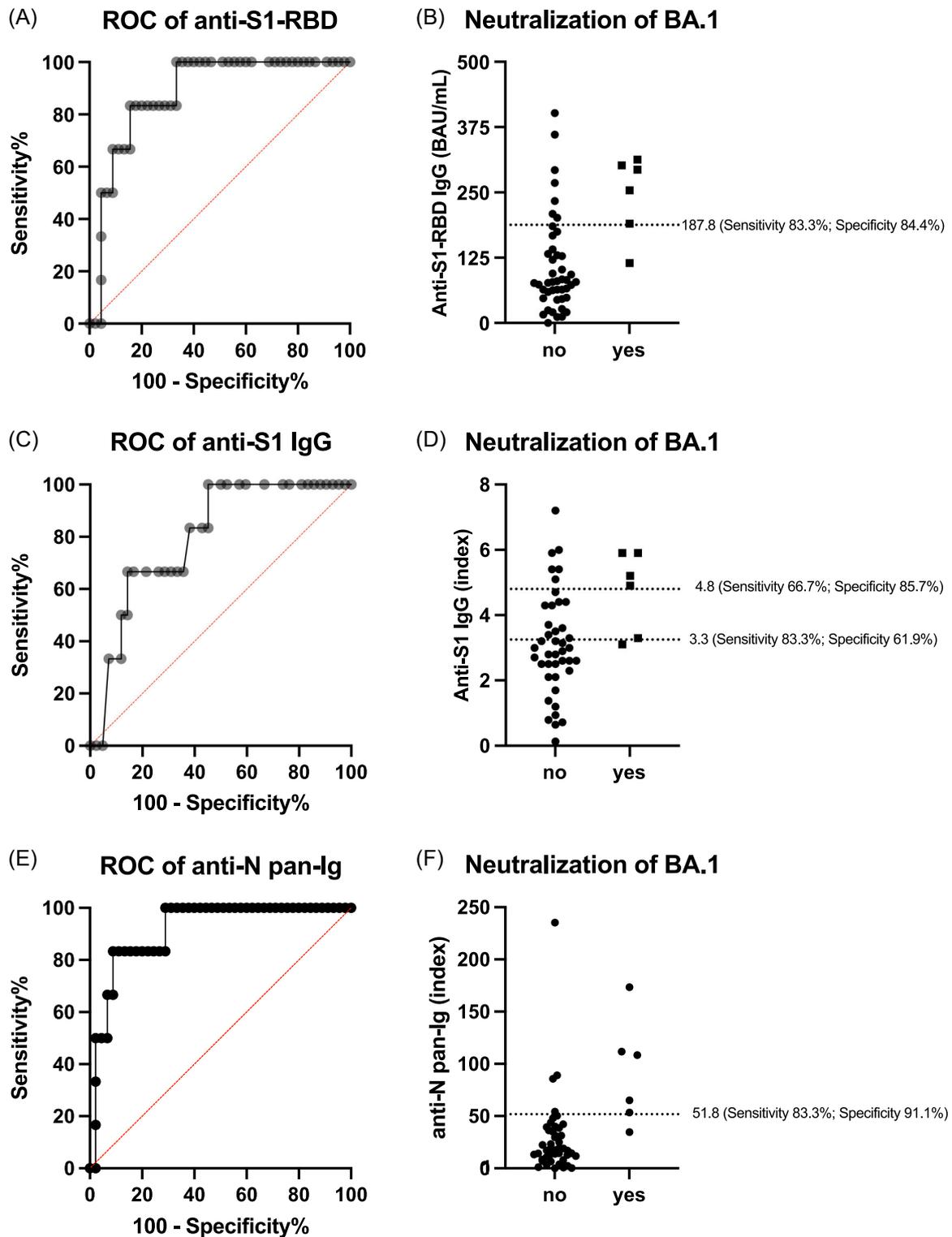
## 4 | DISCUSSION

Immune escape of emerging variants of concern, particularly the omicron variant, and waning humoral immunity after SARS-CoV-2 infection resulted in a high number of reinfections in children and adults. Long-term data on cross-neutralization of variants of concern and particularly of the omicron variant are urgently needed to determine needs and strategies for omicron-adapted booster vaccinations.

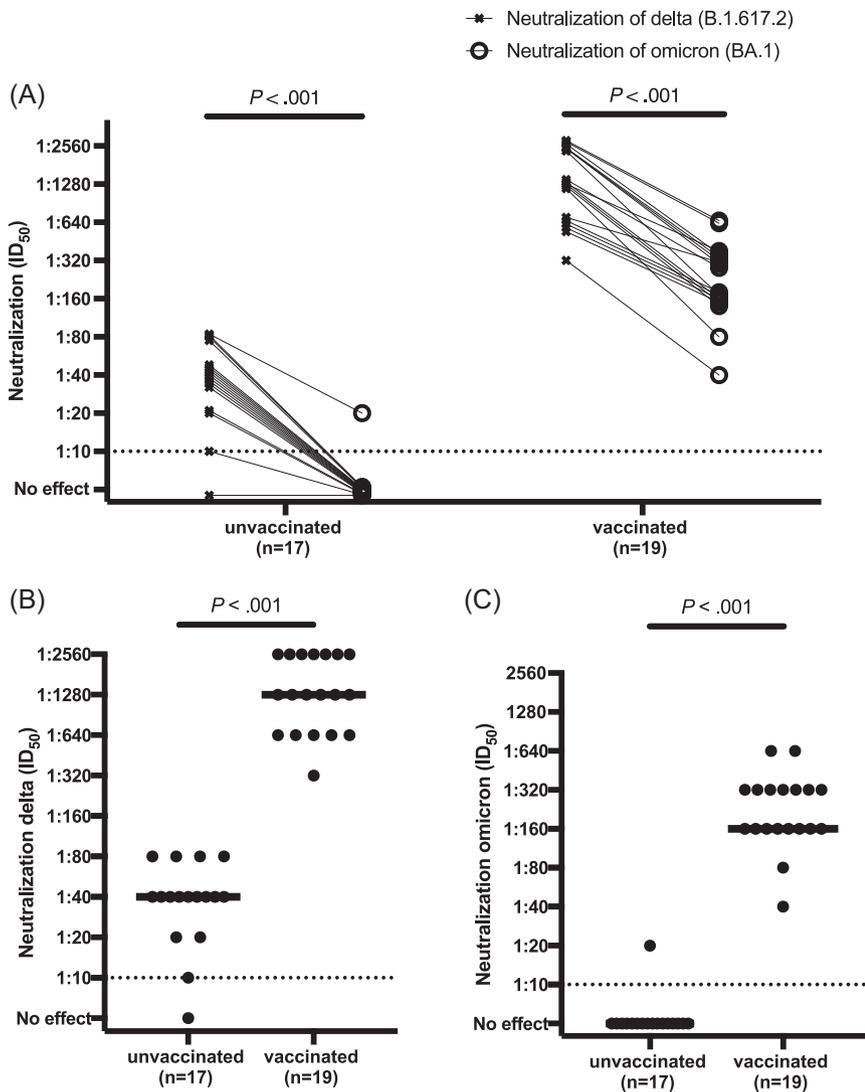
To our knowledge, this is the first study to describe live-virus neutralization against the SARS-CoV-2 BA.1 (omicron) variant in children compared with adults more than 1 year after mild or asymptomatic SARS-CoV-2 infection. We detected immune escape



**FIGURE 3** Live-virus neutralization of SARS-CoV-2 variant B.1.617.2 (delta) in VeroE6 cells in relation to SARS-CoV-2 antibodies against (A) the receptor binding domain of the S1 glycoprotein (RBD) ( $n = 51$ ), (B) the S1 domain (S1) ( $n = 48$ ), and (C) the nucleocapsid (N) ( $n = 51$ ) 14 months after wild-type infection. Three linear regression models (least square regression [LS], robust regression [RR] and least trimmed square regression [LTS]) were applied to calculate the slope (black line, LS; red line, RR and green line, LTS) and min-max accuracy of each model. SARS-CoV-2, Severe acute respiratory syndrome coronavirus type 2.



**FIGURE 4** Anti-SARS-CoV-2 antibody level to predict any ( $ID_{50} \geq 1:10$ ) live-virus neutralization against the BA.1 (omicron) variant in unvaccinated convalescent adults ( $n = 17$ ) and children ( $n = 34$ ). (A, C, E) Receiver operating characteristic (ROC) curve analysis of relative anti-SARS-CoV-2 S1-RBD IgG levels for discrimination of neutralizing serum samples ( $ID_{50} \geq 1:10$ ) against the omicron (BA.1) variant. (B, D, F) shows individual anti-SARS-CoV-2 antibody level in individuals with (yes) or without (no) omicron neutralization including a cut-off (dashed black line) with an at least 80% sensitivity and highest possible specificity for discrimination of neutralizing serum samples. (A, B) anti-S1-RBD IgG (BAU/mL) (ROC-AUC, 0.88; 95% CI, 0.77–0.99,  $p = 0.003$ ), (C, D) anti-S1 IgG (index) (ROC-AUC, 0.80; 95% CI, 0.65–0.95,  $p = 0.02$ ), and, (E, F) anti-N pan-Ig (index) (ROC-AUC, 0.91; 95% CI, 0.82–1.00,  $p = 0.001$ ).



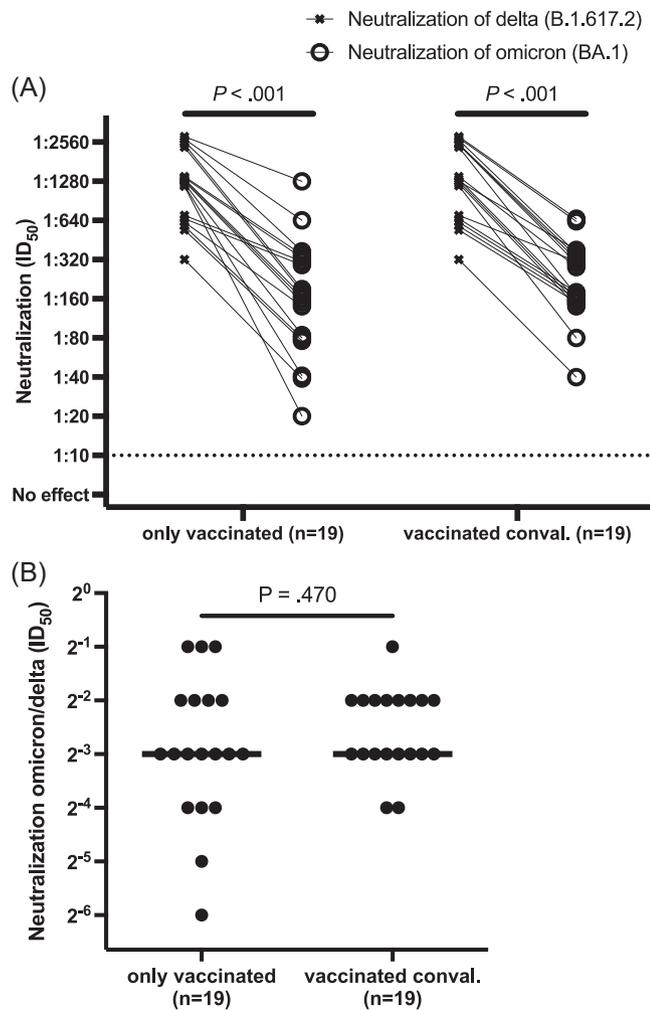
**FIGURE 5** Live-virus neutralization of SARS-CoV-2 variant B.1.617.2 (delta) (A and B) and BA.1 (omicron) (A and C) in VeroE6 cells of sera from adults 14 months after wild-type infection either without additional vaccination (unvaccinated;  $n = 17$ ) or 3 weeks after mRNA vaccination (vaccinated;  $n = 19$ ). Medians are shown in (B and C) as black lines.  $p$  values were calculated using a Wilcoxon signed-rank test (A) and Mann-Whitney U test (B and C). SARS-CoV-2, Severe acute respiratory syndrome coronavirus type 2.

of the SARS-CoV-2 omicron variant compared with the delta variant in all age groups. While neutralization activity against delta was present in most adults and children (94%) and was similar across age groups, live-virus neutralization activity against omicron ranged from 0% (0/16) in adolescents to 27.8% (5/18) in children younger than 12 years, with significantly greater neutralization activity against omicron in the youngest age group of children younger than 12 years compared with adolescents 14 months postinfection.

Previous studies found immune escape of the omicron variant compared to previous variants with SARS-CoV-2 omicron neutralizing activity ranging from 50% (4/8),<sup>25</sup> 26.7% (4/15),<sup>26</sup> 20% (4 of 20)<sup>27</sup> to 16% (8 of 50)<sup>28</sup> 1 month after delta, 2 months after beta and 2 and 12 months after wild-type infection, respectively. However, pseudovirus neutralization assays were used in the majority of patients which may not fully reflect live-virus neutralization activity against BA.1 (omicron); these results can therefore not be readily extrapolated. Only one study also included adults for comparison and demonstrated omicron pseudovirus neutralization in all 13 adults, but only in four of eight children 1 month after delta infection.<sup>25</sup>

Live-virus neutralization is the gold standard for measuring neutralization particularly of immune-evading virus variants; it is highly predictive of protection against SARS-CoV-2 infection.<sup>17,18</sup> We observed that commercially available assays detecting IgG antibodies against S1 and S1-RBD wild-type correlated with live-virus neutralization of the variant B.1.617.2 (delta); but in some individuals similar IgG concentrations were associated with neutralization that differed by several dilution levels. While anti-N pan-Ig was least likely to predict B.1.617.2 (delta) neutralization, it was at least no worse at discriminating samples that neutralized BA.1 (omicron). The SARS-CoV-2 omicron variant carries key mutations in the spike protein that impair binding of neutralizing antibodies to S1 and S1-RBD.<sup>29</sup>

Our observation that children have a comparatively low ability to neutralize the omicron variant 14 months after wild-type infection is important to the current discussion of vaccination strategies involving omicron-adapted vaccines in children. We investigated the effect of booster vaccination on neutralizing live-viruses of SARS-CoV-2 variants B.1.617.2 (delta) and BA.1 (omicron) in adults only, because no SARS-CoV-2 vaccine was licensed by the European



**FIGURE 6** Live-virus neutralization of SARS-CoV-2 variant B.1.617.2 (delta) and BA.1 (omicron) in VeroE6 cells of sera from adults 3 weeks after mRNA vaccination and 14 months after wild-type infection (vaccinated convalescents;  $n = 19$ ) and age- and delta neutralization-matched uninfected adults 3 weeks after a third mRNA vaccine dose (only vaccinated;  $n = 19$ ). Medians are shown in (B) as black lines.  $p$  values were calculated using a Wilcoxon signed-rank test (A) and Mann-Whitney U test (B). SARS-CoV-2, Severe acute respiratory syndrome coronavirus type 2.

Medicines Agency for children <16 years of age at the time the study was conducted. We observed that additional mRNA vaccination 14 months after SARS-CoV-2 wild-type infection resulted in a 32-fold greater delta and at least 32-fold greater omicron neutralization than in unvaccinated, previously infected subjects. Immune escape of new emerging variants, including omicron, has been reported to be stronger for vaccine alone compared to infection plus vaccine-acquired immunity.<sup>23,24,30</sup> In this current study, live-virus neutralization against the omicron variant was 8-fold lower in vaccinated convalescents which was similar to the uninfected healthy control cohort after three doses of mRNA vaccine. These data do not support the previously outlined concept of a stronger “super” or “hybrid” immunity with greater breadth and higher-quality antibodies, especially against variants of concern in convalescent vaccinated

adults.<sup>30–32</sup> This is in line with the conclusion of a recently published systematic review.<sup>33</sup> Cross variant neutralization against omicron has been reported to be even weaker after infection than after standard vaccination in children.<sup>28,34</sup> An increase in SARS-CoV-2 infections among vaccinated children and reports of acute myocarditis in adolescents and young adults following vaccination have led to vaccine hesitancy in children. However, myocarditis after standard vaccination is rare in children aged 5–11 years (1 in 1 million doses)<sup>35</sup>; it was not reported after 650,000 monovalent<sup>36</sup> or 950,000 bivalent booster<sup>37</sup> doses in this age group or in children aged 6 months to 5 years after one million standard vaccinations.<sup>38</sup> Recent studies evaluating the efficacy of wild-type mRNA vaccines in children aged 5–11 years suggest a reduced protection against all omicron infections compared to previous variants, but protection against hospitalization and particularly severe COVID-19 remained high.<sup>39–44</sup>

Our study has several limitations. We do not have data on cellular immunity against SARS-CoV-2. Assessing cellular and particularly SARS-CoV-2-specific T-cell immunity is important for predicting the level of protection from SARS-CoV-2 reinfection and protection from severe COVID-19.<sup>45</sup> A positive correlation of T cell and humoral responses has been shown previously;<sup>46</sup> but T cell immunity, CD4+ and CD8+ T-cells in adults<sup>47–49</sup> and CD4+ T-cells in children,<sup>27</sup> appears to be less affected by immune escape of the omicron variant than antibody responses. Another limitation is the relatively small sample size of our study; the results must therefore be interpreted with caution. Finally, the findings regarding live-virus neutralization of SARS-CoV-2 are applicable to BA.1 and may not be readily extrapolated to more recent omicron variants.

In conclusion, our data indicate that live-virus neutralization of the BA.1 (omicron) variant is not detectable 14 months after mild or asymptomatic wild-type infection in the majority of adults, adolescents and children younger than 12 years, despite higher titers in the youngest age group compared with adolescents. Vaccination of previously infected adults boosted neutralization against delta and omicron 32-fold to a level comparable to the neutralizing capacity of serum from uninfected adults after a third (booster) dose of mRNA vaccine; omicron antibody escape was comparable in both groups. Protection from a previous SARS-CoV-2 wild-type infection is not sufficient to neutralize the omicron variant after more than 1 year. Therefore, children and adolescents who have previously been infected with the wild type may also benefit from an additional omicron-adapted vaccination.

#### AUTHOR CONTRIBUTIONS

Maximilian Stich and Burkhard Tönshoff conceived and designed the study, drafted the first version of the manuscript and collected and analyzed data. Louise Benning and Claudius Speer provided key resources, collected and analyzed data. Sven F. Garbade contributed to drafting sections of the manuscript and did statistical analysis. Marie Bartenschlager, Heeyoung Kim performed laboratory analysis. Kathrin Jeltsch, Julia Tabatabai and Moritz Niesert collected and analyzed data. Aleš Janda, Hanna Renk, Roland Elling, Georg Friedrich Hoffmann, Hans-Georg Kräusslich and Barbara Müller conceived and

designed the study and provided key resources. Ralf Bartenschlager conceived and designed the study, provided key resources and performed laboratory analysis. All authors revised the manuscript for important intellectual content, approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

## ACKNOWLEDGMENTS

We are particularly indebted to all the households that participated in this study. We thank the following staff members for data and sample collection and excellent organizational support: Bettina Haase, Jürgen Grulich-Henn, Kristine Chobanyan-Jürgens, Andreas Ziegler, Julia Euler, Michal Fischer, Iris Schelletter, and Heike Matzkuhn. We thank Christina Klose and Florian Gleich for database assistance. We gratefully acknowledge Stefanie Wolf, Maria Anders-Össwein, Ira Pistorius-Knop, Sylvia Parthé, and Markus Zorn for support in laboratory analyses. The study was funded by the Ministry of Science, Research and the Arts Baden-Württemberg, Germany, within the framework of the special funding line for COVID-19 research, part of the measures to combat the SARS-CoV-2 pandemic in the field of medical research, and the Dietmar Hopp Foundation, St. Leon-Rot, Germany. The funder of the study had no role in design and/or conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. Open Access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Deidentified data will be made available upon publication to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal. Proposals should be submitted to maximilian.stich@med.uni-heidelberg.de.

## ETHICS STATEMENT

The study protocol was approved by the review board and Ethics committee of the Medical Faculty Heidelberg (S-294/2020). The study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all household members and parents or guardians, with assent from children when appropriate for their age.

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## REFERENCES

- Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis.* 2020;20(5):533-534.
- Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 among children in China. *Pediatrics.* 2020;145(6):e20200702.
- Laxminarayan R, Wahl B, Dudala SR, et al. Epidemiology and transmission dynamics of COVID-19 in two Indian states. *Science.* 2020;370(6517):691-697.
- Bundle N, Dave N, Pharris A, Spiteri G, Deogan C, Suk JE. COVID-19 trends and severity among symptomatic children aged 0-17 years in 10 European Union countries, 3 August 2020 to 3 October 2021. *Euro Surveill.* 2021;26(50):2101098
- Accorsi EK, Britton A, Fleming-Dutra KE, et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 omicron and delta variants. *JAMA.* 2022;327(7):639-651.
- Carazo S, Skowronski DM, Brisson M, et al. Estimated protection of prior SARS-CoV-2 infection against reinfection with the omicron variant among messenger RNA-vaccinated and nonvaccinated individuals in Quebec, Canada. *JAMA Netw Open.* 2022;5(10):e2236670.
- CDC. COVID-19 vaccination and case trends by age group, United States. 2022. Accessed October 27, 2022. <https://covid.cdc.gov/covid-data-tracker>
- ECDC. COVID-19 Vaccine Tracker. 2022. Accessed 27 October 2022. <https://vaccinetracker.ecdc.europa.eu>
- Wheatley AK, Juno JA, Wang JJ, et al. Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat Commun.* 2021;12(1):1162.
- Dowell AC, Butler MS, Jinks E, et al. Children develop robust and sustained cross-reactive spike-specific immune responses to SARS-CoV-2 infection. *Nature Immunol.* 2022;23(1):40-49.
- Gentles LE, Kehoe L, Crawford KHD, et al. Dynamics of infection-elicited SARS-CoV-2 antibodies in children over time. *medRxiv.* 2022;1(14):22269235.
- Renk H, Dulovic A, Seidel A, et al. Robust and durable serological response following pediatric SARS-CoV-2 infection. *Nat Commun.* 2022;13(1):128.
- Stich M, Benning L, Speer C, et al. Waning immunity 14 months after SARS-CoV-2 infection. *Pediatrics.* 2022;150(5):e2022057151.
- Garrido C, Hurst JH, Lorang CG, et al. Asymptomatic or mild symptomatic SARS-CoV-2 infection elicits durable neutralizing antibody responses in children and adolescents. *JCI Insight.* 2021;6(17):e150909.
- Bonfante F, Costenaro P, Cantarutti A, et al. Mild SARS-CoV-2 infections and neutralizing antibody titers. *Pediatrics.* 2021;148(3):e2021052173.
- Shen X. Boosting immunity to Omicron. *Nature Med.* 2022;28(3):445-446.
- Chmielewska AM, Czarnota A, Bieńkowska-Szewczyk K, Grzyb K. Immune response against SARS-CoV-2 variants: the role of neutralization assays. *NPJ Vaccines.* 2021;6(1):142.
- Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Med.* 2021;27(7):1205-1211.
- Stich M, Elling R, Renk H, et al. Transmission of severe acute respiratory syndrome coronavirus 2 in households with children,

- southwest Germany, May-August 2020. *Emerging Infect Dis.* 2021;27(12):3009-3019.
20. Benning L, Morath C, Bartenschlager M, et al. Neutralizing antibody activity against the B.1.617.2 (delta) variant 8 months after two-dose vaccination with BNT162b2 in health care workers. *Clin Microbiol Infect.* 2022;28(7):1024.e7-1024.e12.
  21. Tönshoff B, Müller B, Elling R, et al. Prevalence of SARS-CoV-2 infection in children and their parents in southwest Germany. *JAMA Pediatrics.* 2021;175(6):586-593.
  22. Benning L, Morath C, Bartenschlager M, et al. Neutralizing antibody response against the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants after a third mRNA SARS-CoV-2 vaccine dose in kidney transplant recipients. *Am J Transplant (AJT).* 2022;22(7):1873-1883.
  23. Cheng SMS, Mok CKP, Leung YWY, et al. Neutralizing antibodies against the SARS-CoV-2 Omicron variant BA.1 following homologous and heterologous CoronaVac or BNT162b2 vaccination. *Nature Med.* 2022;28(3):486-489.
  24. Gruell H, Vanshylla K, Tober-Lau P, et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. *Nature Med.* 2022;28(3):477-480.
  25. Li J, Wu J, Long Q, et al. Comprehensive humoral and cellular immune responses to SARS-CoV-2 variants in diverse Chinese population. *Research.* 2022;2022:9873831.
  26. Chen LL, Chua GT, Lu L, et al. Omicron variant susceptibility to neutralizing antibodies induced in children by natural SARS-CoV-2 infection or COVID-19 vaccine. *Emerg Microbes Infect.* 2022;11(1):543-547.
  27. Sieber J, Mayer M, Schmidthaler K, et al. Long-Lived immunity in SARS-CoV-2-recovered children and its neutralizing capacity against omicron. *Front Immunol.* 2022;13:882456.
  28. Tang J, Novak T, Hecker J, et al. Cross-reactive immunity against the SARS-CoV-2 Omicron variant is low in pediatric patients with prior COVID-19 or MIS-C. *Nat Commun.* 2022;13(1):2979.
  29. Ou J, Lan W, Wu X, et al. Tracking SARS-CoV-2 Omicron diverse spike gene mutations identifies multiple inter-variant recombination events. *Signal Transduct Target Ther.* 2022;7(1):138.
  30. Chen Y, Tong P, Whiteman N, et al. Immune recall improves antibody durability and breadth to SARS-CoV-2 variants. *Sci Immunol.* 2022;7(78):eabp8328.
  31. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature.* 2021;595(7867):426-431.
  32. Schmidt F, Weisblum Y, Rutkowska M, et al. High genetic barrier to SARS-CoV-2 polyclonal neutralizing antibody escape. *Nature.* 2021;600(7889):512-516.
  33. Buonsenso D, Cusenza F, Passadore L, Bonanno F, De Guido C, Esposito S. Duration of immunity to SARS-CoV-2 in children after natural infection or vaccination in the omicron and pre-omicron era: a systematic review of clinical and immunological studies. *Front Immunol.* 2023;13:1024924.
  34. Bartsch YC, St., St. Denis KJ, Kaplonek P, et al. SARS-CoV-2 mRNA vaccination elicits robust antibody responses in children. *Sci Transl Med.* 2022;14(672):eabn9237.
  35. Hause AM, Shay DK, Klein NP, et al. Safety of COVID-19 vaccination in United States children ages 5 to 11 years. *Pediatrics.* 2022;150(2):2022057313.
  36. Hause AM, Baggs J, Marquez P, et al. Safety monitoring of Pfizer-BioNTech COVID-19 vaccine booster doses among children aged 5-11 years—United States, May 17-July 31, 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71(33):1047-1051.
  37. Hause AM, Marquez P, Zhang B, et al. Safety monitoring of bivalent COVID-19 mRNA vaccine booster doses among children aged 5-11 years—United States, October 12-January 1, 2023. *MMWR Morb Mortal Wkly Rep.* 2023;72(2):39-43.
  38. Hause AM, Marquez P, Zhang B, et al. COVID-19 mRNA vaccine safety among children aged 6 months-5 years—United States, June 18, 2022-August 21, 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71(35):1115-1120.
  39. Lin D-Y, Gu Y, Xu Y, et al. Effects of vaccination and previous infection on omicron infections in children. *N Engl J Med.* 2022;387(12):1141-1143.
  40. Tan SHX, Cook AR, Heng D, Ong B, Lye DC, Tan KB. Effectiveness of BNT162b2 vaccine against omicron in children 5 to 11 years of age. *N Engl J Med.* 2022;387(6):525-532.
  41. Cohen-Stavi CJ, Magen O, Barda N, et al. BNT162b2 vaccine effectiveness against omicron in children 5 to 11 years of age. *N Engl J Med.* 2022;387(3):227-236.
  42. Sacco C, Del Manso M, Mateo-Urdiales A, et al. Effectiveness of BNT162b2 vaccine against SARS-CoV-2 infection and severe COVID-19 in children aged 5-11 years in Italy: a retrospective analysis of January-April, 2022. *Lancet.* 2022;400(10346):97-103.
  43. Fowlkes AL, Yoon SK, Lutrick K, et al. Effectiveness of 2-dose BNT162b2 (Pfizer BioNTech) mRNA vaccine in preventing SARS-CoV-2 infection among children aged 5-11 years and adolescents aged 12-15 years—PROTECT cohort, July 2021-February 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71(11):422-428.
  44. Jang EJ, Choe YJ, Kim RK, Park YJ. BNT162b2 vaccine effectiveness against the SARS-CoV-2 omicron variant in children aged 5 to 11 years. *JAMA Pediatrics.* Published online, January 9, 2023. [10.1001/jamapediatrics.2022.5221](https://doi.org/10.1001/jamapediatrics.2022.5221)
  45. Moss P. The T cell immune response against SARS-CoV-2. *Nature Immunol.* 2022;23(2):186-193.
  46. Zuo J, Dowell AC, Pearce H, et al. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. *Nature Immunol.* 2021;22(5):620-626.
  47. Gao Y, Cai C, Grifoni A, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. *Nature Med.* 2022;28(3):472-476.
  48. Tarke A, Coelho CH, Zhang Z, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell.* 2022;185(5):847-859.e11.
  49. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. *Nature.* 2022;603(7901):488-492.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Stich M, Benning L, Speer C, et al. Live-virus neutralization of the omicron variant in children and adults 14 months after SARS-CoV-2 wild-type infection. *J Med Virol.* 2023;95:e28582. doi:10.1002/jmv.28582