SARS-CoV-2 Delta breakthrough infections in vaccinated patients

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Abstract

The continuous emergence of SARS-CoV-2 variants with increased transmission and immune evasion has caused breakthrough infections in vaccinated population. It is important to determine the threshold of neutralizing antibody titers that permit breakthrough infections. Here we tested the neutralization titers of vaccinated patients who contracted Delta variant. All 75 patients with Delta breakthrough infections exhibited neutralization titers (NT_{50}) of less than 70. Among the breakthrough patients, 76%, 18.7%, and 5.3% of them had the NT_{50} ranges of <20, 20-50, and 50-69, respectively. These clinical laboratory results have implications in vaccine strategy and public health policy.

Main text

The COVID-19 pandemic is umpired by two dynamic factors: (i) the continuous emergence of SARS-CoV-2 variants with improved transmission and/or immune evasion and (ii) the waning immunity post vaccination and infection. This is exemplified by the two recent surges of Delta and Omicron variants, which caused many breakthrough infections in vaccinated individuals. Since antibody neutralization is a key contributor to vaccine protection, it is thus important to define the neutralization levels in patients with breakthrough infections. Such information is essential to guide vaccine strategy and policy. Here we characterized the antibody neutralization in vaccinated patients when they contracted Delta variant infections.

To determine the neutralization titers (NT_{50}) in breakthrough patients when they got infected with Delta variant, we collected sera from 75 patients who were vaccinated and subsequently contracted breakthrough infections. **Table 1** summarizes the patient information and their NT_{50} values. All patients were vaccinated with 2 doses of Pfizer or Moderna vaccine or 1 dose of J&J vaccine. Breakthrough infections were confirmed by positive viral RNA tests.

Although the genotypes of individual infecting viruses were not determined by sequencing, they were most likely Delta variant because all infections had occurred from late July to October, 2021 when Delta was 100% prevalent in our patient population based on the local SARS-CoV-2 surveillance system and about 98% prevalent in the USA (https://covid.cdc.gov/covid-data-tracker/#variant-proportions). All sera were taken 0-5 days before the positive viral nucleic acid tests. We determined the NT_{50} of each serum using a well-established mNeonGreen reporter USA/WA1/2020 SARS-CoV-2 [1]. This neutralization assay has been reliably used to support the BNT162b2 vaccine development [2]. The NT_{50} value was defined as the interpolated reciprocal of the dilution yielding a 50% reduction in mNeonGreen-positive cells. Each specimen was tested in duplicates and the geometric mean of the duplicate results is presented. The first serum dilution of the neutralization test was 1:20. The NT_{50} values of any specimens with no detectable neutralizing activities at 1:20 dilution were treated as 10 for plot and calculation purposes (**Table 1**). The overall results reveal three observations. *First,* all breakthrough patients had low NT_{50}s of <70 (**FIG 1A**). About 76%, 18.7%, and 5.3% of the breakthrough patients exhibited the NT_{50} ranges of <20, 20-50, and 50-69, respectively (**FIG 1B**). The results suggest NT_{50} of 70 as a neutralizing threshold required to prevent Delta breakthrough infections. *Second,* senior people appeared to be more vulnerable to breakthrough infections. Approximately 16%, 25.3%, and 58.7% of the breakthrough cases were in the age groups of 20-40, 41-64, and 65-97, respectively (**FIG 1C**). However, the NT_{50} differences among the three age groups are not all statistically significant (**FIG 1D**). *Third,* almost 90% of the breakthrough patients had received the 2 doses Pfizer or Moderna vaccine or 1 dose J&J vaccine for more than 120 days (**FIG 1E**). However, this observation was not statistically correlated with the NT_{50} differences among different time frames post-vaccination (**FIG 1F**).

One limitation of this study is that the NT_{50} values were measured against the original USA-WA1/2020 SARS-CoV-2 (a strain isolated in late January 2020), not directly against the

delta variant. Previous studies showed that BNT162b2-vaccinated sera (collected at 1 month after dose 2) neutralized Delta variant at an efficiency that was 31-69% lower than the USA-WA1/2020 [3]. Compared with Delta, the newly emerged Omicron is significantly less susceptible to neutralization by vaccinated or non-Omicron infected human sera [4, 5]. The reduced neutralization susceptibility, combined with the increased transmissibility of Omicron, may have accounted for the high breakthrough infections observed in the current Omicron surge. These laboratory investigations, together with the real-world vaccine effectiveness, will provide guidance for future vaccine strategy and public health policy.

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Conflict of Interest: The authors declare competing interests. X.X. and P.-Y.S. have filed a patent on the reverse genetic system. J.Z., H.X., X.X., and P.-Y.S. received compensation from Pfizer for COVID-19 vaccine development. Other authors declare no competing interests.

References:

1. Muruato, A.E., et al., A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. Nat Commun, 2020. **11**(1): p. 4059.
2. Walsh, E.E., et al., Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med, 2020.
3. Liu, J., et al., BNT162b2-elicited neutralization of B.1.617 and other SARS-CoV-2 variants. Nature, 2021.
4. Xia, H., et al., Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2. Cell Host Microbe, 2022.
5. Zou, J., et al., Neutralization against Omicron SARS-CoV-2 from previous non-Omicron infection. Nat Commun, 2022. **13**(1): p. 852.

Table 1. Serum information and NT_{50} values

Serum ID	Age (years)	Gender (F/M)	Ethnicity	Serum collection time (days post positive *NAAT)	Vaccine type	#Days post last vaccination	^NT_{50}
1	94	F	Black	0	Pfizer	137	&10
2	74	F	White	0	Pfizer	176	10
3	74	F	White	-4	Pfizer	193	10
4	91	F	White	0	Pfizer	203	10
5	83	F	White	0	Pfizer	182	10
6	34	M	White	0	Pfizer	139	10
7	66	M	Black	0	Pfizer	178	10
8	60	M	Hispanic	0	Moderna	159	10
9	64	F	Black	0	Pfizer	124	10
10	76	M	White	0	Pfizer	19	10
11	42	F	Black	-5	Pfizer	216	10
12	53	F	White	-4	Pfizer	149	10
13	79	F	Black	0	Pfizer	149	10
14	50	M	Black	-1	Pfizer	101	10
15	85	M	White	0	Pfizer	187	10
16	72	M	Black	0	Pfizer	186	10
17	72	F	Black	0	Pfizer	166	10
18	88	M	White	0	Moderna	207	10
19	34	F	White	-3	Pfizer	128	10
20	56	F	White	0	Pfizer	224	10
21	44	F	White	0	Pfizer	225	10
22	77	F	White	0	†J&J	179	10
23	97	F	White	0	Pfizer	205	10
24	54	F	Black	-1	Pfizer	120	10
25	80	F	Hispanic	0	Pfizer	196	10
26	84	M	White	0	Pfizer	198	10
27	73	F	White	0	Pfizer	190	10
28	68	M	White	-3	Pfizer	231	10
29	69	M	White	0	Pfizer	195	10
30	30	M	White	0	Moderna	156	10
31	52	F	White	0	Pfizer	98	10
32	69	F	Black	0	J&J	188	10
33	75	F	White	0	Pfizer	165	10
34	73	M	White	-3	Pfizer	234	10
35	73	F	White	0	Pfizer	179	10
36	62	F	Hispanic	0	Pfizer	225	10
37	68	F	Black	0	J&J	212	10
38	59	M	Black	0	Pfizer	149	10

39	80	M	Hispanic	0	J&J	170	10
40	77	F	Asian	0	Pfizer	236	10
41	35	M	White	0	Pfizer	138	10
42	72	M	White	0	Pfizer	199	10
43	73	M	White	-5	Pfizer	219	10
44	87	M	White	0	Pfizer	223	10
45	84	F	Black	0	J&J	211	10
46	85	F	White	0	Pfizer	241	10
47	78	F	Hispanic	0	Pfizer	236	10
48	89	M	White	0	Pfizer	162	10
49	56	M	Hispanic	0	Moderna	154	12
50	77	M	White	0	Moderna	238	14
51	77	M	Black	0	J&J	165	14
52	96	F	Black	0	J&J	218	15
53	66	M	White	0	Pfizer	178	15
54	85	F	Black	0	Pfizer	161	16
55	64	M	White	0	Pfizer	156	19
56	65	M	Black	0	Pfizer	113	19
57	30	M	White	-3	Pfizer	223	19
58	85	F	White	0	Pfizer	194	20
59	62	M	White	-1	Pfizer	175	20
60	60	F	White	0	Pfizer	41	21
61	46	F	Black	0	Pfizer	173	21
62	16	F	White	-1	Pfizer	129	21
63	20	F	White	0	Pfizer	119	22
64	41	F	White	-4	Pfizer	190	26
65	34	F	Hispanic	0	Moderna	163	27
66	86	F	White	0	Moderna	187	31
67	59	M	White	0	Pfizer	142	33
68	61	M	Native Hawaiian	-3	Pfizer	175	35
69	80	F	White	0	Pfizer	193	41
70	38	M	Hispanic	0	Pfizer	104	41
71	27	M	Hispanic	0	Pfizer	145	47
72	89	M	White	0	Pfizer	195	51
73	39	F	White	-3	Pfizer	181	52
74	91	F	White	0	Pfizer	166	65
75	38	F	Hispanic	0	Pfizer	69	69

*Nucleic acid amplification test (NAAT)

[#]Days after dose 2 of Pfizer and Moderna vaccine or days after dose 1 of J&J vaccine

^Individual NT_{50} value is the geometric mean of duplicate neutralization test results.

[&]NT_{50} of <20 was treated as 10 for plot purpose and statistical analysis.

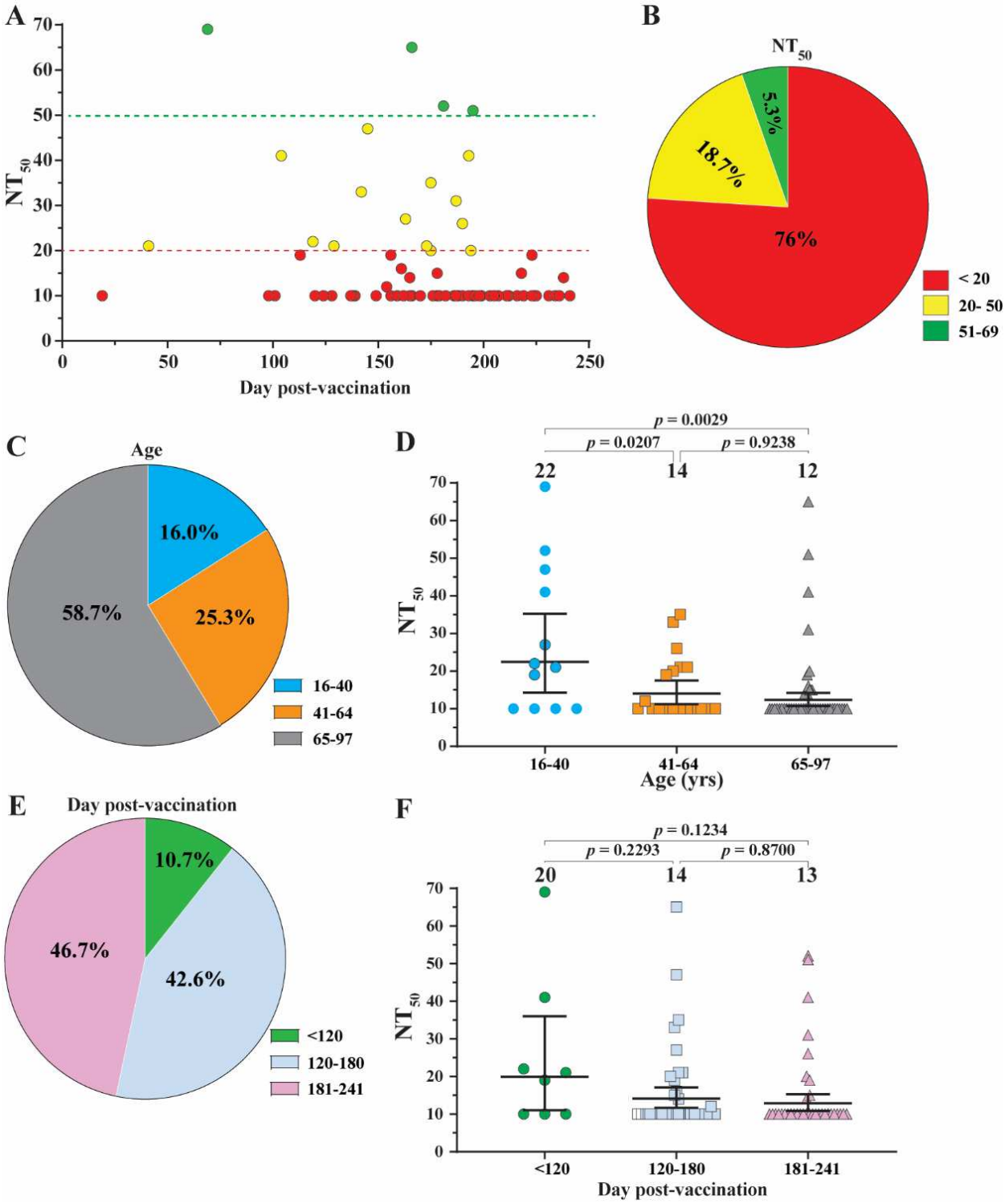

FIG 1. Analysis of Delta breakthrough infections in vaccinated patients. A panel of 75 sera, collected from Delta breakthrough patients, were measured for antibody neutralization titers (NT_{50}) against USA-WA1/202. All patients were immunized with 2 does of Pfizer or Moderna vaccine or 1 dose

of J&J vaccine. All sera were taken 0-5 days before viral RNA-positive test results. All patient information and NT_{50} values are detailed in **Table 1. A.** Plot of NT_{50} values versus days after dose 2 of Pfizer or Moderna vaccine or after 1 dose of J&J vaccine. Each data point represents the geometric mean of NT_{50} for one serum tested in duplicate assays. Different colors represent different NT_{50} ranges. Samples with no detectable neutralizing activities were plotted as 10 for calculation purpose. **B.** Pie presentation of different NT_{50} ranges. **C.** Age distributions. **D.** Plot of NT_{50} of different age groups. **E.** Distribution of breakthrough percentages versus days post-vaccination. **F.** Plot of NT_{50} versus days post-vaccination. In **D** and **F**, the bar heights and the numbers above indicate geometric mean titers. The whiskers indicate 95% confidence intervals. Statistical analysis was performed with the use of the one-way ANOVA with Tukey's correction for multiple comparisons test.