Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Vaccine 39 (2021) 7375–7378

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Absent antibody production following COVID19 vaccination with mRNA in patients under immunosuppressive treatments

Angelika Wagner [a], Joanna Jasinska [a], Elena Tomosel [a], Christoph C. Zielinski [b], Ursula Wiedermann [a,*]

[a] Institute of Specific Prophylaxis and Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Austria
[b] Central European Cancer Center, Wiener Privatklinik, Vienna, Austria, and Central European Cooperative Oncology Group, HQ: Vienna, Austria

ARTICLE INFO

Article history:
Received 21 July 2021
Received in revised form 4 October 2021
Accepted 26 October 2021
Available online 1 November 2021

ABSTRACT

Patients undergoing immunosuppressive treatments have a higher need for protection against coronavirus disease (COVID19) that follows infection with the SARS-CoV-2 virus but their ability to respond sufficiently to COVID vaccines is uncertain.

We retrospectively evaluated SARS-CoV-2 spike subunit 1 (S1)-specific antibody levels after two mRNA doses in 242 patients with underlying chronic inflammatory, hematooncological or metabolic diseases and in solid organ transplant recipients. S1-specific antibodies were measured 30 days after the second dose.

In 15.9% of these patients, no S1-specific antibodies were detectable. Non-responsiveness was linked to administration of B-cell depleting therapies as well as to ongoing therapies that block lymphocyte trafficking (Fingolimod) or inhibit T cell proliferation (Tacrolimus).

Thus, it is important to inform immunosuppressed patients about the risk of vaccine non-responsiveness and the necessity to maintain non-pharmaceutical protection measures. In these risk patients antibody testing and cellular analysis are helpful to estimate the benefit/responsiveness to further booster vaccinations.

Introduction

COVID19 mRNA vaccines consist of a lipid nanoparticle-formulated nucleoside-modified mRNA which encodes for the receptor-binding domain of the SARS-CoV-2 spike protein [1]. In fully vaccinated healthy individuals, mRNA vaccines induce a robust anti-spike antibody response resulting in ~ 95% efficacy against COVID19 [1].

Considering the extent of the SARS-CoV-2 pandemic, such observations on vaccine efficacy in healthy individuals were of utmost importance, but information on efficacy in vulnerable populations has been largely lacking [2]. We wondered whether treatments known to directly reduce B-cell numbers or impair T-lymphocyte function would inhibit vaccine-induced antibody production.

Thus, we performed a retrospective study of S1-antibody production following mRNA vaccination in patients undergoing various immunosuppressive treatment regimens.

Methods Study population

We analysed S1-specific antibody results in a total of 214 patients (117 females, 97 males; mean age: 53. 4 (51.4–55.4 95 % CI) at our outpatient vaccination clinic for high-risk patients after intramuscular administration of two doses BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna). Both vaccines contain mRNA encoding for the spike protein in lipid nanoparticles that were administered into the deltoid muscle with no other vaccines administered concomitantly. Among these were patients suffering from chronic inflammatory diseases (including rheumatoid arthritis, intestinal bowel disease or multiple sclerosis; n = 104), hematooncological diseases (n = 66), solid tumors (n = 14), patients with solid organ transplants (n = 22) or metabolic disorders (n = 8) Additionally, antibody results from 26 healthy individuals (50% females; mean age: 48.7 (41.5–55.9 95 %CI) served as controls.

Serologic testing

S1-specific IgG titers were assessed by ELISA (Quantivac®, Euroimmune) following the manufacturer's instructions at an average of 31.7 (30.2–33.3 95 %CI) days after the second dose. We included test results received between January 2021 and June

* Corresponding author at: Institute of Specific Prophylaxis and Tropical Medicine, Centre for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Kinderspitalgasse 15, Vienna A-1090, Austria.
E-mail address: ursula.wiedermann@meduniwien.ac.at (U. Wiedermann).

https://doi.org/10.1016/j.vaccine.2021.10.068
0264-410X/

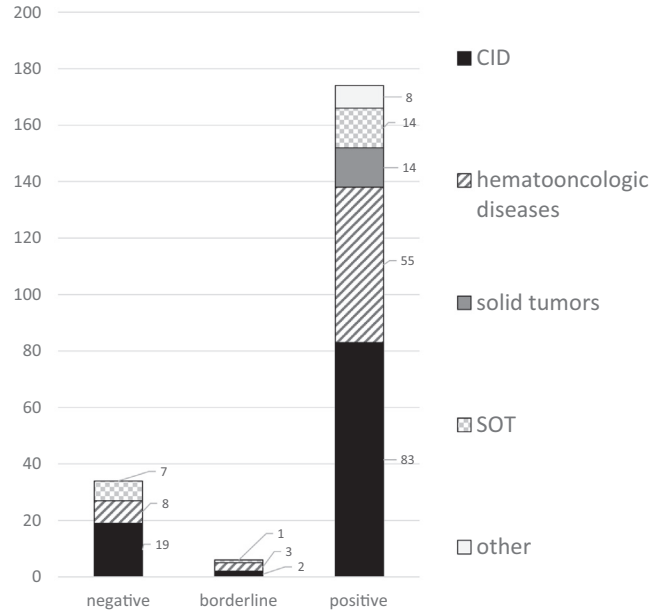

Fig. 1. S1-specific SARS-CoV-2 IgG antibody titer results according to underlying disease category (chronic inflammatory diseases (CID), hematooncological diseases, solid tumors, solid organ transplant (SOT) and other disorders such as metabolic diseases) in the overall study population (n = 214).

23rd 2021. Ethics approval for this study was obtained from the Ethics committee of the Medical University of Vienna (EK 1586/2021).

Results & Discussion

In our study population, we were able to evaluate baseline S1-specific antibody titers prior to the first dose in 89.1% (191/214) of patients. Of those 1.05% (n = 2) were positive and 0.52% (n = 1) was in borderline range already before receiving the first dose of an mRNA vaccine indicating a prior asymptomatic infection. After the two dose vaccination schedule we observed that anti-SARS-CoV-2 antibodies were undetectable (i.e. < 25.6 BAU/ml) in 15.89% (34/214; mean age 56.3 (50.7–61.9 95 %CI); 58.8% females) of the mentioned entity of patients(Fig. 1; Table 1). Borderline titer results (i.e. 25.6–35.2 BAU/ml) according to the manufactureŕs reference values were obtained in 2.8% (6/214; mean age 64.9 (53.6–76.3 95 %CI); 16.7% females; Fig. 1, Table 2) of patients and positive antibody results were received in 81.31% (n = 174; mean age) of patients. In contrast, among the healthy individuals, that had requested an antibody titer measurement, all (n = 26) had positive antibody titer (i.e. > 35.2 BAU/ml) results after two doses of the mRNA vaccines. Furthermore, geometric mean titers were significantly higher in the healthy individuals compared to the seropositive patients (GMT 706.7 versus 517.1; $p < 0.05$, Students *t*-test).

Table 1
patient characteristics of those without antibody responses after COVID19 mRNA vaccine.

	Age band	gender	diagnosis	Immunosuppressive treatment	Last dose RTX before 1st mRNA in months	Vaccine	Interval between 1st and 2nd dose
1	21–30	m	Multiple sclerosis	Fingolimod		BNT162b2	21
2	31–40	f	Multiple sclerosis	Fingolimod		BNT162b2	21
3	31–40	f	Multiple sclerosis	Fingolimod		BNT162b2	21
4	41–50	f	Multiple sclerosis	Fingolimod		BNT162b2	21
5	41–50	m	Multiple sclerosis	Fingolimod		BNT162b2	21
6	51–60	f	Multiple sclerosis	Fingolimod		BNT162b2	24
7	51–60	f	Rheumatoid arthritis	Rituximab		BNT162b2	21
8	71–80	m	Rheumatoid arthritis	Methotrexate, Tofacitinib		BNT162b2	22
9	51–60	f	Myositis	Rituximab	7	BNT162b2	21
10	51–60	f	Myositis	Rituximab		BNT162b2	21
11	61–70	m	Vasculitis	Rituximab, cortisone Mycophenolate Mofetil	4	BNT162b2	21
12	71–80	m	Myasthenia gravis	Mycophenolate Mofetil		BNT162b2	21
13	18–20	m	Goodpasture syndrome,	Rituximab	9	BNT162b2	21
14	71–80	f	Vasculitis	Rituximab, cortisone	12	BNT162b2	22
15	61–70	m	Pemphigus	Mycophenolate Mofetil, cortisone		BNT162b2	21
16	61–70	f	Systemic lupus erythematosus	Mycophenolate Mofetil		BNT162b2	21
17	51–60	f	Scleroderma	Rituximab	8	BNT162b2	21
18	31–40	f	Collagenosis	Rituximab, Cortisone	>12	BNT162b2	n.d.
19	51–60	f	Sarcoid	Ebetrexat		BNT162b2	21
20	71–80	m	Multiple myeloma, autologus stem cell transplantation	Lenalidomid		BNT162b2	21
21	51–60	m	Multiple Myeloma, autologous stem cell transplantation	Pomalidomid, cortisone		BNT162b2	21
22	61–70	m	Stem cell transplantation 2.20 with GvHD	Ruxolitinib		BNT162b2	21
23	21–30	f	Kidney transplantation	Mycophenolate Mofetil, Tacrolimus		BNT162b2	21
24	31–40	m	Kidney transplantation	Sirolimus, Mycophenolate		BNT162b2	21
25	51–60	f	Kidney transplantation	Tacrolimus, Mycophenolate Mofetil, Prednisolon		BNT162b2	21
26	61–70	f	Kidney transplantation	Tacrolimus, Azathioprin, Prednisolon		BNT162b2	21
27	61–70	m	Kidney transplantation	Tacrolimus, Mycophenolate, cortisone		BNT162b2	21
28	51–60	f	Lung transplantation	Mycophenolate Mofetil		BNT162b2	21
29	61–70	f	Heart transplantation, multiple	Everolimus, Tacrolimus,		BNT162b2	21

Table 1 (*continued*)

	Age band	gender	diagnosis	Immunosuppressive treatment	Last dose RTX before 1st mRNA in months	Vaccine	Interval between 1st and 2nd dose
			myeloma	Daratumumab,			
30	71–80	m	Lymphoma	Rituximab	8	BNT162b2	21
31	81–90	f	Lymphoma	Rituximab, Bendamustin	1	BNT162b2	22
32	81–90	m	Lymphoma	Rituximab	n.d.	BNT162b2	21
33	61–70	m	chronic lymphocytic leukemia	anti-CD20	8	BNT162b2	21
34	21–30	f	Aplastic anemia	(Immunglobuline substitution)		BNT162b2	21

n.d. not documented.

Table 2
patient characteristics of those with borderline results after COVID19 mRNA vaccine.

	Age band	gender	diagnosis	Immunosuppressive treatment	Vaccine	Interval between 1st and 2nd dose
1	61–70	m	polymyositis	Mycophenolate mofetil	BNT162b2	21
2	81–90	m	CIDP (chronic inflammatory demyelinating polyneuropathy)	Mycophenolate mofetil	BNT162b2	21
3	71–80	m	Heart transplantation	Mycophenolate mofetil	BNT162b2	21
4	5160-	m	Multiple myeloma, autologous stem cell transplantation	Carfilzomib,Daratumumab, Dexamethason, Pomalidomide	BNT162b2	21
5	71–80	f	Multiple myeloma	lenalidomide	BNT162b2	21
6	31–40	m	Acute myeloid leukemia, allogenous stem cell transplantation	corticoide	BNT162b2	21

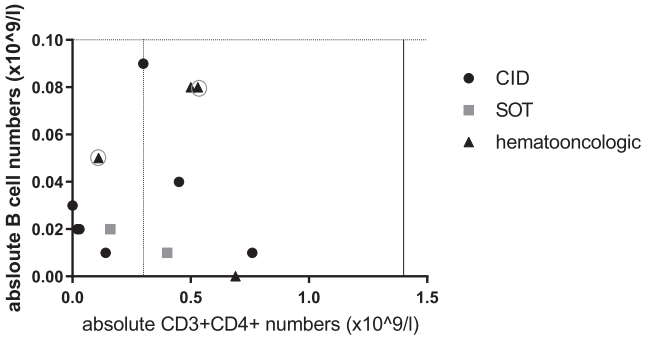

Fig. 2. Correlation of B cell counts with CD3 + CD4 + T cell counts in non-responders. Results were available from 13 participants (Reference values for CD19 + B cells: 0.1–0.5 x10^9/l and for CD3 + CD4 + T cells: 0.3–1.4 x10^9/l). Results from two hematooncological patients with borderline antibody results are marked with a circle.

With regard to non-responsiveness, further analysis showed that a lack of antibody production was preferentially linked to an ongoing treatment with Rituximab in patients with B-cell malignancies or autoimmune disorders, Fingolimod in patients with multiple sclerosis or Calcineurine inhibitors (Tacrolimus) in renal transplant patients (Table 1). Due to the retrospective character of this study B and T cell counts are not available from all study participants, However, among 13 non-responders B and T cell counts between 34 days before and up to 115 days after the first mRNA dose were available and revealed that none of them showed normal B cell counts according to the reference values, irrespective of the type of immunosuppressive therapies (Fig. 2). Part of these patients displayed CD4 + T cell counts in normal range, however, we do not have further information on the functionality of these cells. It is proposed that various mechanisms seem to be involved in the inability to mount an antibody response which include B cell depletion (<1%) by Rituximab[3], prevention of lymphocyte trafficking from lymphoid tissue by Fingolimod[4] or inhibition of lymphocyte proliferation by Tacrolimus[5] thereby hindering also T-cell help for B-cell antibody production. The Rituximab-induced defect in antibody production persisted for at least 11 months after the termination of treatment and was normalized only after this period resulting in an antibody response in 4 out of 14 Rituximab-treated patients. Of note, two patients with the last Rituximab administration longer than 11 months ago were still not able to mount an antibody response and remained seronegative, indicating individual recovery rates after this treatment. Recent data now show that part of these B cell depleted patients and also other immunosuppressed patients are able to mount a cellular response to the COVID19 vaccine [6–9].

In the group of responders, the underlying treatment regimens were diverse (Fig. 3). The data further show that 2 out of 8 patients on Fingolimod were able to mount an antibody response. In SOT patients, particularly kidney-transplanted patients (as well as one with a heart and one with a liver transplant on Tacrolimus) did not show any antibody responses, whereas the other SOT patients - even when treated with Tacrolimus - mounted S1-specific antibody levels. This illustrates that immune responsiveness/non-responsiveness cannot easily be predicted in patients with high-grade immunosuppressive treatments, and therefore immunologic testing of antibodies and cellular responses may help to anticipate vaccine-responsiveness.

Conclusion

We conclude that both, patients undergoing such immunosuppressive treatments and physicians prescribing these therapies should be informed about the potential lack of anti-SARS CoV-2 antibody formation following vaccination. Patients with the mentioned diseases and treatments need counseling for alternative protection methods including social distancing, use of masks and – most importantly – the inclusion of their immediate contacts into vaccination programs. However, also in patients mounting an immune response, one should be aware of the fact that antibody titers are often lower than in the healthy population and it is necessary to follow the kinetics of these antibody levels over time as they may wane more quickly in the immunosuppressed. Application of additional vaccine doses seem justified if an immune response at least at the cellular level can be expected and has been shown promising in SOT patients to increase seroconversion rates and antibody levels [10,11]. Importantly, these doses should be discussed in relation to lymphocyte typing results, and whenever

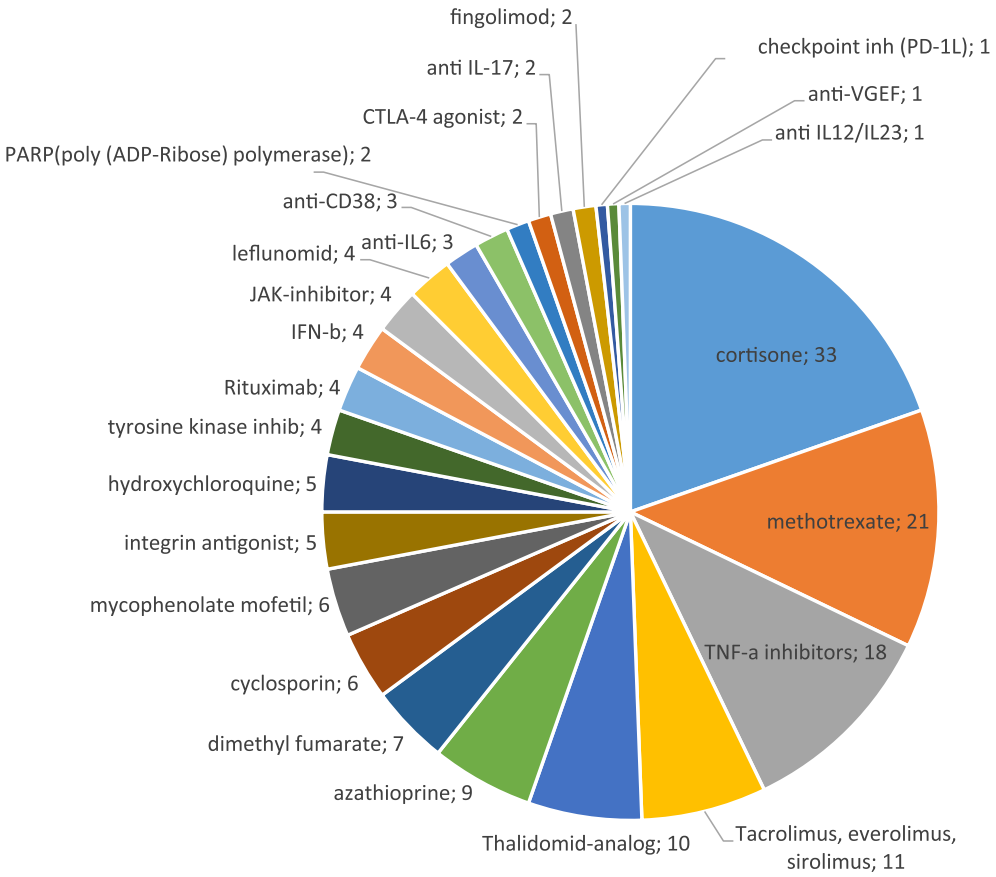

Fig. 3. Underlying immunosuppressive treatment in responders. Among seroresponders, 33 participants had already terminated immunosuppressive treatment before the first mRNA dose. Among those treated with corticoids 25 out of 33 had a dose of below 20 mg per day.

applicable, by lymphocyte functionality tests as well as monitoring of humoral and cellular responses. Wherever feasible, the timing of the booster application should be planned to utilize therapeutic cycles/intervals with lower immunosuppressive drug levels.

Authors contributions:

Literature search: AW, CZ, UW; figures: AW, ET; study design: AW, CZ, UW; data collection: AW, ET, JJ, UW; data analysis: AW, ET, JJ, UW; data interpretation: AW, ET, JJ, CZ, UW; writing: AW, CZ, UW

No funding was received for this retrospective data analysis.

CRediT authorship contribution statement

Angelika Wagner: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Joanna Jasinska:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Elena Tomosel:** Data curation, Formal analysis, Investigation, Visualization, Writing – review & editing. **Christoph C. Zielinski:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ursula Wiedermann:** Conceptualization, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

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.

References

[1] Lombardi A, Bozzi G, Ungaro R, Villa S, Castelli V, Mangioni D, et al. Mini Review Immunological Consequences of Immunization With COVID-19 mRNA Vaccines: Preliminary Results. Front Immunol 2021;12(). https://doi.org/10.3389/fimmu.2021.657711.
[2] Righi E, Gallo T, Azzini AM, Mazzaferri F, Cordioli M, Merighi M, et al. A Review of Vaccinations in Adult Patients with Secondary Immunodeficiency. Infect Dis Ther 2021;10(2):637–61.
[3] Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. Nat Rev Drug Discov 2021;20(3):179–99.
[4] Pelletier D, Hafler DA. Fingolimod for multiple sclerosis. N Engl J Med 2012;366(4):339–47.
[5] Bendickova K, Fric J. Roles of IL-2 in bridging adaptive and innate immunity, and as a tool for cellular immunotherapy. J Leukoc Biol 2020;108(1):427–37.
[6] Mrak D, Tobudic S, Koblischke M, Graninger M, Radner H, Sieghart D, et al. SARS-CoV-2 vaccination in rituximab-treated patients: B cells promote humoral immune responses in the presence of T-cell-mediated immunity. Ann Rheum Dis 2021;80(10):1345–50.
[7] Asplund Hogelin K, Ruffin N, Pin E, et al. Development of humoral and cellular immunological memory against SARS-CoV-2 despite B cell depleting treatment in multiple sclerosis. *iScience* 2021; **24**(9): 103078.
[8] Apostolidis SA, Kakara M, Painter MM, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat Med* 2021.
[9] Prendecki M, Clarke C, Edwards H, McIntyre S, Mortimer P, Gleeson S, et al. Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. Ann Rheum Dis 2021;80(10):1322–9.
[10] Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three Doses of an mRNA Covid-19 Vaccine in Solid-Organ Transplant Recipients. N Engl J Med 2021;385(7):661–2.
[11] Benotmane I, Gautier G, Perrin P, Olagne J, Cognard N, Fafi-Kremer S, et al. Antibody Response After a Third Dose of the mRNA-1273 SARS-CoV-2 Vaccine in Kidney Transplant Recipients With Minimal Serologic Response to 2 Doses. JAMA 2021;326(11):1063. https://doi.org/10.1001/jama.2021.12339.