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Neutralization of Severe Acute Respiratory Syndrome Coronavirus 2 Omicron and Other Variants in Serum From Children With Vaccination-Induced Myocarditis

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1 **Neutralization of SARS-CoV-2 Omicron and other variants in serum from children**
2 **with vaccination-induced myocarditis**

3
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22 **Running Title:** Omicron and vaccine-induced myocarditis.

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ABSTRACT

Our study demonstrates that children who developed SARS-CoV-2 vaccination-induced myocarditis and may not receive another vaccination, could be susceptible to infection with Omicron and emerging variants. We observed higher neutralizing antibody titers in myocarditis patients vs. healthy vaccinated children, but significantly lower neutralization titers against Omicron in both groups.

Keywords: COVID-19, SARS-CoV-2, Omicron, Vaccination, Myocarditis.

ACCEPTED MANUSCRIPT

1 INTRODUCTION

2 The emergence of Omicron variant of SARS-CoV-2 resulted in rapid spread
3 across the globe. As of February 2022, the World Health Organization had defined five
4 SARS-CoV-2 variants of concern (VOCs); Alpha, Beta, Gamma, Delta, Omicron) as well
5 as two variants of interest (VOIs); Lambda, and Mu[1]. Viral spike protein of Omicron
6 contains over 30 mutations, raising concerns that Omicron could be resistant to
7 neutralizing antibodies generated following SARS-CoV-2 vaccination[2], and a third
8 vaccine dose may be required to boost immunity[3]. Even though SARS-CoV-2 mRNA
9 vaccines have been safe and effective in providing protection against COVID-19, under
10 rare circumstances SARS-CoV-2 mRNA vaccination may result in temporary, self-
11 resolving vaccine-induced myocarditis in some younger individuals[4, 5].

12 Booster vaccine doses are approved in United States for individuals >12 years of
13 age. Children who developed rare SARS-CoV-2 vaccination-induced myocarditis are
14 potentially at risk of another reactive cardiac episode following vaccination, and
15 therefore, may not receive a third vaccine dose. However, there is no information on
16 neutralization response to Omicron variant in children with vaccine-induced myocarditis.
17 Moreover, the capacity of children with vaccine-induced myocarditis during the acute-
18 phase myocarditis and post-acute recovery stage to elicit neutralizing antibodies against
19 the prototype vaccine strain and variants of concern (VOCs), especially against the fast-
20 spreading Omicron variant, is unknown. In absence of a vaccine booster dose, these
21 children could be at risk for breakthrough infection, especially from the Omicron variant.
22 Hence, it is important to determine the capacity of vaccine-induced antibodies to
23 neutralize SARS-CoV-2 VOCs/VOIs in these children.

1 Therefore, we assessed the spike-binding and SARS-CoV-2 neutralizing
2 antibodies induced following mRNA-based vaccination against vaccine-homologous
3 WA1/2020 and the VOCs/VOIs in two different cohorts of fully vaccinated children: one
4 with vaccination-induced myocarditis and a second cohort of age-matched, healthy
5 children.

6 **MATERIALS AND METHODS**

7 **Pediatric serum samples**

8 All pediatric participants were ≤ 21 years old (age range 4.8 to 18.7 years old)
9 who received mRNA (Pfizer-BioNTech's BNT162b2 or Moderna mRNA-1273) vaccine
10 between May-September 2021 (Supplementary Table S1). All children prior to
11 vaccination were SARS-CoV-2 naïve by SARS-CoV-2 PCR and antibody-negative
12 against SARS-CoV-2 nucleocapsid protein at the time of sample collection. Serum
13 samples from 13 of vaccination-induced myocarditis children were collected within 2-5
14 days post- vaccination (acute phase). One child had myocarditis and serum collected
15 after his first vaccination and 12 children developed myocarditis after the second
16 vaccination. A later sample (post-acute) was collected between 2-5 weeks after
17 resolution of myocarditis from 10 of these children (Supplementary Table S2). Post-
18 second vaccination serum samples were also obtained between 2-4 weeks post-second
19 vaccination from 10 healthy pediatric controls.

20 **Neutralization assay**

21 Sera were evaluated in a qualified SARS-CoV-2 pseudovirion neutralization
22 assay (PsVNA) using SARS-CoV-2 WA-1 strain and the five variants of concern
23 (VOCs): Alpha, Gamma, Beta, Delta and Omicron and two variants of interest: Lambda,

1 and Mu (Table S3). SARS-CoV-2 neutralizing activity measured by pseudovirion
2 neutralization assay (PsVNA) correlated with PRNT (plaque reduction neutralization test
3 with authentic SARS-CoV-2 virus) in previous studies[6].

4 Pseudovirions were produced as previously described[6]. The neutralization
5 assays were performed as previously described [7] [8, 9]. Briefly, 50 μ L of SARS-CoV-2
6 S pseudovirions (~200,000 relative light units) were pre-incubated with 3-fold serial
7 dilutions (20-fold starting dilution) of heat-inactivated serum for 1h. Then virus-antibody
8 mixtures were added to 293T-ACE2-TMPRSS2 cells (10^4 cells/50 μ L) [6] [5]. Cells were
9 lysed 24 h later, and luciferase activity was measured using One-Glo luciferase assay
10 system (Promega, Cat# E6130). The assay of each serum was performed in duplicate,
11 and the 50% neutralization titer was calculated using Prism 9 (GraphPad Software). The
12 limit of detection for the neutralization assay is 1:20. Two independent biological
13 replicate experiments were performed for each sample and variation in PsVNA50 titers
14 was <9% between replicates.

15 **Seroreactivity of pediatric samples to SARS-CoV-2 RBD by ELISA**

16 96-well Immulon plates were coated with 50 ng/100 μ L of recombinant spike-
17 RBD either from WA1/2020 or Omicron. Starting at 1:100 dilution, 5-fold serially diluted
18 serum samples were added and bound human IgG was detected with 1:5000 dilution of
19 HRP-conjugated anti-human IgG Fc-specific antibody (Jackson Immuno Research).
20 End-point titer was determined as 2-fold above the average absorbance values of
21 serum binding to blank control wells. The end-point titer is reported as the last serum
22 dilution that was above this cutoff.

23

1 **Quantification and statistical analysis**

2 Descriptive statistics were performed to determine the geometric mean titer
3 (GMT) neutralizing values or mean end-point titers and were calculated using
4 GraphPad. All experimental data to compare differences among groups were analyzed
5 using lme4 and emmeans packages in R (RStudio version 1.1.463). Since age and sex
6 can be biologically plausible confounders, data were analyzed for statistical significance
7 between groups to control for age and sex as covariates (predictor variables) using a
8 multivariate linear regression model, and the sample size was small. To ensure
9 robustness of the results, absolute measurements were log₂-transformed before
10 performing the analysis. For comparisons between the vaccine groups (factor variable),
11 pairwise comparisons were extracted using 'emmeans' and Tukey-adjusted p values
12 were used for denoting significance to reduce Type 1 error due to multiple testing. The
13 tests were two-sided tests. The differences were considered statistically significant with
14 a 95% confidence interval when the p value was less than 0.05.

15 **RESULTS**

16 **Capacity of vaccination induced antibodies to neutralize SARS-CoV-2 and** 17 **variants in children**

18 Two different cohorts of children fully vaccinated with mRNA-based vaccines
19 were evaluated in the study: thirteen children who developed vaccination-induced
20 myocarditis and another 10 age-matched healthy children with no history of COVID-19
21 (Figure 1A). We measured SARS-CoV-2 neutralizing antibodies to distinguish
22 neutralization capacity against the prototype vaccine WA1/2020 strain as well as

1 individual VOC/VOIs that previously or are currently circulating around the globe
2 (Supplementary Table S3).

3 Virus neutralization assays performed with sera collected between 2-4 weeks
4 post-second vaccination from 10 healthy children showed the highest neutralizing
5 antibody response against vaccine homologous WA1/2020 with geometric mean titer
6 (GMT) of 1:735 (Fig. 1B). Neutralizing antibody response was reduced against SARS-
7 CoV-2 variants ranging from 1.2-fold for Alpha (GMT 1:604) to 3.3-fold against Beta
8 (GMT 1:222) compared with vaccine-homologous WA1/2020. Neutralization titers
9 against Omicron were reduced by 27.2-fold (GMT 1:27) and 50% of the 10 healthy
10 children had no measurable neutralization titers (Fig. 1B and Supplementary Table S2).

11 Post-vaccination sera obtained from 13 children who developed vaccine-induced
12 myocarditis demonstrated neutralizing antibodies early (2-5 days) post-vaccination
13 against WA1/2020 (GMT 1:911) during the acute phase of myocarditis (Fig. 1C). After
14 resolution of myocarditis, the neutralizing antibody response increased by ~5-fold in
15 sera collected at 2-5 weeks post-vaccination (GMT 1:3529). Similar trend for increase in
16 neutralizing GMTs of 2 to 8-fold from acute to post-acute (2-5 weeks) time-point
17 following vaccination was observed across all SARS-CoV-2 variants (Fig. 1C). At 2-5
18 weeks post-vaccination, neutralization of Omicron (GMT 1:111) was reduced by 31.8-
19 fold compared with the vaccine-homologous WA1/2020 (GMT 1:3529). A correlation
20 was observed for neutralization titers between vaccine-homologous WA1/2020 and
21 variants (Supplementary Figure S1).

22 Post-vaccination neutralization titers were significantly higher for children with
23 vaccination-induced myocarditis (2-5 weeks post-vaccination) compared with healthy

1 control children (2-4 weeks post-vaccination) against vaccine-homologous WA1/2020
2 as well as trended higher for Omicron (not statistically significant), possibly suggesting
3 higher immunogenicity of mRNA vaccines in the vaccine-induced myocarditis children
4 (Figure 1D). The neutralization titers in both groups were significantly lower against the
5 Omicron variant.

6 **SARS-CoV-2 RBD-binding antibodies to vaccine-homologous WA1/2020 and** 7 **Omicron variant following vaccination in children**

8 To further explore the differences in neutralizing antibodies observed between
9 WA1/2020 and Omicron variant, we measured binding IgG antibodies to the SARS-
10 CoV-2 spike receptor binding domain (RBD) of both WA1/2020 and Omicron for serum
11 from vaccination-induced myocarditis (Myo; 2-5 weeks post-vaccination) and healthy
12 control children (C; 2-4 weeks post-vaccination). The end-point titer of serum IgG
13 binding to WA1/2020-RBD was 13.7-fold higher for vaccination-induced myocarditis and
14 55-fold higher for healthy children compared with their corresponding IgG binding to
15 Omicron-RBD (Fig. 1E). RBD-binding IgG trended higher in serum from vaccination-
16 induced myocarditis (Myo) than healthy controls (C) against both WA1/2020 (1.5-fold)
17 and Omicron (5.5-fold), but this difference did not reach statistical significance between
18 the two pediatric cohorts.

19 **DISCUSSION**

20 Our data demonstrate that neutralizing antibodies elicited by two doses of
21 vaccine could provide protection against the highly mutated Omicron variant in some
22 children but suggests the need for vaccine boosters in children for maximum protection.
23 Children with vaccination-induced myocarditis made a robust antibody response that

1 trended higher than healthy children. However, both populations remain potentially
2 susceptible to infection with the Omicron variant if immunity wanes over time. We
3 recognize the small sample size as a limitation of this study.

4 **CONCLUSION**

5 Clinical studies for vaccine effectiveness against Omicron will determine the role
6 of booster vaccination in pediatric populations.

7 **NOTES:**

8 **Author Contributions:**

9 **Designed research:** S.K., J.C.B.

10 **Clinical specimens and unblinded clinical data:** J.C.B., C.S., K.D.

11 **Performed assays:** F.Z., G.G. and, S.K.

12 **Contributed to Writing:** S.K., J.C.B., C.S., K.D.

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14 the manuscript. We thank Carol Weiss (FDA) and NIH Vaccine Research Center for
15 providing plasmid clones expressing SARS-CoV-2 spike variants.

16 **Data sharing.** All data needed to evaluate the conclusions in the article are present in
17 the manuscript.

18 **Ethics Statement:** The study protocol was approved by U.S. Food and Drug
19 Administration's (FDA) Research Involving Human Subjects Committee [252-
20 Determination-CBER-2020-04-02] and was conducted with de-identified samples. This

1 study complied with all relevant ethical regulations for work with human participants.

2 The Institutional Review Board of the University of California San Diego approved the

3 protocol for collection of samples (UCSD #140220) and written informed consent and

4 assent was obtained from the parents or legal guardians and the patient as appropriate.

5 **Disclaimer:** The content of this publication does not necessarily reflect the views or

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15 **Conflict of Interest:** J.C.B. reports support outside of this work from NICHD:

16 R61HD105590. All other authors declare that they have no other competing interests.

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1 **Figure Legend**

2 **Figure 1: Demographics, antibody binding and neutralization by post-vaccination**
3 **serum from children against SARS-CoV-2 WA1/2020 strain and variants.**

4 (A). Clinical and demographic characteristics of children with vaccine-induced
5 myocarditis and healthy control children used in the study. (B-C) Neutralization assays
6 were performed with the use of pseudoviruses harboring the SARS-CoV-2 spike
7 proteins of the WA1/2020 vaccine strain, VOIs: Lambda and Mu or VOCs: Alpha, Beta,
8 Gamma, Delta, or the Omicron variants as described before[7]. Serum samples
9 following two doses of SARS-CoV-2 mRNA vaccination were obtained from ten healthy
10 children without any co-morbidities (Panel B), and 13 children who developed post-
11 vaccination myocarditis 2-5 days (early acute time-point) after first dose (n=1) or post-
12 second (n=12) vaccination (Panel C). A later subacute sample was collected from 10 of
13 these children between 2-5 weeks post-second vaccination after resolution of
14 myocarditis (Panel C). (D) Comparative SARS-CoV-2 neutralization post-2nd vaccination
15 serum samples from 10 children who developed vaccination-induced myocarditis at 2-5
16 weeks (Myo; in red) versus 10 healthy children without any co-morbidities (C; in black)
17 against vaccine-homologous WA1/2020 or the Omicron variant. The assay of each
18 serum sample was performed in duplicate to determine the 50% neutralization titer.
19 Each data point represents an individual sample (circles) and indicates the PsVNA50
20 titer (serum dilution that resulted in 50% virus neutralization) obtained with each sample
21 against the indicated pseudovirus. The heights of the bars and the numbers over the
22 bars indicate the geometric mean titers, and the whiskers indicate 95% confidence
23 intervals. The fold-change indicates the average decrease in neutralization titer of the

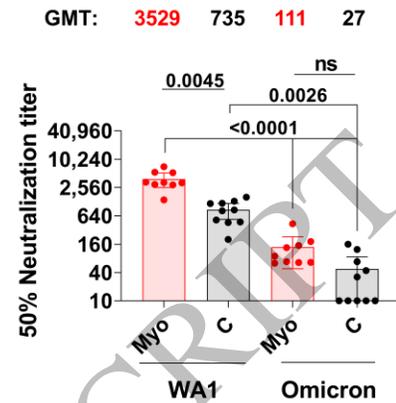
1 indicated SARS-CoV-2 variant as compared with that of the vaccine-homologous
2 WA1/2020 virus. The horizontal dashed line indicates the limit of detection for the
3 neutralization assay (PsVNA50 of 20). The samples that did not neutralize SARS-CoV-2
4 at 1:20 serum dilution was given a PsVNA50 value of 10 for graphic representation and
5 statistical analysis. Differences between SARS-CoV-2 strains were analyzed by lme4
6 and emmeans packages in R using Tukey's pairwise multiple comparison test and the
7 p-values are shown for significant differences only. (E) SARS-CoV-2 RBD-binding IgG
8 to WA1/2020 and Omicron variant by post-vaccination serum from children. IgG binding
9 to SARS-CoV-2 RBD either from vaccine-homologous WA1/2020 or Omicron variant in
10 serum samples obtained at 2-4 weeks following two doses of SARS-CoV-2 mRNA
11 vaccination from ten healthy children without any co-morbidities (C; in black), or
12 between 2-5 weeks post-second vaccination from 10 children who developed post-
13 vaccination myocarditis (Myo; in red), after resolution of myocarditis. Each serum
14 sample was evaluated in IgG-ELISA in duplicate to determine the RBD-binding IgG end-
15 point titer against RBD of either WA1/2020 or the Omicron variant. The height of bars
16 and numbers over the bars indicate the mean IgG titers, and the whiskers indicate 95%
17 confidence intervals. The horizontal dashed line indicates the limit of detection for IgG
18 ELISA (1:100). Statistical differences between pediatric groups were analyzed by lme4
19 and emmeans packages in R using Tukey's pairwise multiple comparison test and the
20 p-values are shown.

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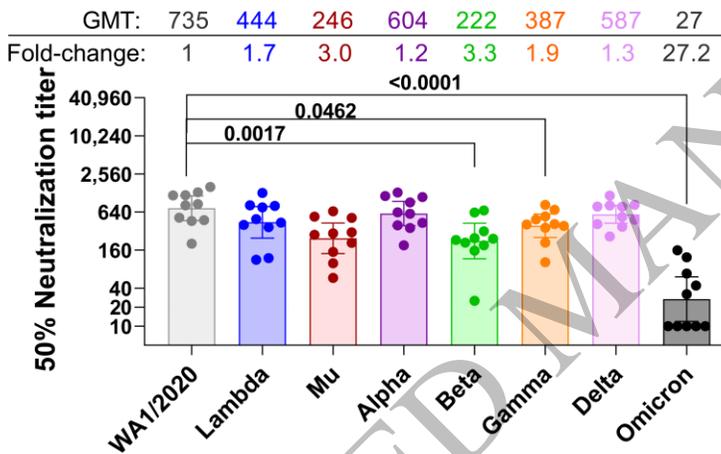
A. Clinical and demographic characteristics of children

	Post-vaccine myocarditis (n=13)	Healthy Controls (n=10)
Age, yrs ¹ , median (IQR)	14.6 (13.8-15.7)	16.6 (15.4-17.8)
Male, n (%)	13 (100)	10 (100)
Race/Ethnicity, n (%)		
Asian	1 (8)	2 (20)
White	4 (30)	5 (50)
Hispanic	7 (54)	2 (20)
> 2 races or other	1 (8)	1 (10)
Vaccine Type, n (%)		
Vaccine Type, Pfizer	12 (92)	9 (90)
Vaccine Type, Moderna	1 (8)	1 (10)

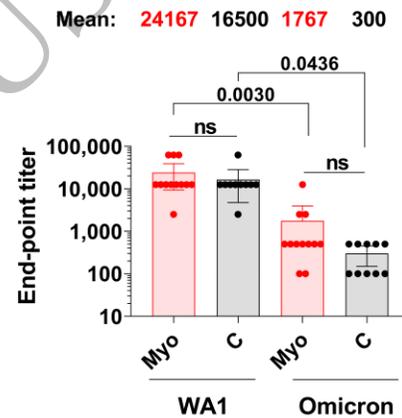
D. Neutralization (Myocarditis vs Healthy)



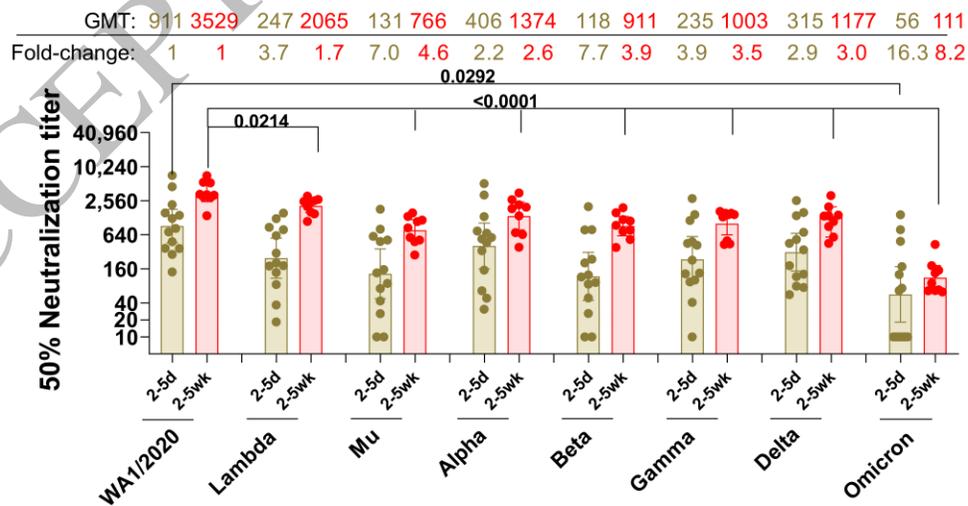
B. Neutralization assay with Healthy children vaccine serum (n=10)



E. SARS-CoV-2 RBD-binding IgG



C. Neutralization assay with Post-vaccination Myocarditis serum (n=13)



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Figure 1
165x216 mm (3.6 x DPI)