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Immunogenicity and Tolerability of COVID-19 mRNA Vaccines in PID patients with functional B-cell defects

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PII: S0091-6749(21)02667-1

DOI: <https://doi.org/10.1016/j.jaci.2021.11.022>

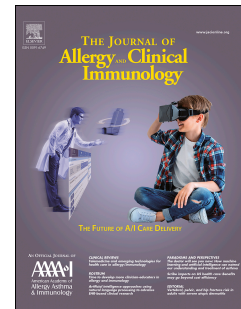
Reference: YMAI 15393

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 5 August 2021

Revised Date: 29 October 2021

Accepted Date: 5 November 2021



Please cite this article as: Pham MN, Murugesan K, Banaei N, Pinsky BA, Tang M, Hoyte E, Lewis DB, Gernez Y, Immunogenicity and Tolerability of COVID-19 mRNA Vaccines in PID patients with functional B-cell defects, *Journal of Allergy and Clinical Immunology* (2022), doi: <https://doi.org/10.1016/j.jaci.2021.11.022>.

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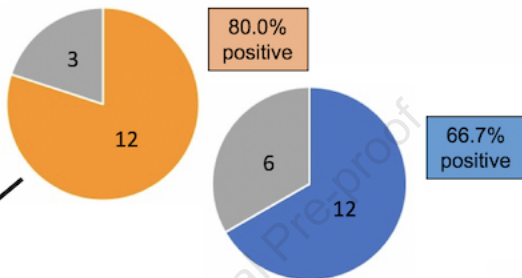
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Immunogenicity and Tolerability of COVID-19 mRNA Vaccines in PID Patients with Functional B Cell Defects

SARS-CoV-2 specific T-cell response

assessed by interferon gamma release assay

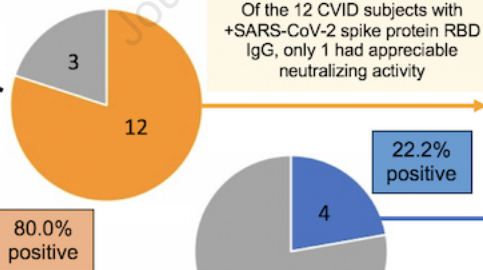


33 patients with humoral defect

- 15 patients with CVID
- 18 patients with other antibody deficiencies

63.6% received BNT162b2
30.3% received mRNA-1273

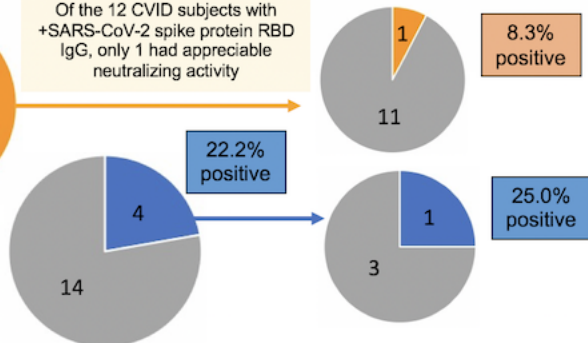
SARS-CoV-2 spike protein RBD IgG



Of the 12 CVID subjects with +SARS-CoV-2 spike protein RBD IgG, only 1 had appreciable neutralizing activity

Neutralizing activity

ACE2 receptor blocking activity $\geq 50\%$



ACE2 – angiotensin-converting enzyme 2; CVID – common variable immunodeficiency; RBD – receptor binding domain; SAD – specific antibody deficiency; XLA – X-linked agammaglobulinemia

Immunogenicity and Tolerability of COVID-19 mRNA Vaccines in PID patients with functional B-cell defects

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Conflict of Interest statement: The authors declare that they have no relevant conflicts of interest.

Funding Sources: none

Manuscript information:

Words: 1,561/ 1,500

Figures: 2, Tables: 2

Supplementary material:

eTables: 1

Abstract:

Background: Data on the safety and efficacy of COVID-19 vaccination in people with a range of primary immune deficiencies are lacking since these patients were excluded from COVID-19 vaccine trials. This information may help in clinical management of this vulnerable patient group.

Objective: To assess humoral and T-cell immune responses after two doses of SARS-CoV-2 mRNA vaccines in patients with PIDs and functional B-cell defects.

Methods: A double-center retrospective review of patients with PID who completed COVID-19 mRNA vaccination and who had humoral responses assessed through SARS-CoV-2 spike protein receptor binding domain (RBD) IgG antibody levels with reflex assessment of the antibody to block RBD binding to ACE2 (hereafter referred to as ACE2 receptor blocking activity, as a surrogate test for neutralization) and T-cell response evaluated by an interferon-gamma release assay (IGRA). Immunization reactogenicity was also reviewed.

Results: A total of 33 patients with humoral defect were evaluated. 69.6% received BNT162b2 vaccine (Pfizer-BioNTech) and 30.3% received mRNA-1273 (Moderna). The mRNA vaccines were generally well tolerated without severe reactions. The IGRA was positive in 77.4% of our patients (24 of 31). About half of our subjects (16 of 33) had detectable RBD-specific IgG responses but only two of these 16 subjects had an ACE2 receptor blocking activity level of $\geq 50\%$.

Conclusion: Vaccination of this cohort of PID patients with COVID-19 mRNA vaccines was safe and cellular immunity was stimulated in a majority. However, antibody responses to the spike protein RBD were less consistent, and, when detected, was not effective at ACE2 blocking.

Clinical Implication: mRNA vaccination may be less effective at preventing acquisition of SARS-CoV-2 in our cohort of PID patients with functional B-cell defects. The Induction of SARS-CoV-2 spike protein-specific T-cell immunity by vaccination might help reduce the severity of disease in these patients.

Capsule summary: mRNA vaccines were well tolerated in our cohort of PID patients with functional B-cell defects. T-cell responses to the mRNA vaccine were elicited in most of our subjects with PID whereas antibody responses associated with protection from SARS-CoV-2 infection were not reliably induced.

Key words: SARS-CoV-2; SARS-CoV-2 vaccination; primary immunodeficiency; ACE2 blocking antibody; SARS-CoV-2 spike protein antibody; antibody deficiency; common variable immunodeficiency, Good syndrome; Monoclonal antibody, SARS-CoV-2 interferon-gamma release assay

Abbreviations:

ACE2 – angiotensin-converting enzyme 2

CLIA: clinical laboratory improvement amendments

CID: combined immunodeficiency

COVID-19 - coronavirus disease 2019

CTLA-4: cytotoxic T-lymphocyte associated protein 4

CVID – common variable immunodeficiency

Def: deficiency

- 84 IFN- γ - interferon-gamma
- 85 Ig Tx: immunoglobulin therapy
- 86 IGRA - interferon-gamma release assay
- 87 mRNA – messenger ribonucleic acid
- 88 PID – primary immunodeficiency
- 89 SAD: specific antibody deficiency
- 90 SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2
- 91 XLA: X-linked agammaglobulinemia
- 92

Manuscript Text:**Introduction**

SARS-CoV-2 remains a serious threat to global health and a significant cause of morbidity and mortality, especially in patients with primary immunodeficiencies (PIDs). (1) Two safe and effective mRNA vaccines targeting the spike protein of SARS-CoV-2 have been granted Emergency Use Authorization (EUA). (2) SARS-CoV-2-specific humoral and T-cell responses both contribute to protection against COVID-19 infection. (3-5) Although about 10 million people in the US are immunocompromised, patients with immunodeficiencies including PIDs were excluded from the SARS-CoV-2 vaccine trials leading up to the EUAs. Thus, data on safety, and immune responses to COVID-19 vaccination in recipients with immune deficiencies and dysregulation syndromes are limited. Recent publications have suggested good tolerance and immunogenicity in patients with PID but more and larger studies are needed, (6-8) that include evaluation of antibody responses that predict protection from infection.

Here, 33 patients with diverse PIDs ranging in age between 19 and 79 years (mean 50.2 ± 18.35 years) followed at the Allergy and Immunology clinics at Stanford University and the University of California, San Francisco were studied. All had received two doses of either mRNA-1273 (Moderna) or BNT162b2 (Pfizer-BioNTech) SARS-CoV-2 mRNA vaccines (Table 1). We focused on the evaluation of safety and efficacy of mRNA vaccination for PID patients with humoral defects, including patients with moderately low to normal levels of B cells and impaired or absent specific antibody responses as well as those with low or absent B cells and globally reduced antibody production. To evaluate the immunogenicity of the vaccine, we measured the spike protein-specific antibody response using a SARS-CoV-2 IgG antibody

ELISA coating with S1 receptor binding domain (RBD) antigen, with reflex to SARS-CoV-2 ACE2 receptor blocking activity, (9) which correlates well with antibody viral neutralization. (10) Spike-protein-specific T-cell responses were evaluated using a SARS-CoV-2 interferon-gamma release assay (IGRA). (11) These assays were performed at Stanford Health Care Clinical Virology Laboratory, a CLIA certified laboratory.

Results and discussion

Testing was performed a mean of 10.9 ± 5.3 weeks after the second vaccine dose and most subjects had positive immune results to some degree (Figure 1). Twenty-four [77.4% (24 of 31)] of our patients had positive IGRA results. About half of our subjects (16 of 33) had detectable RBD-specific IgG responses but only two had an ACE2 receptor blocking activity level of $\geq 50\%$. Our subjects had impaired antibody responses as their predominant clinical immunodeficiency [such as common variable immunodeficiency (CVID) (N=15), hypogammaglobulinemia (N=4), selective antibody deficiency (SAD) (N=2), Good's Syndrome with absent B cells (n=4), agammaglobulinemia (N=2), hyper-IgM syndrome (N=2), PIK3R1 deficiency (N=1), CTLA-4 haploinsufficiency (N=1) and combined immunodeficiency [Ataxia telangiectasia (N=1) and ATP6AP1 gene/immunodeficiency 47(N=1)](Table 1). Thirty-two subjects (96.9%) were on immunoglobulin replacement. Sixty-nine percent of the patients received the BNT162b2 (Pfizer-BioNTech) vaccine, whereas the remainder received the mRNA-1273 (Moderna) vaccine. Five had a SARS-CoV-2 spike protein IgG level checked prior to COVID-19 vaccination, which was undetectable in all cases. None of our patients had a known history of SAR-CoV-2 infection prior to vaccination and none developed a SARS-CoV-2 infection during the study period. Clinical data for up to nine months post-vaccination are reported.

Tolerability/reactogenicity information was gathered through chart review and revealed that the vaccines were well tolerated (Table 2). There were no severe reactions.

All our patients had an antibody deficiency, which is the most common general category of PID. As expected, our 4 patients with inborn errors that markedly impair IgG antibody production (two with agammaglobulinemia and two with hyper-IgM syndrome secondary to CD40-ligand deficiency) had negative SARS-CoV-2 IgG antibody results (Table 1, Figure 2). IGRA results were positive for all four of these patients, consistent with the relative selectivity of these immune defects and their largely sparing T-cell immunity. In our subjects with humoral defects as part of CVID (N=15), 80% had a positive SARS-CoV-2 spike protein RBD-specific IgG result and 80% had a positive IGRA result. CVID patients with a positive RBD-specific IgG level had a statistically significant higher average of circulating CD3 T-cell level than those who had a negative result ($1317 \pm 431.9/\mu\text{L}$ versus $531 \pm 157.0/\mu\text{L}$, respectively), (T-test P-value = 0.029). The mean baseline IgG levels were also higher in CVID patients who had a positive RBD-specific IgG responses than those who did not ($364.7 \pm 102.0 \text{ mg/dL}$ versus $91.0 \pm 58.0 \text{ mg/dL}$, respectively, (T-Test P-value = 0.004). A striking finding was that only one of the 15 CVID patients who mounted a positive RBD IgG-specific antibody response also had functional antibodies that blocked the interaction of the RBD with ACE2, as assessed in the ACE2 blocking antibody assay. (9) As this blocking activity correlates well with antibody that effectively neutralizes SARS-CoV-2 for entry into host cells, (10) this indicated that most CVID patients did not mount antibody responses that would be protective from SARS-CoV-2 infection.

The responsiveness of PID patients to COVID-19 vaccination might be potentially difficult to assess if the patient is on Ig replacement with product that contains SARS-CoV-2 antibody derived from donors who have either had SARS-CoV-2 infection and/or who have

received COVID-19 vaccines. We anticipated that this was unlikely to account for the presence of 46.9% of our 32 patients who were on Ig replacement having any spike protein-specific antibody given the usual lag between seroprevalence in the blood donor population and the specific antibody in manufactured Ig products. (12-16) To evaluate the potential impact of Ig therapy on SARS-CoV-2 spike protein RBD-specific humoral responses, we evaluated two patients (#24 and #32 Table 1) for SARS-CoV-2 ACE2 receptor blocking antibody levels pre- and post-IVIG therapy. For Patient#24, both pre- and post-IVIG therapy ACE2 receptor blocking activity was <10% and for Patient#32, the post-IVIG ACE2 receptor blocking activity minimally changed from <10% pre-infusion to 14%, post-infusion. Thus, in these two subjects, the IVIG products they received in September 2021 (over 1.5 years since the start of the global COVID-19 pandemic) did not appreciably alter their levels of protective neutralizing antibody.

Our study of PID patients with functional B-cell defects is, to the best of our knowledge, the first to evaluate the ACE2 receptor blocking activity after two doses of the SARS-CoV-2 mRNA vaccines. The receptor blocking activity competition assay evaluates the ability of the antibody in serum or plasma to bind to the spike protein RBD and prevent its interaction with ACE2 (9). The level of receptor blocking activity may correlate with antibody-mediated neutralization assays employing viruses pseudotyped with the spike protein. (10) Thus, our finding that only one out of 15 CVID patients had an ACE2 blocking level of $\geq 50\%$ and that such activity was undetectable in most of these patients raises the possibility that mRNA vaccination may provide minimal protection from SARS-CoV-2 infection for CVID patients. It is also important to consider that the ACE2 receptor blocking assay employed the RBD similar to that encoded by the current EUA approved mRNA vaccines, and protection might be even further reduced with SARS-CoV-2 variants that have amino acid changes in the RBD domain.

Similar to Hagin *et al.* (7), 80% of our patients with CVID had a spike protein-RBD specific IgG response. Additionally, 80% of our CVID patients had spike protein-specific T-cell immune response. In the antibody deficient patients in our cohort, and in contrast to the study of Hagin *et al.* (7), there was no difference between age or IgG at baseline and a positive SARS-CoV2-2 spike protein result but those with a positive IGRA result were younger, mean age 48.2+17.7 versus 64.3+12.4 years (T-test P=0.032).

This study has several limitations, including the relatively small size of our cohort and that the vaccine observations only covered a limited period of time. We plan to measure and report on additional data including our patients' responses to a third vaccine dose given new recommendations by the Centers for Disease Control and Prevention for a third mRNA dose in patients with moderate and severe immunodeficiencies (<https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html>). (17) We also did not include any patients with hemophagocytic lymphohistiocytosis or autoinflammatory conditions. Additionally, the ability to interpret the clinical significance of individual patient ACE2 receptor blocking activity for providing protection will require additional clinical studies to establish validated cut-off values. The threshold of ACE2 receptor blocking activity of $\geq 50\%$ for a positive result was chosen for this report but further studies are needed to more precisely establish protective ranges.

Currently, SARS-CoV-2 monoclonal antibody therapies are granted EUA for use in older and high-risk individuals, such as some PID patients, for post-exposure prophylaxis or infection with SARS-CoV-2. In patients with humoral defects where functional antibody protection is not achieved either through vaccination or immunoglobulin replacement therapy, it would be reasonable to expand monoclonal antibody therapy to serve as a prophylactic in this high-risk patient population. In fact, an EUA has recently been requested for a monoclonal antibody

cocktail (AstraZeneca) to serve as pre-exposure prophylaxis in vulnerable populations, such as the immunocompromised. Studying vaccinated PID patients and their neutralizing antibody, may help determine those who can benefit from such prophylactic therapy.

In our cohort of PID patients with functional B-cell defects, mRNA vaccines were well tolerated and although antibody responses to the spike protein that are associated with protection were not reliably induced in most of our subjects, T-cell responses were elicited in most of our patients. These T-cell immune responses are anticipated to be helpful in limiting viral replication in cases of established infection. (5) Further long-term studies will aid in determining effective therapies and recommendations in patients with PID during this SARS-CoV-2 pandemic.

216 **Acknowledgements:**

217 We thank the patients and their families for participating in research studies. We would like to
218 thank Cristina R. Cortales for her invaluable technical help.

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266 Immunocompromised People 2021 [updated Updated Oct. 8, 2021].

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Figure Legends & Tables:

Figure 1: Immunogenicity of the SARS-CoV-2 Vaccines in PID patients with functional B-

cell defects Legend: PID – primary immunodeficiency, IGRA- interferon-gamma (IFN- γ)

release assay. SP RBD IgG antibody to the SARS-CoV-2 RBD domain of the spike protein. Ab

blocking activity $\geq 50\%$ - ACE2 blocking antibody activity $\geq 50\%$. Numbers in bars signify

number of subjects. Unless otherwise noted, sample size of that group is 33.

*denominator is N=31

Figure 2: SARS-CoV-2 ACE2 blocking activity

Legend: SARS-Cov-2 antibody ACE2 blocking activity in PID patients with B-cell functional

defect (N=33). Subdividing patients according to different disease categories. “Other PID”

includes XLA patients (N=2), Good syndrome (N=4), CTLA-4 haploinsufficiency (N=1);

PIK3R1 (N=1), AT (N=1) and ATP6AP1 (N=1)

Table 1: Subject Characteristics and Test Results

Legend: CTLA-4: cytotoxic T-lymphocyte associated protein 4; CVID: common variable

deficiency; Def: deficiency; Ig Tx: immunoglobulin therapy; SAD: specific antibody deficiency;

XLA: X-linked agammaglobulinemia

Table 2 – Symptoms post-COVID vaccination.

*Flare occurred at 1-week post-vaccination

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eTable 1. Subject Characteristics

Subjects	Age	Sex	PID Dx	ALC	B cells	T cells (CD3)	T cells (CD4)	T cells (CD8)	Immunosuppressant	SARS-CoV-2 spike protein, IgG	SARS-CoV-2 ACE2 Blocking Activity	SARS - CoV-2 IGRA	Baseline IgG	Baseline IgM	Baseline IgA	IgG trough	Genetic info
	yrs			K/uL	/uL	/uL	/uL	/uL					mg/dL	mg/dL	mg/dL	mg/dL	
1	21	M	Agammaglobulinemia	980	20	862	666	118	None	Negative	-	Positive	-	20	<8	738	PID Invitae Panel neg
2	30	M	XLA	2158	0	2072	928	1079	None	Negative	-	Positive	-	8	<8	1040	BTK c.1349+2dup (Spice site)
3	30	F	CVID	1008	71	796	504	131	None	Positive	50-60%	Positive	300	28.1	<8	876	PID: VUS in PRKCD and VSP13B
4	32	F	CVID	467	37	420	266	126	Azathioprine	Negative	-	Positive	-	26	0	1127	Negative WES
5	38	F	CVID	1788	215	1570	1091	411	Budesonide	Positive	40-50%	Positive	335	37	23	977	PID Panel neg
6	40	M	CVID	1344	40	981	524	417	None	Positive	40-50%	Positive	542	75	90	90	-
7	41	F	CVID	1566	266	1237	626	407	None	Positive	<10%	Positive	-	-	-	926	CVID Panel neg
8	53	M	CVID	774L	77L	642L	317	302	Adalimumab, ustekinumab	Negative	-	Positive	<60	<11	<15	968	-
9	56	M	CVID	3320	1162	1594	1328	199	Tocilizumab	Positive	<10%	Negative	288	15	<7	985	NOD2 and VUS in ODCK8, JAK3, and TERT
10	58	F	CVID	1870	449	1253	804	411	None	Positive	<10%	Positive	361	26	14	818	-
11	59	M	CVID	1300	-	-	-	-	Budesonide, hydrocortisone, vedolizumab	Negative	-	Negative	132	17.4	23.3	1053	c.2104C>T (p.Arg702Trp) - was

																	identified in NOD2.
12	60	F	CVID	1887	226	1189	774	396	none	Positive	30-40%	Positive	399	<1	32	1310	-
13	63	F	CVID	1822	109	1330	875	474	Budesonide	Positive	30-40%	Positive	413	52	67	1150	-
14	71	F	CVID	2982	209	2117	1700	388	None	Positive	20-30%	Positive	473	33	141	857	-
15	72	M	CVID	2242	157	1995	650	1300	None	Positive	-	Positive	150	<5	<5	1020	-
16	73	F	CVID	1495	194	912	628	254	hydroxychloroquine	Positive	<10%	Positive	377	34	396	Not on Ig Tx	-
17	79	F	CVID	1066	11	831	725	117	None	Positive	<10%	Negative	374	1110	48	866	VUS in RECQL4
18	39	F	hypogammaglobulinemia	947	30	821	442	359	None	Positive	60-70	Positive	658	44	57	916	-
19	55	F	hypogammaglobulinemia	1670	117	1386	1052	251	hydroxychloroquine, mycophenolate	Negative	-	Positive	573	21	115	958	PID Panel: VUS FOXP3, ATM, EPG5, AND TTC7A
20	67	F	hypogammaglobulinemia	1359	82	1182	761	408	None	Positive	<10%	Positive	611	57	79	1900	-
21	75	M	hypogammaglobulinemia	1930	251	1583	656	965	None	Negative	-	Negative	661	-	-	1110	PID Panel: VUS ATM
22	53	F	SAD	1749	175	1294	857	367	None	Positive	40-50%	Positive	1080	78	163	1410	-
23	74	F	SAD	1600	48	1312	1136	176	None	Positive	10-20%	Positive	1130	206	334	1200	-
24	43	M	Good syndrome with hypogammaglobulinemia	1579	0	1,392	199	1,132	Everolimus, prednisone	Negative	-	Negative	259	0	69	754	-
25	65	F	Good syndrome with hypogammaglobulinemia	1642	0	1412	755	903	None	Negative	-	Positive	118	22	11	656	-

26	68	F	Good syndrome with hypogammaglobulinemia	858	0	849	223	601	Tacrolimus	Negative	-	Negative	<8	705	<6	1034	-
27	70	F	Good syndrome with hypogammaglobulinemia	230	2	177	62	122	Cyclosporine, prednisone	Negative	-	Negative	-	-	-	1203	-
28	39	M	Hyper IgM Syndrome	-	-	-	-	-	None	Negative	-	Positive	-	301	0	933	CD40L: c.491+1G>c (splice donor)
29	40	M	Hyper IgM Syndrome	1340	107	1179	402	616	None	Negative	-	Positive	-	-	-	854	CD40L: 530 A>G
30	19	M	CTLA-4 Deficiency	2502	500	1902	1001	626	Sirolimus	Negative	-	Positive	521	31	23	1030	CTLA-4: 567+5G>C
31	29	M	PIK3R1	795	215	517	231	278	None	Negative	-	-	-	305	0	805	PIK3R1:c.1425+1G.A (splice donor)
32	26	F	Ataxia Telangiectasia	468	42	295	164	122	None	Negative	-	-	925	88	45	1250	-
33	20	M	ATP6AP1 gene/immunodeficiency 47	1376	537	743	523	179	None	Negative	-	Positive	40	7	12	708	ATP6AP1: p.E346K; (c.1036G>A)

Legend: AI: autoimmunity; ALC: absolute lymphocyte count; BTK: Burton tyrosine kinase; CLL: chronic lymphoid leukemia; CIU: chronic idiopathic urticaria; Dx: diagnosis; Hx: history; IBD: inflammatory bowel disease; IILD: Interstitial lung disease; ITP: idiopathic

thrombocytopenia; SAD: specific antibody deficiency; VUS: variants of unknown significance; Yrs: years; WES: whole exome; WBC: white blood count; XLA: X-linked agammaglobulinemia

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Subjects	Age (Years)	Sex	PID Dx	Antibody Deficiency	Ig Tx	SARS- CoV-2 mRNA Vaccine	Weeks between second vaccine dose and serology	SARS- CoV-2 spike protein IgG - post vaccine	SARS- CoV-2 ACE2 Blocking Activity	SARS- CoV-2 IGRA
1	21	M	Agammaglobulinemia	Yes	Yes	Pfizer-BioNTech	4.43	Negative	-	Positive
2	30	M	XLA	Yes	Yes	Moderna	4.00	Negative	-	Positive
3	30	F	CVID	Yes	Yes	Pfizer-BioNTech	5.86	Positive	50-60%	Positive
4	32	F	CVID	Yes	Yes	Pfizer-BioNTech	8.71	Negative	-	Positive
5	38	F	CVID	Yes	Yes	Pfizer-BioNTech	4.14	Positive	40-50%	Positive

6	40	M	CVID	Yes	Yes	Moderna	5.57	Positive	40-50%	Positive
7	41	F	CVID	Yes	Yes	Pfizer-BioNTech	9.14	Positive	<10%	Positive
8	53	M	CVID	Yes	Yes	Moderna	9.43	Negative	-	Positive
9	56	M	CVID	Yes	Yes	Pfizer-BioNTech	15.00	Positive	<10%	Negative
10	58	F	CVID	Yes	Yes	Pfizer-BioNTech	4.86	Positive	<10%	Positive
11	59	M	CVID	Yes	Yes	Pfizer-BioNTech	7.00	Negative	-	Negative

12	60	F	CVID	Yes	Yes	Pfizer-BioNTech	9.57	Positive	30-40%	Positive
13	63	F	CVID	Yes	Yes	Moderna	9.86	Positive	30-40%	Positive
14	71	F	CVID	Yes	Yes	Moderna	10.71	Positive	20-30%	Positive
15	72	M	CVID	Yes	Yes	Moderna	17.57	Positive	N/A	Positive
16	73	F	CVID	Yes	No	Pfizer-BioNTech	24.71	Positive	<10%	Positive
17	79	F	CVID	Yes	Yes	Pfizer-BioNTech	11.29	Positive	<10%	Negative

18	39	F	hypogammaglobulinemia	Yes	Yes	Moderna	9.57	Positive	60-70%	Positive
19	55	F	hypogammaglobulinemia	Yes	Yes	Pfizer-BioNTech	6.85	Negative	-	Positive
20	67	F	hypogammaglobulinemia	Yes	Yes	Pfizer-BioNTech	9.43	Positive	<10%	Positive
21	75	M	hypogammaglobulinemia	Yes	Yes	Moderna	16.77	Negative	-	Negative
22	53	F	SAD	Yes	Yes	Pfizer-BioNTech	6.57	Positive	40-50%	Positive
23	74	F	SAD	Yes	Yes	Moderna	14.43	Positive	10 -20%	Positive

24	43	M	Good syndrome with hypogammaglobulinemia	Yes	Yes	Pfizer-BioNTech	9.86	Negative	-	Negative
25	65	F	Good syndrome with hypogammaglobulinemia	Yes	Yes	Pfizer-BioNTech	5.86	Negative	-	Positive
26	68	F	Good syndrome with hypogammaglobulinemia	Yes	Yes	Moderna	19.00	Negative	-	Negative
27	70	F	Good syndrome with hypogammaglobulinemia	Yes	Yes	Pfizer-BioNTech	19.14	Negative	-	Negative
28	39	M	Hyper IgM Syndrome	Yes	Yes	Pfizer-BioNTech	15.71	Negative	-	Positive
29	40	M	Hyper IgM Syndrome	Yes	Yes	Pfizer-BioNTech	13.14	Negative	-	Positive

30	19	M	CTLA-4 Deficiency	Yes	Yes	Pfizer- BioNTech	6.43	Negative	-	Positive
31	29	M	PIK3R1	Yes	Yes	Pfizer- BioNTech	18.25	Negative	-	-
32	26	F	Ataxia Telangiectasia	Yes	Yes	Pfizer- BioNTech	5.71	Negative	-	-
33	20	M	ATP6AP1 gene/immunodef iciency 47	Yes	Yes	Pfizer- BioNTech	4.43	Negative	-	Positive

Table 1 Legend: Ab: antibody; assoc: associated; btwn: between; CTLA-4: cytotoxic T-lymphocyte associated protein 4; CVID: common variable deficiency; Def: deficiency; ICL: idiopathic CD4 lymphocytopenia; Ig Tx: immunoglobulin therapy; SAD: specific antibody deficiency; XLA: X-linked agammaglobulinemia

Table 2 – Symptoms post-COVID vaccination

Side Effects after SARS-CoV-2 Vaccination		
Subjects total who had symptoms	14	42% (14/33)
Symptoms		
Sore arm	6	18.2%
Fatigue	4	12.1%
Headache	5	15,1%
Local reaction/rash	2	6%
Fever/chills	1	3%
Myalgias	1	3%
Neck stiffness	1	3%
Vertigo/paresthesia	1	3%
Nausea/vomiting	1	3%
Flare of enteropathy*	1	3%
Flare of chronic urticaria	1	3%

*Flare occurred 1-week post-vaccination

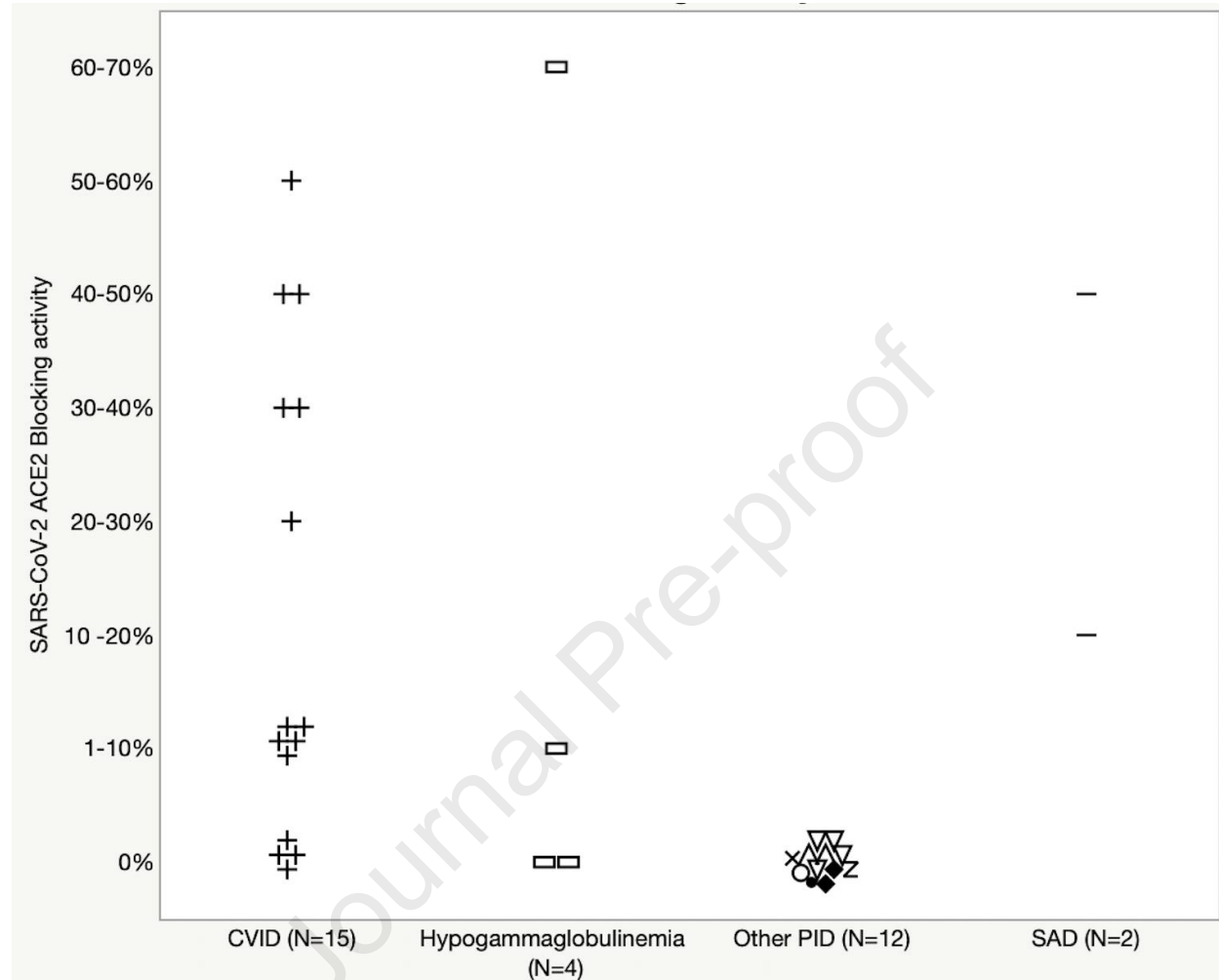
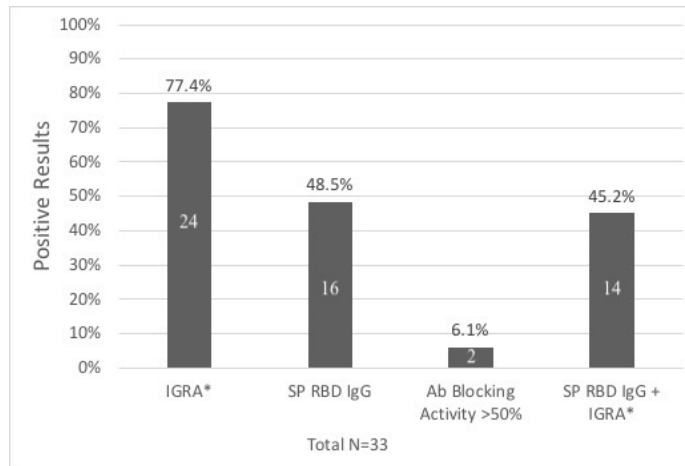
Figure 2: SARS-CoV-2 ACE2 blocking activity

Figure 1. Immunogenicity of the SARS-CoV-2 Vaccines in PID patients with functional B-cell defects



Immunogenicity and Tolerability of COVID-19 mRNA Vaccines in PID patients with functional B-cell defects – Supplementary Material

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Supplementary text:

X-linked agammaglobulinemia (XLA) is a humoral immunodeficiency caused by mutations in Bruton's tyrosine kinase (Btk) a key signal transduction molecule required for B-cell development. Patients have low to absent B-cells (most patients have a small number of B-cells), and reduced levels of all immunoglobulin classes. Common variable immunodeficiency

(CVID) is an antibody deficiency syndrome characterized by decreased serum IgG (IgM and IgA are often decreased) with impaired specific antibody responses. CTLA-4 haplo-insufficiency is an immunodysregulatory disorder, that can present like CVID-like phenotype, when there is hypogammaglobulinemia. Immunodeficiency 47 (IMD47) is a complex immunodeficiency syndrome characterized by hypogammaglobulinemia, recurrent bacterial infections, defective glycosylation of serum proteins, and liver disease with neonatal jaundice and hepatosplenomegaly. Good's syndrome (GS) with immunodeficiency is a rare condition in which thymoma is associated with hypogammaglobulinemia. It is characterized by increased susceptibility to bacterial, viral, and fungal infections, as well as autoimmunity. Most patients have no circulating B cells. X-linked hyper-IgM syndrome is a combined immune deficiency that is characterized by antibody deficiency as well as an impaired ability of T cells to activate monocytes and dendritic cells. This disease is caused by mutations in CD40 ligand (CD40L), a molecule that is expressed on the surface of activated T cells. CD40L interacts with CD40 on the surface of B cells to activate immunoglobulin class-switching (shifting antibody production from IgM to IgG, IgA, or IgE) and to establish B cell memory. PIK3R1 deficiency is also considered a predominantly antibody deficiency. Patients with XLA, CVID, GS, X-linked hyper IgM syndrome, PIK3R1 deficiency, Immunodeficiency 47 (IMD47) and CTLA-4 deficiency require uninterrupted immunoglobulin replacement therapy for the antibody deficiency component of their disease.

Supplementary method: ELISA to detect anti-SARS-CoV-2 and anti-SARS-CoV antibodies in plasma samples **Competition ELISA to detect antibodies blocking the binding of ACE2 to RBD.** This assay (and its references) was performed at Stanford Health Care

Clinical Virology Laboratory, a CLIA certified laboratory. In brief, 96-well Corning Costar high binding plates (Thermo Fisher: cat. 9018) were coated with SARS-CoV-2 spike RBD protein in PBS at a concentration of 0.1 µg per well overnight at 4°C. All competition ELISA steps were carried out on the next day at RT. Wells were washed 3x with PBS-T and blocked with PBS-T containing 3% non-fat milk powder for 1 hour. Wells were then incubated with plasma samples from our cohort of patients at a dilution of 1:10 in PBS-T containing 1% non-fat milk for 1 hour. Two quality controls (Access SARS-CoV-2 IgG QC, QC1-QC2, 2 levels, catalog no. C58964, Beckman Coulter) and two blank wells incubated with PBS-T containing 1% non-fat milk were included on each plate. ACE2-mFc diluted to 0.5 µg/ml in 1% non-fat milk powder was added without washing steps and incubated for an additional 45 min. After washing 3x with PBS-T, horseradish peroxidase conjugated goat anti-mouse IgG (Fc specific, catalog no. 31439, Invitrogen, 1:10'000 dilution) in PBS-T containing 1% non-fat milk was added and incubated for 45 min. Wells were washed 3x with PBS-T and dried by vigorous tapping of plates on paper towels. TMB substrate solution was added, and the reaction was stopped after 12 min by addition of 0.16 M sulfuric acid. The OD at 450 nanometers was measured with an EMax Plus microplate reader (Molecular Devices, San Jose, CA). OD values were converted to percentage of blocking using the following formula: $100 * (1 - (\text{sample OD} - 0.2) / (\text{QC1 OD} - 0.2))$, taking into account the background noise of the assay of 0.2 as determined after testing pre-pandemic control plasma samples.

Please refer to reference (3) for detail description.

The interferon-gamma (IFN-γ) release assay (IGRA) used here measured IFN-γ released by antigen-specific T cells after overnight stimulation with a commercially available peptide pool

consisting of spike, S1, nucleocapsid, and membrane proteins (Miltenyi Biotec, Bergisch Gladbach, Germany). (4) This assay (and its references) was performed at Stanford Health Care Clinical Virology Laboratory, a CLIA-certified laboratory. In brief, freshly collected blood in lithium heparin tube was (1) left unstimulated as negative control; stimulated with (2) peptide pool consisting of spike, S1, nucleocapsid, and membrane proteins (Miltenyi Biotec, Bergisch Gladbach, Germany) or 3) mitogen as positive control. The IFN- γ concentration in the plasma fraction was measured with an automated enzyme-linked immunosorbent assay (ELISA) instrument in international units per milliliter (IU/mL). IFN- γ response was defined as peptide stimulated minus unstimulated. The Mann-Whitney *U* test was used to compare median IFN- γ responses between groups. The Wilcoxon signed-rank test of medians was used to compare differences between paired results. The receiver operating characteristic curve was used to derive an IFN- γ response cutoff at the Youden maximum index value, which assigns equal weight to sensitivity and specificity. Criteria for Positive: When Nil is ≤ 8.0 AND SARS-COV-2 Antigen minus Nil is ≥ 0.35 . Criteria for Negative: When Nil is ≤ 8.0 AND SARS-COV-2 Antigen minus Nil is < 0.35 AND Mitogen-Nil is ≥ 0.5 . Criteria for Indeterminate: 1) Nil is ≤ 8.0 AND SARS-COV-2 Antigen minus Nil is < 0.35 AND Mitogen minus Nil is < 0.5 OR 2) Nil is > 8 . Criteria for borderline Result: Although a borderline range has not been defined, various sources of variability can cause a positive result with SARS-COV-2 Antigen minus Nil between 0.35 and 0.7. (5) It should be noted that the IFN-gamma measured by interferon-gamma release assay (IGRA) is a signature cytokine for the Th1 subpopulation while mitogen assays measure a much wider panoply of T cell related responses. So, it is expected that only the T cells that can recognize specifically SARS-COV-2 peptides will produce ITN-gamma ($\sim < 1$ % of the T cell population) (6).

Analysis and Data Visualization

JMP®, Version 15, SAS Institute Inc., Cary, NC, 1989–2021 and Microsoft Corporation.

(2018). *Microsoft Excel* were used for data analysis and visualization. Chisquare test was performed using JMP, version 15.

Normality of the data was determined and established through using the Kolmogorov-Smirnov

Test of Normality from website: <https://www.socscistatistics.com/tests/kolmogorov/default.aspx>

(last accessed October 12, 2021).

Two-tailed, unpaired T-tests were calculated using the GraphPad QuickCalcs Web

site: <https://www.graphpad.com/quickcalcs/ttest1/?format=C> (last accessed October 13, 2021).

Supplementary References

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134

135 **eTable 1: Subject Characteristics**

136

137 **Legend:** AI: autoimmunity; ALC: absolute lymphocyte count; BTK: Burton tyrosine
138 kinase; CLL: chronic lymphoid leukemia; CIU: chronic idiopathic urticaria; Dx:
139 diagnosis; Hx: history; IBD: inflammatory bowel disease; ILD: Interstitial lung
140 disease; ITP: idiopathic thrombocytopenia; MGUS : gammopathy of unknown
141 significance; SAD: specific antibody deficiency; VUS: variants of unknown
142 significance; WES: whole exome; WBC: white blood count; XLA: X-linked
143 agammaglobulinemia.

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