

Stronger and durable SARS-CoV-2 immune response to mRNA vaccines in 5–11 years old children with prior COVID-19

Costanza Di Chiara^{a,b,1,*}, Anna Cantarutti^{c,1}, Maria Raffaella Petrara^d, Francesco Bonfante^e, Elisa Benetti^k, Riccardo Boracchini^c, Luca Bosa^a, Francesco Carmona^f, Chiara Cosma^g, Nicola Cotugno^{h,i}, Marthe Le Prevost^j, Giorgia Martini^a, Alessandra Meneghel^a, Matteo Pagliari^e, Paolo Palma^{h,i}, Elena Ruffoni^f, Annachiara Zin^a, Anita De Rossi^{d,f}, Carlo Giaquinto^{a,b}, Daniele Donà^{a,b,1}, Andrea Padoan^{k,1}

^a Department for Women's and Children's Health, University of Padova, Via Giustiniani, 3 - 35128 Padua, Italy

^b Penta - Child Health Research, Corso Stati Uniti, 4 - 35127 Padua, Italy

^c Department of Statistics and Quantitative Methods, Division of Biostatistics, Epidemiology and Public Health, Laboratory of Healthcare Research and Pharmacoepidemiology, University of Milano-Bicocca, Piazza dell'Ateneo Nuovo, 1 - 20126 Milan, Italy

^d Oncology and Immunology Section, Department of Surgery, Oncology and Gastroenterology, University of Padova, Via Giustiniani, 2 - 35124 Padua, Italy

^e Division of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università, 10 - 35020 Legnaro (Padua), Italy

^f Immunology and Diagnostic Molecular Oncology Unit, Veneto Institute of Oncology IOV-IRCCS, Via Gattamelata, 64 - 35128 Padua, Italy

^g Department of Laboratory Medicine, University-Hospital of Padova, Via Giambattista Belzoni, 160 - 35121 Padua, Italy

^h Unit of Clinical Immunology and Vaccinology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

ⁱ Department of Systems Medicine, University of Rome "Tor Vergata", Piazza Sant'Onofrio, 4 - 00165 Rome, Italy

^j Medical Research Council Clinical Trials Unit at University College London, 90 High Holborn, WC1V 6LJ London, United Kingdom

^k Department of Medicine-DIMED, University of Padova, Via Giustiniani 2, 35128 Padua, Italy

ARTICLE INFO

Keywords:

BNT162b2 mRNA COVID-19 vaccine
Comirnaty
5–11-year-old children
T and B regulatory cells
SARS-CoV-2 anti-S-RBD antibody
SARS-CoV-2 neutralizing antibodies

ABSTRACT

Background and objectives: mRNA vaccines elicit a durable humoral response to SARS-CoV-2 in adults, whereas evidence in children is scarce. This study aimed to assess the early and long-term immune response to the mRNA vaccine in children with or without previous SARS-CoV-2 infection.

Methods: In a multicentre prospective observational study, we profiled the immune response to the Pfizer BioNTech (BNT162b2) vaccine in 5–11-year-old children attending the University Pediatric Hospital of Padua and Bambino-Gesù Hospital in Rome (Italy) from December-2021 to February-2023. Blood samples were collected pre-, 1-, and 6-months after vaccination. Neutralizing antibodies (NAbs) and anti-spike-receptor-binding-domain (anti-S-RBD) IgG titers were analyzed through Plaque Reduction Neutralization Test (PRNT) and chemiluminescent immune-enzymatic assay (CLIA), respectively. Immune cell phenotypes were analyzed by flow cytometry.

Results: Sixty children (26 [43 %] female, median age = 8 years [IQR = 7–10.7]) were enrolled in the study, including 46 children with a laboratory-confirmed previous COVID-19 (SARS-CoV-2-recovered) and 14 SARS-

Abbreviations: mRNA, Messenger RNA; Tregs, Regulatory T and B (Bregs); VOCs, Variants of concern; BNT162b2, Pfizer BioNTech mRNA COVID-19 vaccine; NPS, Nasopharyngeal swab; HC, Healthy children; IC, Immunocompromised children; SOT, Solid organ transplant; MIS-C, Multisystem inflammatory syndrome in children; anti-S-RBD, Binding IgG antibodies to SARS-CoV-2 spike protein; Nabs, Neutralizing antibodies; WT, Wild-type; B.1.1.529 BA.2, Omicron; PBMCs, Peripheral blood mononuclear cells; dRT-PCR, Direct real time PCR; WHO, World Health Organization; IQR, Interquartile range; GMT, Geometric mean titer.

* Corresponding author at: Division of pediatric infectious diseases, Department for Women's and Child's health, University of Padua, Via Giustiniani 3, Padua, Italy

E-mail addresses: costanza.dichiara@phd.unipd.it (C. Di Chiara), anna.cantarutti@unimib.it (A. Cantarutti), raffaella.petrara@unipd.it (M. Raffaella Petrara), FBonfante@izsvenezie.it (F. Bonfante), elisa.benetti@aopd.veneto.it (E. Benetti), riccardo.boracchini@unimib.it (R. Boracchini), luca.bosa@phd.unipd.it (L. Bosa), francesco.carmona@iov.veneto.it (F. Carmona), chiara.cosma@aopd.veneto.it (C. Cosma), nicola.cotugno@opbg.net (N. Cotugno), m.leprevost@ucl.ac.uk (M. Le Prevost), giorgia.martini64@gmail.com (G. Martini), alessandra.meneghel@aopd.veneto.it (A. Meneghel), MPagliari@izsvenezie.it (M. Pagliari), paolo.palma@opbg.net (P. Palma), elena.ruffoni@iov.veneto.it (E. Ruffoni), annachiara.zin@gmail.com (A. Zin), anita.derossi@unipd.it (A. De Rossi), carlo.giaquinto@unipd.it (C. Giaquinto), daniele.dona@unipd.it (D. Donà), andrea.padoan@unipd.it (A. Padoan).

¹ Contributed as co-first and co-senior authors.

<https://doi.org/10.1016/j.vaccine.2023.12.006>

Received 14 August 2023; Received in revised form 8 November 2023; Accepted 1 December 2023

Available online 8 December 2023

0264-410X/© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

CoV-2-naïve participants defined as the absence of antigen-specific antibodies before vaccination. SARS-CoV-2-recovered participants recorded higher anti-S-RBD IgG and Wild-type and Omicron BA.2 NAbs titers than SARS-CoV-2-naïve participants at both 1- and 6-months after vaccination. Antibody titers correlated with T (Tregs) and B (Bregs) regulatory cell frequencies in SARS-CoV-2-recovered children. Both SARS-CoV-2-recovered and SARS-CoV-2-naïve participants decreased antibody titers by approximately 100 to 250 % from 1 to 6 months. While children with immunocompromising underlying conditions developed immune responses comparable to those of healthy children, solid organ transplant recipients exhibited lower levels of NAbs and anti-S-RBD IgG titers, as well as reduced frequencies of Tregs and Bregs.

Conclusions: mRNA vaccination triggered a higher production of specific anti-SARS-CoV-2 antibodies along with increased levels of regulatory cells in children with previous SARS-CoV-2 infection up to the following 6 months. These findings provide insights into boosting pre-existing immunity.

1. Background

Messenger RNA (mRNA) COVID-19 vaccines have been widely reported to induce a strong specific humoral and cellular response to SARS-CoV-2 [1,2], with individuals who had a prior SARS-CoV-2 infection exhibiting higher immune response than those who were SARS-CoV-2-naïve and vaccinated [3–8]. However, a progressive decline in antigen-specific antibody levels was observed over the six months post-vaccination, regardless of previous SARS-CoV-2 infection [1,3,9].

To date, research has predominantly focused on adolescents and adults, and there is a knowledge gap on the long-term immune response to COVID-19 vaccination in children.

Studies on individuals who recovered from COVID-19 have shown an inverse correlation of antigen-specific antibody titers with age, with more intense humoral responses to SARS-CoV-2 infection in young children than adults [10,11]. Additionally, children who recovered from SARS-CoV-2 infection had a higher expansion of regulatory T (Tregs) and B (Bregs) cells than adults, likely by mitigating the viral-induced inflammation/chronic immune activation, leading to a higher specific production of anti-SARS-CoV-2 antibodies [12].

The emergence of multiple SARS-CoV-2 variants of concern (VOCs) has raised alarms about the potential increased transmission and evasion from vaccine-conferred immunity [13]. Therefore, a better understanding of the antigen-specific immune response and durability after mRNA vaccination in healthy and immunocompromised children with or without previous SARS-CoV-2 infection is necessary to inform public health policies and optimize vaccination strategies in the pediatric population.

2. Materials and methods

2.1. Study design and data collection

A prospective, observational study was conducted from December/2021 to February/2023 to profile the longitudinal immunogenicity of mRNA COVID-19 vaccine on 5–11-year-old children attending the Pediatric University Hospital of Padua and the Bambino Gesù Children's Hospital in Rome (Italy).

Children met the following criteria for enrollment: a) aged between 5 and 11 years at the time of vaccination and b) had received a 2-doses primary series of the Pfizer BioNTech mRNA COVID-19 vaccine (BNT162b2) (10- μ g doses with an interval between doses of 21 days) or c) had received at least one dose of the BNT162b2 within 12 months after previous laboratory-confirmed SARS-CoV-2 infection [14].

Whole blood samples were collected at three time points: pre-vaccination, \sim 1 month after vaccination, and \sim 6 months after vaccination. A detailed description of the blood sample collection procedure is reported in the eMethods in the supplement.

Patient follow-up was stopped in the event of a new probable or confirmed close contact with a COVID-19 case and/or a laboratory-confirmed SARS-CoV-2 infection.

Participants' anonymized demographic and clinical characteristics

as well as laboratory findings from enrollment and follow-up were entered into a web-based database using the REDCap® platform (Vanderbilt University, Tennessee). The study protocol is approved by the local Ethics Committee (Prot. N° 0,070,714 of November 24th, 2020; last amendment Prot. N° 0,024,018 del 5/4/2022). Parents/legally authorized representatives were informed of the research proposal and provided written consent to use the routinely collected patient data.

2.2. Case identification and definitions

Children who had evidence of SARS-CoV-2 infection (i.e., a molecular or antigenic COVID-19 nasopharyngeal swab (NPS) and/or positive SARS-CoV-2 serology test at the pre-vaccination assessment) preceding vaccination were identified as SARS-CoV-2-recovered. For each SARS-CoV-2-recovered participant, the index date of infection was established based on the date of the first positive NPS. SARS-CoV-2 infections before December 11, 2021, or after January 7, 2022, were classified as pre-Omicron or Omicron infections, respectively, based on Italy's predominant circulating SARS-CoV-2 VOC [15]. Children were identified as SARS-CoV-2-naïve if they had no analytical evidence of SARS-CoV-2 infection at the pre-vaccination assessment.

Children were further classified into four groups based on the presence, or not, of comorbidities: 1) healthy children (i.e., no comorbidities) (HC), 2) immunocompromised children (i.e., an immune-inflammatory chronic renal and rheumatological disease requiring an immunomodulatory therapy) (IC), 3) solid organ transplant recipients (i.e., liver or renal organ transplant recipients on anti-rejection regimen) (SOT), and 4) having a previous diagnosis of multisystem inflammatory syndrome in children (MIS-C).

2.3. Serological assays

Binding IgG antibodies to SARS-CoV-2 spike protein (anti-S-RBD) and neutralizing antibodies (NAbs) to Wild-type (WT) and Omicron (B.1.1.529 BA.2) VOC were measured as previously described (eMethods in the supplemental) [16,17].

2.4. Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were thawed, washed, and stained for 20 min in the dark with the Live/Dead Fixable Near-IR Dead Cell Stain Kit (Life Technologies, Carlsbad, CA, USA) and with monoclonal antibodies, as previously described (eMethods and eFigure1 in the supplemental) [12].

2.5. CXCL10 mRNA expression

T-cell activation was assessed following an incubation period of 16–18 h by measuring the CXCL10 mRNA expression by direct real time PCR (dRT-PCR) (bKIT™ Immunofinder dqTACT [Ref. HK032X300]), a method already validated with respect to an IGRA assay, quantitatively measuring IFN γ . The assay used allow to amplify the mRNA of the gene CXCL10, which is a proxy of the activation of CD8 + cells and the detail

of the procedure is reported in eMethods in the supplemental [18]. Every sample (whole blood from every patient) was stimulated in a single biological replicate and tested in a single technical replicate.

2.6. Statistical analyses

Descriptive statistics were used to compare the distribution of gender, age, COVID-19 WHO clinical classification [19], COVID-19 vaccination, and pediatric comorbidities (HC, IC, SOT, and MIS-C), overall and among SARS-CoV-2-recovered and SARS-CoV-2-naïve participants.

The production and long-term persistence of antibody and immune cells titers following mRNA vaccination were assessed by comparing: 1) the median and interquartile range (IQR) of anti-S-RBD IgG antibody titers and immune cells, and 2) the geometric mean titer (GMT) and the 95 % confidence interval (95 % CI), of NAbs against WT virus and BA.2 VOC. Median and IQR and GMT and 95 % CI of antibodies and immune cells titers were evaluated overall and stratified by SARS-CoV-2-recovered and SARS-CoV-2-naïve participants at 1- and 6-months after vaccination. The Wilcoxon Rank Sum test and the sample *t*-test were performed, respectively.

To describe the antibodies and immune cells' kinetics over time, we conducted a longitudinal analysis on an intra-participant-paired sample from a sub-cohort of 39 SARS-CoV-2-recovered and 5 SARS-CoV-2-naïve children tested at 1- and 6-months after vaccination. The decay of the Abs was evaluated as $\frac{Absvalueat6mo - Absvalueat1mo}{Absvalueat6mo} * 100$. The Signed Rank test and the paired *t*-test were performed, where appropriate, to compare values at 1- and 6-months after vaccination among SARS-CoV-2-recovered and SARS-CoV-2-naïve children, respectively.

The association between anti-S-RBD IgG and NAbs titers at 6-months follow-up was assessed with a linear regression model. The strength of associations between variables was evaluated by the Pearson correlation coefficient, using the logarithm (base 2) of the antibody titers given data skew.

Moreover, to better investigate the effect of mRNA vaccination in subjects with pre-existing immunity from a prior SARS-CoV-2 infection, we conducted a longitudinal analysis on an intra-subject-paired sample from a sub-cohort of 18 SARS-CoV-2-recovered children tested at pre-vaccination, 1-, and 6-months after vaccination to evaluate the trend over time of antibodies and immune cells. The increase from pre- to 6-months assessment was evaluated as $\frac{Absvalueat6mo - preAbsvalue}{Absvalueat6mo} * 100$.

Finally, Tregs and Bregs frequencies were correlated with levels of NAbs to WT and BA.2, and anti-S-RBD IgG antibody titers evaluating the Spearman correlation due to data skew.

All analyses were also performed, stratifying by HC, IC, SOT, and MIS-C. However, since the multiple stratifications and comparison, we performed a descriptive analysis only.

Analyses were performed using the Statistical Analysis System software (version 9.4; SAS Institute, Cary, North Carolina). Statistical significance was set at the level of 0.05. All P values were 2-sided. Graphs were made using GraphPad Prism version 9 (GraphPad Software, La Jolla, CA).

3. Results

3.1. Cohort design and participants' characteristics

In total, 135 longitudinal samples were collected from 60 children aged 5–11 years receiving the BNT162b2 vaccine at 3-time points, ranging from pre-vaccination to 6-months after vaccination. Table 1 (eFigure2, eTable1) shows the characteristics of the 60 participants, of which 26 (43 %) were females, 46/60 (77 %) were SARS-CoV-2-recovered, and 14/60 (23 %) SARS-CoV-2-naïve. All SARS-CoV-2-recovered children had been infected during the pre-Omicron era.

Table 1

Demographic and clinical characteristics of the study population by SARS-CoV-2-recovered and SARS-CoV-2-naïve cases.

	Overall	SARS-CoV-2-recovered	SARS-CoV-2-naïve
Cases, N (%)	60	46 (77)	14 (23)
Age in years, median (P25 - P75)^a	9 (7 – 11)	9 (7 – 10)	10.5 (8 – 11)
Gender, N (%)			
Female	26 (43)	17 (37)	9 (64)
Male	34 (57)	29 (63)	5 (36)
COVID-19 WHO clinical classification, N (%)^b			
asymptomatic	–	18 (38)	–
mild	–	21 (45)	–
moderate	–	0 (0)	–
severe	–	0 (0)	–
MIS-C	–	8 (17)	–
Comorbidities categories, N (%)			
Healthy children [HC]	40 (67)	36 (77)	4 (31)
Immunocompromised children [IC]	8 (13)	2 (4)	6 (46)
Solid organ transplant recipients [SOT]	4 (7)	1 (2)	3 (23)
COVID-19 vaccination, N (%)			
1-dose primary series	9 (15)	6 (13)	3 (21)
2-dose primary series	51 (85)	40 (87)	11 (100)
Time from infection to vaccination, months, median (IQR)	–	13 (7–14)	–

^a Subject's age at vaccination.

^b The severity of COVID-19 was scored according to the World Health Organization (WHO) definition as mild, moderate, severe, critical, or Multisystem inflammatory syndrome in children (MIS-C) [18].

3.2. Antibody response and antibody kinetics up to 6 months following COVID-19 mRNA vaccination

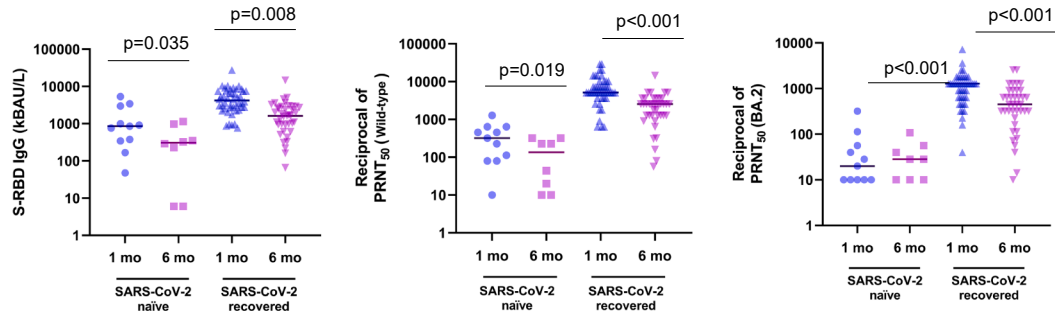
COVID-19 mRNA vaccination induced higher anti-S-RBD IgG antibody titers in SARS-CoV-2-recovered compared to SARS-CoV-2-naïve children at both 1- ($p = 0.03$) and 6-months ($p = 0.01$) post-vaccination (Fig. 1A, eTable2 in the supplemental). SARS-CoV-2-recovered children also recorded higher WT NAbs titers compared to SARS-CoV-2-naïve participants at both 1- ($p = 0.02$) and 6-months ($p < 0.001$) after vaccination (Fig. 1A, eTable2 in the supplemental). Similarly, SARS-CoV-2-recovered showed higher BA.2 NAbs titers compared to SARS-CoV-2-naïve participants at both 1- ($p < 0.001$) and 6-months ($p < 0.001$) post-vaccination (Fig. 1A).

Fig. 1B (eTable2 in the supplemental) shows the antibody kinetics over time. All individuals showed decreased anti-S-RBD IgG and WT and BA.2 NAbs titers, regardless of a previous COVID-19. Further cross-sectional analysis of 6-month antibody titers demonstrated that anti-S-RBD IgG remained highly correlated with NAbs, indicating that anti-S-RBD IgG antibodies retain their functional characteristics and neutralization capacity over time (eFigure3 in the supplemental).

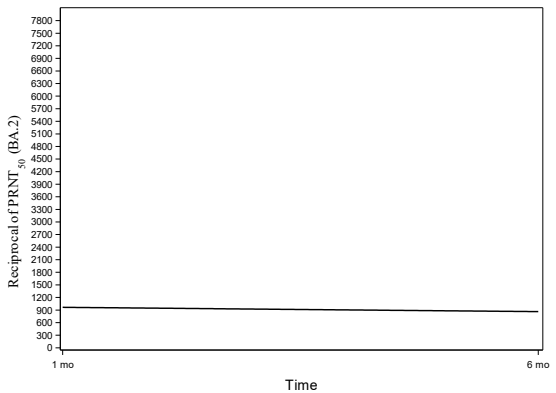
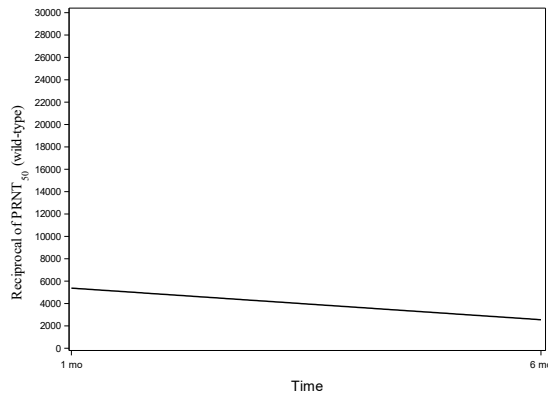
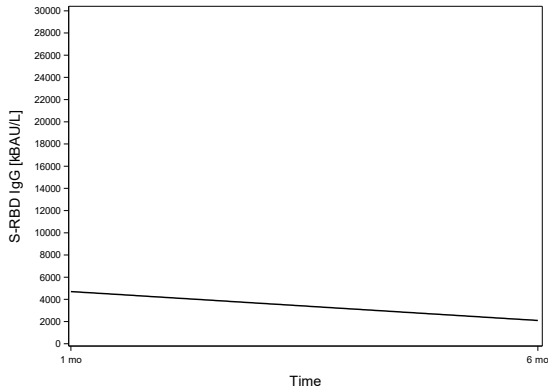
Fig. 1C (eTable3 in the supplemental) shows the antibodies kinetics from pre-vaccination, 1-, and 6-months post-vaccination in SARS-CoV-2-recovered participants. Both anti-S-RBD IgG antibodies and NAbs titers showed a robust increase at a 1-month time point post-vaccination compared to the pre-vaccination titer. In addition, despite a decay in antibody titers over time, both anti-S-RBD IgG antibodies and NAbs titers at 6-months remained higher than the pre-vaccination levels.

In an analysis assessing the humoral response to SARS-CoV-2 vaccination stratifying participants by comorbidity (i.e., HC, IC, SOT, and MIS-C), both the SARS-CoV-2-recovered SOT and the SARS-CoV-2-naïve SOT recipient showed a 3.5-fold and 5.5-fold lower anti-S-RBD IgG titers at 1-month after vaccination compared to SARS-CoV-2-recovered and SARS-CoV-2-naïve HC, respectively. Similarly, SOT recipients achieved a lower neutralizing response than HC against both WT and BA.2 strains at 1-month after vaccination, regardless of a previous SARS-CoV-2 infection. Moreover, SOT recipients showed lower antibody responses

Panel A



Panel B



Panel C

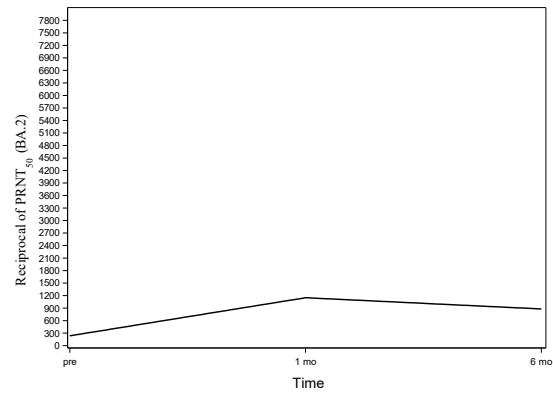
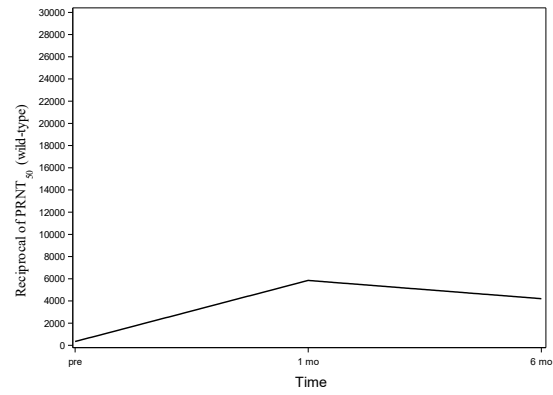
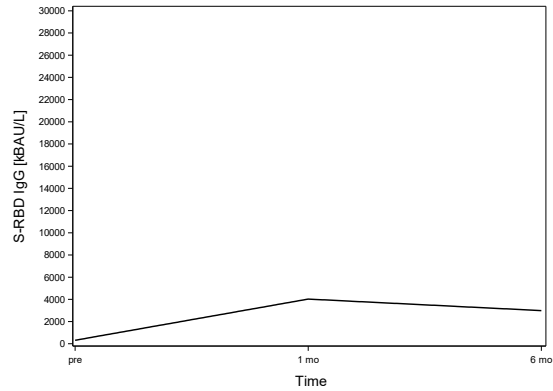


Fig. 1. Magnitude and kinetics of the neutralizing antibody titers to Wild-type (WT) and Omicron BA.2 strains and anti-S-RBD IgG antibody titers following mRNA vaccination in SARS-CoV-2-recovered cases compared to SARS-CoV-2-naïve cases. *Panel A* shows titers of anti-S-RBD IgG, NAbS to WT, and NAbS to Omicron BA.2 in SARS-CoV-2-recovered and SARS-CoV-2-naïve cases at 1- (blu dots) and 6-months (purple dots) after vaccination. The Signed Rank test and the paired *t*-test were performed, appropriately. *Panel B* shows the individual (grey lines) and overall (black lines) kinetics of anti-S-RBD IgG, NAbS to WT, and NAbS to Omicron BA.2 titers between 1- and 6-months after vaccination in 39 SARS-CoV-2-recovered and 5 SARS-CoV-2-naïve cases who were tested at both time points. *Panel C* shows the individual (grey lines) and overall (black lines) boosting effect of mRNA vaccination on anti-S-RBD IgG, NAbS to WT, and NAbS to Omicron BA.2 titers in 18 subjects with pre-existing immunity from a prior SARS-CoV-2 infection who were tested at 3 time points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compared to HC at 6-months after vaccination, irrespective of prior SARS-CoV-2 infection (eFigure4, eTable4 in the supplemental).

Conversely, MIS-C participants recorded similar anti-S-RBD IgG antibody titers to HC, at both 1- and 6-months post-vaccination. WT and BA.2 NAb titers were also similar between MIS-C and HC at both 1- and 6-months after vaccination (eFigure4, eTable4 in the supplemental).

3.3. Cellular immune profile up to 6-months following COVID-19 mRNA vaccination

Fig. 2 (eTable5 in the supplemental) shows the immunological parameters compared between SARS-CoV-2-recovered and SARS-CoV-2-naïve cases at 1- and 6-months after vaccination. No difference was observed in the frequencies of activated and senescent T (CD4 and CD8) and B cells at both time points. The frequencies of Tregs were higher in SARS-CoV-2-recovered than in SARS-CoV-2-naïve children both at 1- ($p < 0.001$) and 6-months after vaccination ($p = 0.02$). Similarly, the frequencies of Bregs were higher in SARS-CoV-2-recovered than in SARS-CoV-2-naïve children both at 1- ($p = 0.02$) and 6-months after vaccination ($p = 0.004$) (Fig. 2). Within the SARS-CoV-2-recovered participants, Tregs and Bregs frequencies were positively correlated with levels of anti-S-RBD IgG antibody, WT NAb, and BA.2 NAb titers at both 1- and 6-months after vaccination (eFigure5, eFigure6 in the supplemental). No correlation was found between regulatory cells and NAb in SARS-CoV-2-naïve children.

Immune-activated CD4 cells decreased in SARS-CoV-2-recovered cases ($p = 0.03$) between 1 and 6-months post-vaccination. Similarly, Tregs decreased their percentage between 1- and 6-months follow-up in SARS-CoV-2-recovered participants ($p = 0.01$) (eTable6 in the supplemental). Conversely, the rate of senescent CD4 cells increased in SARS-CoV-2-recovered cases between 1- and 6-months after vaccination ($p < 0.001$). No differences in the kinetics of T and B subpopulations in SARS-CoV-2-naïve children were found (eTable6 in the supplemental).

The longitudinal analysis on a subject-paired sample from a sub-cohort of 18 SARS-CoV-2-recovered participants tested at pre-vaccination, 1-, and 6-months after vaccination showed that both Tregs and Bregs were boosted at 1-month postvaccination compared to the pre-vaccination assessment, and decreased at 6-months after vaccination (eTable7 in the supplemental).

Immune profile assessed stratifying participants by comorbidities showed similar percentages of activated and senescent T and B cells among the HC, IC, SOT, and MIS-C groups, regardless of a previous SARS-CoV-2 infection. The SARS-CoV-2-recovered SOT recipient showed lower percentages of Tregs and Bregs compared to HC, IC, and MIS-C at both 1- and 6-months after vaccination (eTable8 in the supplemental).

3.4. T-cell immunoreactivity against SARS-CoV-2 peptides at 6-month after mRNA vaccination

Similar proportions of children who had a positive CXCL10 mRNA expression were found between SARS-CoV-2-recovered (36/38, 95 %) and SARS-CoV-2-naïve children (8/9, 89 %) (eTable9 in the supplemental).

4. Discussion

To the best of our knowledge, our study represents one of the first immunological studies evaluating the long-term immune response to the BNT162b2 vaccine in 5–11-years-old children. We evaluated the dynamic changes of the antigen-specific humoral and cellular responses to mRNA COVID-19 vaccination in a prospective cohort of 60 children aged 5–11 years with or without a previous asymptomatic or mild SARS-CoV-2 infection up to 6 months following vaccination. We observed that mRNA vaccination induced robust circulating antibody responses to SARS-CoV-2 with a distinct response profile in both SARS-CoV-2-

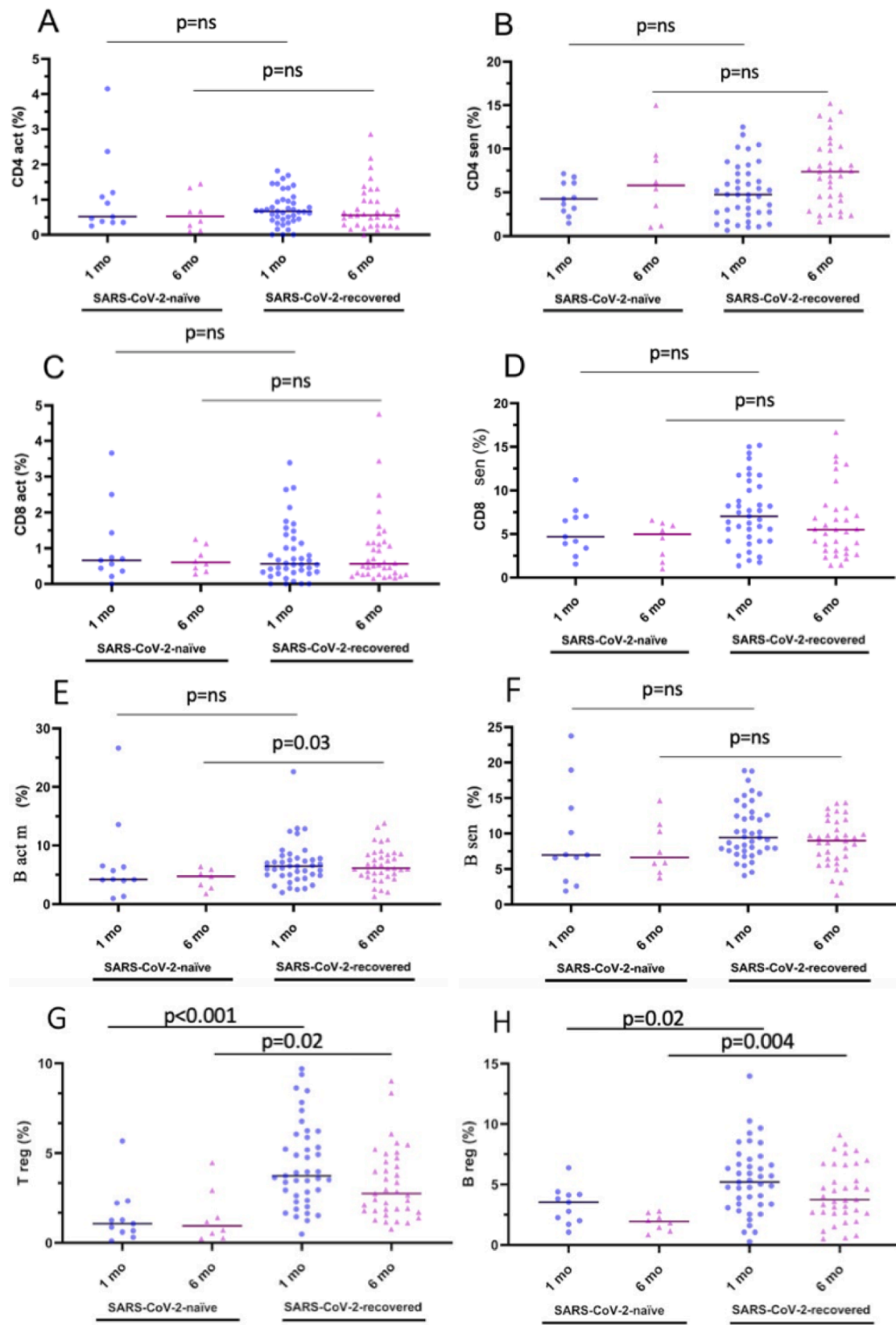
recovered and SARS-CoV-2-naïve children. We documented that mRNA vaccines elicited higher humoral responses and higher Tregs and Bregs frequencies in SARS-CoV-2-recovered compared to naïve-vaccinated children at both 1- and 6-months after vaccination. Our findings are similar to several studies documenting a stronger and more durable immune response to BNT162b2 vaccine in adults [1,20,21]. Additionally, these results are in line with recently published studies, showing higher levels of binding antibody and Omicron-targeted NAb titers after mRNA vaccination in children with hybrid immunity compared to SARS-CoV-2-naïve subjects [22–24]. These observations offer valuable insights into enhancing pre-existing immunity and lend support to recent research demonstrating the significance of administering the BNT162b2 vaccine to SARS-CoV-2-recovered children to prevent subsequent reinfection [25].

In this study, we strengthened and extended upon previous findings documenting the persistence of detectable specific NAb and binding antibody titers up to 6-months after vaccination in both SARS-CoV-2-recovered and SARS-CoV-2-naïve children, despite an observed decline in their titers over time. Moreover, a cross-sectional analysis of 6-months humoral response also demonstrated that binding antibodies remained highly correlated with neutralizing titers to WT, indicating that S-RBD-specific IgG responses retain their functional characteristics and neutralizing capacity over time. The persistence of higher titers of specific antibodies at 6-months post-vaccination compared to pre-vaccination, as well as the correlation of anti-S-RBD IgG with neutralizing titers, were also documented in adults, highlighting the importance of boosting a pre-existing immunity to confer a longer durable protection against SARS-CoV-2 reinfection [2].

The repeated exposure upon vaccination boosts SARS-CoV-2-specific T CD4 cells, responsible for recall and expansion of memory B cells, resulting in a more robust humoral response in SARS-CoV-2-recovered compared to naïve-vaccinated subjects. Specifically, hybrid immunity, marked by the presence of both spike and non-spike memory T cells, exhibits heightened and broader production of binding and variant-neutralizing antibodies, leading to a cross-reactive humoral response to Omicron VOC [21,26].

In this study, we found higher levels of Tregs and Bregs in SARS-CoV-2-recovered than SARS-CoV-2-naïve participants at both 1- and 6-months after vaccination. This suggests that the greater expansion of Tregs and Bregs in SARS-CoV-2-recovered children might have resulted in a higher specific production of anti-SARS-CoV-2 antibodies after vaccination in these children compared to SARS-CoV-2-naïve children. Given the lack of disparities in the frequencies of immune activation between SARS-CoV-2-recovered and SARS-CoV-2-naïve children, it is plausible to hypothesize that vaccination does not trigger cytokine storm or aspecific immune activation, resulting in a reduced occurrence of regulatory cells in SARS-CoV-2-naïve children compared to SARS-CoV-2-recovered children following vaccination. Consequently, the greater levels of regulatory cells observed in hybrid immunity, attributed to prior infection rather than vaccination *per se*, may play a key role in enhancing a greater specific humoral response against the virus, likely through their suppressive activity of the aspecific immune response [12]. Changes in circulating Tregs have been investigated after vaccination against pathogens other than SARS-CoV-2 [27]. An increased frequency of Tregs was observed after the live attenuated yellow fever vaccine and the Hepatitis B vaccine [28]. In contrast, adjuvanted or non-adjuvanted influenza vaccines did not affect the expansion of regulatory cells [28]. The effects of different vaccines on Tregs dynamics may depend on the properties of the vaccine. Furthermore, the simultaneous administration of multiple vaccines may trigger complex immunologic milieu where cytokines, B cells and possibly Tregs could contribute to either the enhancement or the dampening of immunologic responses [28].

Interestingly, IC children, who were treated with immunosuppressor/immunomodulant regimens developed a comparable humoral and cellular response to HC, regardless of previous SARS-CoV-2-



(caption on next page)

Fig. 2. Immune cell profile in SARS-CoV-2-recovered and SARS-CoV-2-naïve cases at 1- (blu dots) and 6-months (purple dots) after mRNA COVID-19 vaccination. The Signed Rank test was performed appropriately. *Panel A* shows %CD4 activation (CD4 act) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel B* shows %CD4 senescence (CD4 sen) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel C* shows %CD8 activation (CD8 act) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel D* shows %CD8 senescence (CD8 sen) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel E* shows %B activated memory (B act m) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel F* shows %B senescence (B sen) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel G* shows %T-regulatory cells (Tregs) cells at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants; *Panel H* shows %B-regulatory cells (Bregs) at 1- and 6-months after vaccination in SARS-CoV-2-naïve and SARS-CoV-2-recovered participants. ns: not significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

infection. On the other hand, both SARS-CoV-2-recovered and SARS-CoV-2-naïve SOT recipients showed significantly lower antibody and cell titers compared to HC after 2-dose mRNA vaccination. Similarly, SOT recipients showed lower Tregs and Bregs frequencies than HC. These results might suggest the need for an additional dose of primary series [29], a higher dosage of vaccines [24], or additional preventive strategies such as monoclonal antibodies to prevent severe diseases in these fragile patients. Our findings are in line with several studies demonstrating a lower magnitude of immune response to mRNA vaccines, especially in SOT recipient adults [30–32].

Antibody titers and immune cell profiles were boosted with vaccination in MIS-C cases, with a higher anti-S-RBD IgG response than HC, despite a lower neutralization power of their sera compared to HC. Similarly, Perez et al. [33] recently demonstrated that BNT162b2 primary series boosted broadly cross-reactive binding antibodies in children with a previous MIS-C which were maintained for up to 3 months. However, the NAbs levels were low, especially against the Omicron variant.

Our study has several limitations. First, the overall number of subjects was small, which limited our ability to thoroughly explore the impact of an immunocompromising condition on the immune response to mRNA vaccination. Second, the time points used in this study did not allow us to capture the full kinetics of the immune response. An additional sampling timepoint 3 months post-vaccination could have allowed for a better assessment of antibody and cell decays over time. Lastly, the lack of evaluation of memory B cell responses meant we were not able to describe the prolonged germinal centre reactions nor evaluate the durability of cell protection against reinfection.

In conclusion, we demonstrated the magnitude and kinetics of humoral and cellular responses to mRNA vaccination in 5–11 years old children up to 6 months post-vaccination and showed higher antibody peaks for SARS-CoV-recovered children at every follow-up time point.

Previous studies have demonstrated a correlation between SARS-CoV-2-specific antibody levels and protection from reinfection, thus implying the presence of humoral correlates of protection [34]. Therefore, the decline in vaccine-induced immunity might be reflected in an increased susceptibility to reinfection 5-months post-immunization, underscoring the importance of booster vaccination schedules in the pediatric population [21].

This study provides valuable insights into enhancing preexisting immunity with mRNA COVID-19 vaccines in children with a prior SARS-CoV-2 infection. Moreover, these findings can aid in establishing vaccination schedules for non-previously infected children and determining the optimal timing for booster immunization in pediatric patients who have already had COVID-19.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The corresponding author would like to thank Dr. Bertilla Ranzato for her support in patient enrollment. The authors thank all the family pediatricians collaborating with the project. The authors thank all families who attended the CovFC of the University Hospital of Padova.

Funding/support

This work is partially supported by ORCHESTRA, a three-year international research project aimed at tackling the coronavirus pandemic, funded by the European Union's Horizon 2020 research and innovation program (H2020-RIA GA No.101016167). The views expressed in this document are the sole responsibility of the author and the Commission is not responsible for any use that may be made of the information contained therein.

The evaluation of the neutralizing humoral response against different SARS-CoV-2 viral variants is part of the VERDI project (101045989), which is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

Contributors statement

Dr. Costanza Di Chiara designed the study, provided reagents and samples, conducted data interpretation, and wrote the original draft of the manuscript. Dr. Anna Cantarutti performed the statistical analysis and wrote the original draft of the manuscript. Dr. Maria Raffaella Petrara performed the investigations, conducted data interpretation, and contributed to the writing of the original draft of the manuscript. Dr. Francesco Bonfante designed the study, performed the investigations, conducted data interpretation, and critically reviewed the manuscript for important intellectual content. Dr. Riccardo Boracchini performed the statistical analysis and contributed to drafting the manuscript. Drs. Elisa Benetti, Luca Bosa, Nicola Cotugno, Giorgia Martini, Alessandra Meneghel, and Annachiara Zin provided reagents and samples and critically reviewed the manuscript. Drs. Francesco Carmona, Chiara Cosma, and Elena Ruffoni performed the investigations and contributed to drafting the manuscript. Dr. Marthe Le Prevost supervised the project, contributed to data analysis, and drafted the manuscript. Drs. Anita De Rossi, Carlo Giaquinto, Daniele Donà, and Andrea Padoan designed the study, supervised the project, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2023.12.006>.

References

- [1] Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. 374(6572):abm0829 Science 2021. <https://doi.org/10.1126/science.abm0829>.
- [2] Painter MM, Mathew D, Goel RR, et al. Rapid induction of antigen-specific CD4+ T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity* 2021;54(9):2133–2142.e3. <https://doi.org/10.1016/j.immuni.2021.08.001>.
- [3] Goel RR, Apostolidis SA, Painter MM, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals following mRNA vaccination. 6(58):eabi6950 *Sci Immunol* 2021. <https://doi.org/10.1126/sciimmunol.abi6950>.
- [4] Bates TA, McBride SK, Leier HC, et al. Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and antibodies that effectively neutralize variants. 7(68):eabn8014 *Sci Immunol* 2022. <https://doi.org/10.1126/sciimmunol.abn8014>.
- [5] Tejedor Vaquero S, de Campos-Mata L, Ramada JM, et al. The mRNA-1273 vaccine induces cross-variant antibody responses to SARS-CoV-2 with distinct profiles in individuals with or without pre-existing immunity. *Front Immunol*. 2021;12:737083. Published 2021 Sep 3. doi:10.3389/fimmu.2021.737083.
- [6] Oberhardt V, Luxenburger H, Kemming J, et al. Rapid and stable mobilization of CD8+ T cells by SARS-CoV-2 mRNA vaccine. *Nature* 2021;597(7875):268–73. <https://doi.org/10.1038/s41586-021-03841-4>.
- [7] Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol* 2022;23(2):186–93. <https://doi.org/10.1038/s41590-021-01122-w>.
- [8] La Gualana F, Maiorca F, Marrapodi R, et al. Opposite effects of mRNA-based and adenovirus-vectored SARS-CoV-2 vaccines on regulatory T cells: a pilot study. *Biomedicines* 2023. <https://doi.org/10.3390/biomedicines11020511>. Published 2023 Feb 10, 11(2):511.
- [9] Padoan A, Cosma C, Bonfante F, et al. Neutralizing antibody titers six months after Comirnaty vaccination: kinetics and comparison with SARS-CoV-2 immunoassays. *Clin Chem Lab Med*. 2021;60(3):456–463. Published 2021 Dec 16. doi:10.1515/cclm-2021-1247.
- [10] Yang HS, Costa V, Racine-Brzostek SE, et al. Association of age with SARS-CoV-2 antibody response. *JAMA Netw Open* 2021. <https://doi.org/10.1001/jamanetworkopen.2021.4302>. Published 2021 Mar 1, 4(3):e214302.
- [11] Di Chiara C, Cantarutti A, Costenaro P, et al. Long-term immune response to SARS-CoV-2 infection among children and adults after mild infection. *JAMA Netw Open*. 2022;5(7):e2221616. Published 2022 Jul 1. doi:10.1001/jamanetworkopen.2022.21616.
- [12] Petrara MR, Bonfante F, Costenaro P, et al. Asymptomatic and mild SARS-CoV-2 infections elicit lower immune activation and higher specific neutralizing antibodies in children than in adults. *Front Immunol*. 2021;12:741796. Published 2021 Sep 30. doi:10.3389/fimmu.2021.741796.
- [13] Zhou D, Dejnirattisai W, Supasa P, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* 2021;184(9):2348–2361.e6. <https://doi.org/10.1016/j.cell.2021.02.037>.
- [14] Food and Drug Administration. FDA authorizes Pfizer-BioNTech COVID-19 vaccine for emergency use in children 5 through 11 years of age [cited 2021 Dec 27], accessed on May 21, 2023. <https://www.fda.gov/news-events/press-announcements/fda-authorizes-pfizer-biontech-covid-19-vaccine-emergency-use-children-5-through-11-years-age> External Link.
- [15] Chen C, Nadeau S, Yared M, et al. CoV-spectrum: analysis of globally shared SARS-CoV-2 data to identify and characterize new variants. *Bioinformatics* 2022;38(6):1735–7. <https://doi.org/10.1093/bioinformatics/btab856>.
- [16] Bonfante F, Costenaro P, Cantarutti A, et al. Mild SARS-CoV-2 infections and neutralizing antibody titers. *Pediatrics* 2021;148(3). <https://doi.org/10.1542/peds.2021-052173>. e2021052173.
- [17] Padoan A, Bonfante F, Cosma C, et al. Analytical and clinical performances of a SARS-CoV-2 S-RBD IgG assay: comparison with neutralization titers. *Clin Chem Lab Med* 2021;59(8):1444–52. <https://doi.org/10.1515/cclm-2021-0313>. Published 2021 Apr 14.
- [18] Cosma C, Galla L, Padoan A, et al. SARS-CoV-2 specific T-cell humoral response assessment after COVID-19 vaccination using a rapid direct real-time PCR amplification (published online ahead of print, 2023 Mar 24). 10.1515/cclm-2023-0129 *Clin Chem Lab Med* 2023. <https://doi.org/10.1515/cclm-2023-0129>.
- [19] Clinical management. https://apps.who.int/iris/bitstream/handle/10665/338871/WHO-2019-nCoV-clinical-web_annex-2021.1-eng.pdf (2021).
- [20] Spinardi JR, Srivastava A. Hybrid immunity to SARS-CoV-2 from infection and vaccination—evidence synthesis and implications for new COVID-19 vaccines. *Biomedicines*. 2023;11(2):370. Published 2023 Jan 27. doi:10.3390/biomedicines11020370.
- [21] Pooley N, Abdoal Karim SS, Combadière B, et al. Durability of vaccine-induced and natural immunity against COVID-19: A narrative review. *Infect Dis Ther* 2023;12(2):367–87. <https://doi.org/10.1007/s40121-022-00753-2>.
- [22] Ludwikowska KM, Popiel A, Matkowska-Kocjan A, et al. COVID-19 mRNA BNT162b2 vaccine immunogenicity among children with a history of paediatric multisystem inflammatory syndrome temporally associated with COVID-19 (PIMS-TS). *Vaccine* 2023;41(21):3317–27. <https://doi.org/10.1016/j.vaccine.2023.04.035>.
- [23] Suntronwong N, Kanokudom S, Assawakosri S, et al. Neutralizing antibodies against Omicron BA.5 among children with infection alone, vaccination alone, and hybrid immunity [published online ahead of print, 2023 May 18]. *Int J Infect Dis* 2023;134:18–22. <https://doi.org/10.1016/j.ijid.2023.05.005>.
- [24] Bartsch YC, St Denis KJ, Kaplonek P, et al. SARS-CoV-2 mRNA vaccination elicits robust antibody responses in children. 14(672):eabn9237 *Sci Transl Med* 2022. <https://doi.org/10.1126/scitranslmed.abn9237>.
- [25] Gazit S, Saciuk Y, Perez G, et al. Hybrid immunity against reinfection with SARS-CoV-2 following a previous SARS-CoV-2 infection and single dose of the BNT162b2 vaccine in children and adolescents: a target trial emulation [published online ahead of print, 2023 Apr 13]. *Lancet Microbe* 2023;S2666–5247(23):00103–9. [https://doi.org/10.1016/S2666-5247\(23\)00103-9](https://doi.org/10.1016/S2666-5247(23)00103-9).
- [26] Crotty S. Hybrid immunity. *Science* 2021;372:1392–3. <https://doi.org/10.1126/science.abj2258>.
- [27] Ferlito C, Visco V, Biselli R, et al. Safety of multiple vaccinations and durability of vaccine-induced antibodies in an Italian military cohort 5 years after immunization. *Biomedicines*. 2021;10(1):6. Published 2021 Dec 21. doi:10.3390/biomedicines10010006.
- [28] de Wolf ACMT, van Aalst S, Ludwig IS, et al. Regulatory T cell frequencies and phenotypes following anti-viral vaccination. *PLoS One*. 2017;12(6):e0179942. Published 2017 Jun 28. doi:10.1371/journal.pone.0179942.
- [29] Morgans HA, Bradley T, Flebbe-Rehwaldt L, et al. Humoral and cellular response to the COVID-19 vaccine in immunocompromised children [published online ahead of print, 2022 Nov 14]. *Pediatr Res*. 2022;1-6. doi:10.1038/s41390-022-02374-4.
- [30] Lee ARYB, Wong SY, Chai LYA, et al. Efficacy of covid-19 vaccines in immunocompromised patients: systematic review and meta-analysis. *BMJ* 2022. <https://doi.org/10.1136/bmj-2021-068632>. Published 2022 Mar 2, 376:e068632.
- [31] Mehrabi Nejad MM, Shobeiri P, Dehghanbanadaki H, et al. Seroconversion following the first, second, and third dose of SARS-CoV-2 vaccines in immunocompromised population: a systematic review and meta-analysis. *Virol J*. 2022;19(1):132. Published 2022 Aug 8. doi:10.1186/s12985-022-01858-3.
- [32] Giannella M, Righi E, Pascale R, et al. Evaluation of the kinetics of antibody response to COVID-19 vaccine in solid organ transplant recipients: the prospective multicenter ORCHESTRA cohort. *Microorganisms* 2022. <https://doi.org/10.3390/microorganisms10051021>. Published 2022 May 12, 10(5):1021.
- [33] Perez MA, Hsiao HM, Chen X, et al. Serologic responses to COVID-19 vaccination in children with history of multisystem inflammatory syndrome (MIS-C). *Vaccine* 2023;41(17):2743–8. <https://doi.org/10.1016/j.vaccine.2023.03.021>.
- [34] Perry J, Osman S, Wright J, et al. Does a humoral correlate of protection exist for SARS-CoV-2? A systematic review. *PLoS One* 2022. <https://doi.org/10.1371/journal.pone.0266852>. Published 2022 Apr 8, 17(4):e0266852.