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Short Communication

SARS-CoV-2 antibody determination in a vaccinated and recovered cohort in Austria

Elisabeth Mara*, Tobias Mader, Johannes Gratzer, Stefanie Hochegger, Thomas Pekar

Biomedical Science, University of Applied Sciences Wiener Neustadt, Austria

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ABSTRACT

Since December 2019 the world has been dealing with a severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic. The first SARS-CoV-2 vaccine was made available in Europe at the end of 2020. 202 volunteers from the vicinity of the University of Applied Sciences Wiener Neustadt took part in this study; their IgG levels recognizing the RBD of SARS-CoV-2 were determined. The aim was to evaluate the SARS-CoV-2 titer levels of vaccinated, recovered and vaccinated plus recovered persons. We could show that there is a significant difference in the antibody levels of vaccinated, vaccinated plus recovered and only recovered probands. Additionally, the highest antibody levels were found in triple vaccinated persons. Furthermore, the Moderna vaccine seems to have a higher immune response.

Introduction

The world has been dealing with the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic since December 2019. Many measures have been taken to stem the spread of the virus, such as lockdowns, social distancing and wearing face masks. At the end of 2020, the European Medicines Agency (EMA) approved the first SARS-CoV-2 vaccine in Europe [1,2]. Currently five vaccines are approved in Europe, which are the vector based Vaxzevria (AstraZeneca) and COVID-19-Vaccine Jansen (Johnson&Johnson), the mRNA vaccines Comirnaty (BioNTech/Pfizer) and Spikevax (Moderna) and the protein based, recombined Nuvaxovid (Novavax) [3].

The first efforts to develop a vaccine against SARS-CoV were made after the SARS outbreak of 2002-2004, but research was halted due to the eradication of the virus. However, research for MERS-CoV continued. In these earlier studies it was discovered that the spike protein found in most coronaviruses is responsible for receptor binding and membrane fusion. For SARS-CoV and SARS-CoV-2 the spike protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cells. It was shown that antibodies targeting the spike protein, and especially the receptor binding domain (RBD), prevented the binding of the virus to the cell and therefore neutralized the virus [1].

Most studies dealing with the analysis of vaccine effectiveness included mRNA vaccines, CoronaVac and the vaccine from AstraZeneca. It was shown that these vaccines could prevent severe disease, hospitalization or death from variants of concern present at that time point (Alpha, Beta, Gamma, Delta). The vaccines showed different effectiveness against the various variants, ranging from 47.3-100% for mRNA vaccines and 67-74.5% for the vaccine from AstraZeneca [4].

Generally speaking, the vaccinations offer good protection against a corona infection or a severe course. The effectiveness against the Delta variant is 85% on average [5].

A recent metastudy showed that the effectiveness of vaccination against infection with the Omicron variant is reduced by a factor of 4 compared to infection with the Delta variant [6]. This reduction in effectiveness can probably be traced back to the high number of mutations in the Omicron variant, including mutations in the receptor-binding domain of the spike protein [7].

A comparison by Shenai et al. between fully vaccinated and recovered individuals shows no difference in the risk of infection [8]. However, it has also been shown that SARS-CoV-2 infection before or after vaccination gives a significantly larger boost to the neutralizing antibody response compared to vaccination alone [9].

Additional knowledge must be gained about the effectiveness of the vaccination schemes used.

In this study, the IgG levels recognizing the RBD of SARS-CoV-2 were determined within 202 volunteers from a university's vicinity. The aim was to evaluate the SARS-CoV-2 titer levels of vaccinated, recovered and vaccinated plus recovered persons.

Method

Serum samples were collected through venipuncture from 202 participants between 15/2/2022 and 20/3/2022 and frozen in Eppendorf® tubes at -20°C until further procedure. The IgG level recognizing the

* Corresponding author.
E-mail address: emara@gmx.at (E. Mara).

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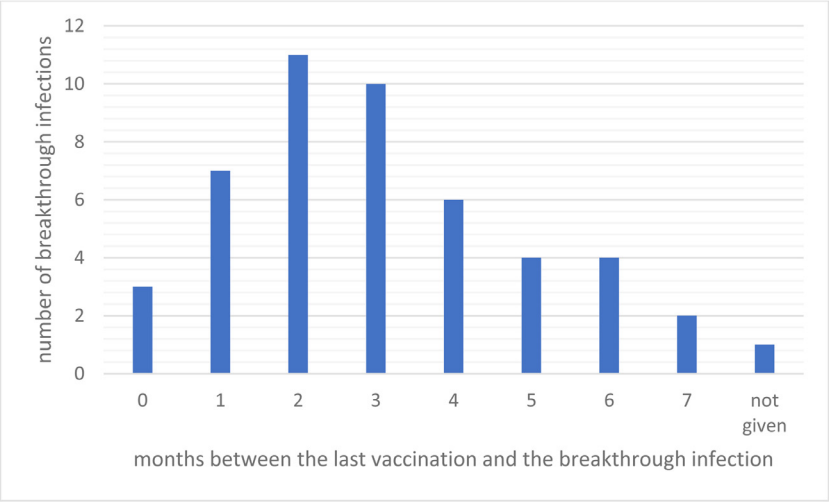

Fig. 1. Months between the last vaccination and the breakthrough infection

RBD of the S protein of SARS-CoV-2 was determined through ELISA (DRG Instruments GmbH). A questionnaire was completed to survey the COVID-19 anamnesis (e.g. if and how often the probands were tested, which tests were used, if they were tested positive, which symptoms they had etc.). A positive titer was assumed above 55 IU/ml. Written, informed consent was obtained from all participants in accordance with the inhouse ethics committee. The data gathered was analyzed using Microsoft® Excel® and IBM® SPSS® Statistics 26.

Results

A total of 202 probands took part in this study; 143 (70.8%) of the probands were female and 59 (29.2%) male. The ages ranged from 19 to 67 years with a mean age of 39,26 years. 63 of the 202 (31.2%) participants were tested positive with PCR, whereas 57 (90.5%) of them were tested positive once, 6 (9.5%) twice. 33 (52.4%) of the participants were between 18-35 years, 21 (30.3%) between 36-50 years and 9 (14.3%) between 51-67 years. Most of the 52 probands with a symptomatic illness suffered from a headache (66.1%), followed by tiredness (62.7%), sore throat (57.6%), cough (49.2%) and ageusia/anosmia (37.3%). 14 of the 63 (22.2%) of the recovered persons complained about symptoms after recovering, such as breathing difficulties, reduced stamina, or limited ageusia/anosmia.

193 of the 202 probands (95.5%) were already vaccinated at the time of sampling, of which 163 (84.5%) had received three doses, 29 (15%) two doses and only one (0.5%) a single dose.

An examination of the inoculants used for vaccination showed 3 Pfizer shots (45.6%) was the most frequent vaccination schemata, followed by 2 times AstraZeneca and 1 time Pfizer (20.7%). The exact distribution of the vaccination schemes is listed in Table 1.

128 out of the 193 (66.32%) vaccinated persons had side effects after the vaccination, for example fever, tiredness, melalgia/muscle pain and/or headache.

48 (24.9%) of the already vaccinated probands were infected with SARS-CoV-2 after vaccination. 44 (91.7%) of them had a mild course of disease, whereas four (8.3%) had an asymptomatic course. The time period between the last vaccination and breakthrough infection was between zero and seven months; in two probands the breakthrough infection occurred after the first dose, in 17 (35.42%) after the second dose and in 48 (58.33%) after the third dose. In Table 2 the vaccination regime of breakthrough infections is seen.

Fig. 1 shows that most breakthrough infections occur about two and three months after last contact with the pathogen.

Table 1
Vaccination schemes according to their frequency

Inoculant	Frequency	Percentage
3x Pfizer	88	45.6%
2x AstraZeneca 1x Pfizer	40	20.7%
2x Pfizer 1x Moderna	19	9.8%
2x Pfizer	18	9.3%
2x AstraZeneca 1x Moderna	8	4.1%
1x Johnson & Johnson 1x Pfizer	7	3.6%
3x Moderna	4	2.1%
2x AstraZeneca	4	2.1%
2x Moderna 1x Pfizer	2	1.0%
1x Johnson & Johnson 2x Pfizer	2	1.0%
1x Johnson & Johnson	1	0.5%
Total	193	100.0%

Table 2
Vaccination regime of breakthrough infections

Inoculant	Frequency	Percentage
3x Pfizer	20	41.7%
2x Pfizer	11	22.9%
2x AstraZeneca 1x Pfizer	7	14.6%
2x Pfizer 1x Moderna	4	8.3%
2x AstraZeneca	3	6.3%
1x Johnson&Johnson 1x Pfizer	2	4.2%
1x Johnson&Johnson	1	2.1%
Total	48	100%

The measurement of the antibody titer showed that 198 of the 202 (98%) had a qualitatively positive antibody titer against SARS-CoV-2. All vaccinated and vaccinated/recovered probands had a positive antibody titer, whereas only five of the nine (55.6%) of only recovered probands showed a positive result. A difference in the titer could be observed between recovered, vaccinated and vaccinated/recovered probands. The lowest titer was found in the group of only recovered persons (1.8 IU/ml-1532.5 IU/ml, median 154.2 IU/ml). The highest titer was found in the group of vaccinated/recovered probands with titers ranging from 166.6 IU/ml to >3200 IU/ml (median 3056.8 IU/ml). The antibody titer from only vaccinated probands ranged from 83.5 IU/ml to >3200 IU/ml (median 1270 IU/ml) (Fig. 2).

A correlation in time between the vaccination or recovery and the antibody titer could be observed ($p<0.001$) (Fig. 3).

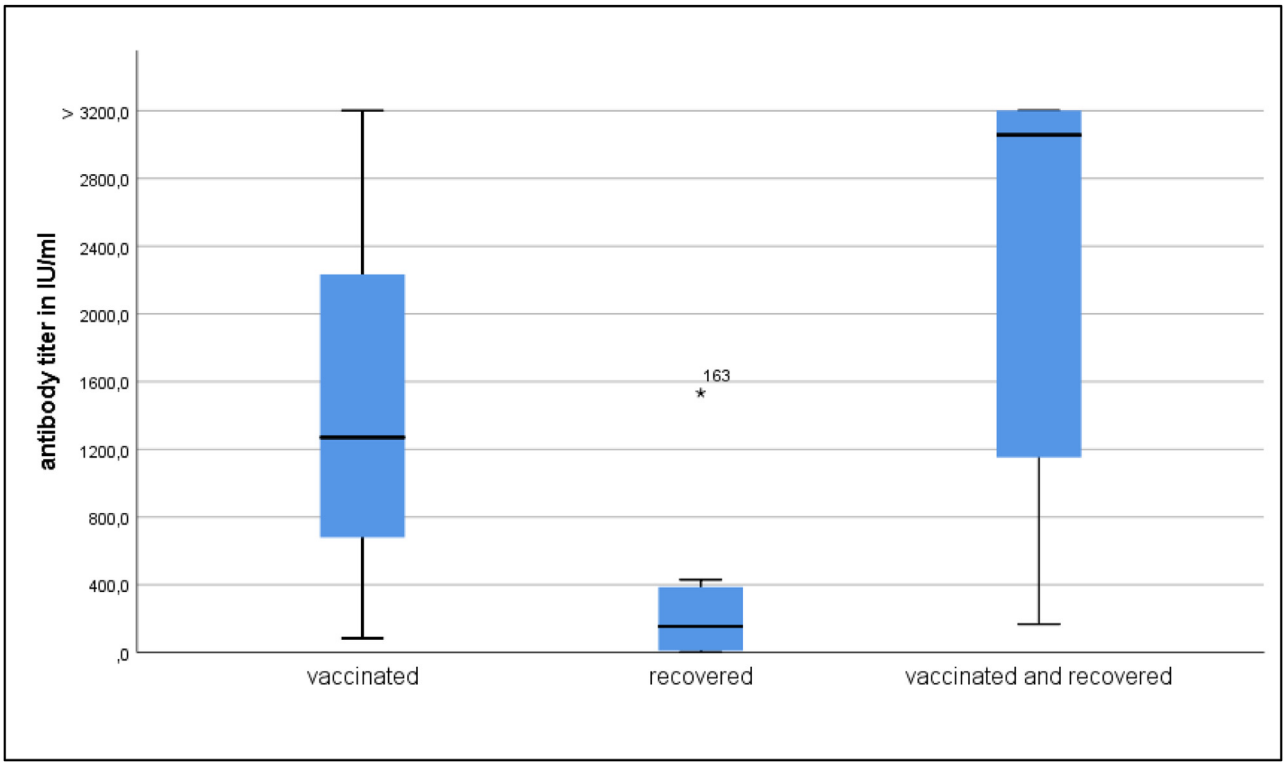

Fig. 2. Boxblot of the titer of recovered, vaccinated and recovered/vaccinated probands

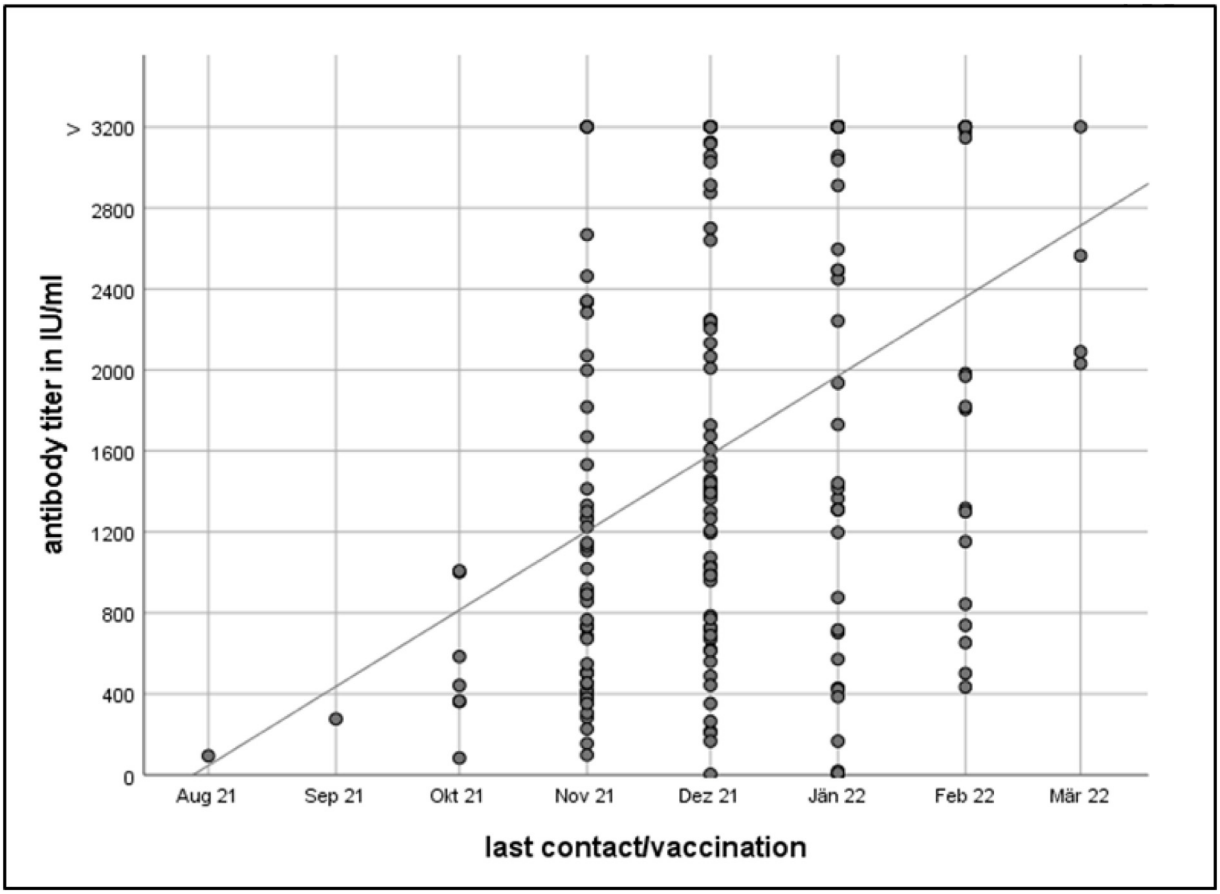

Fig. 3. Antibody titer in relation to the timepoint of the last contact with the pathogen or the last vaccination, respectively.

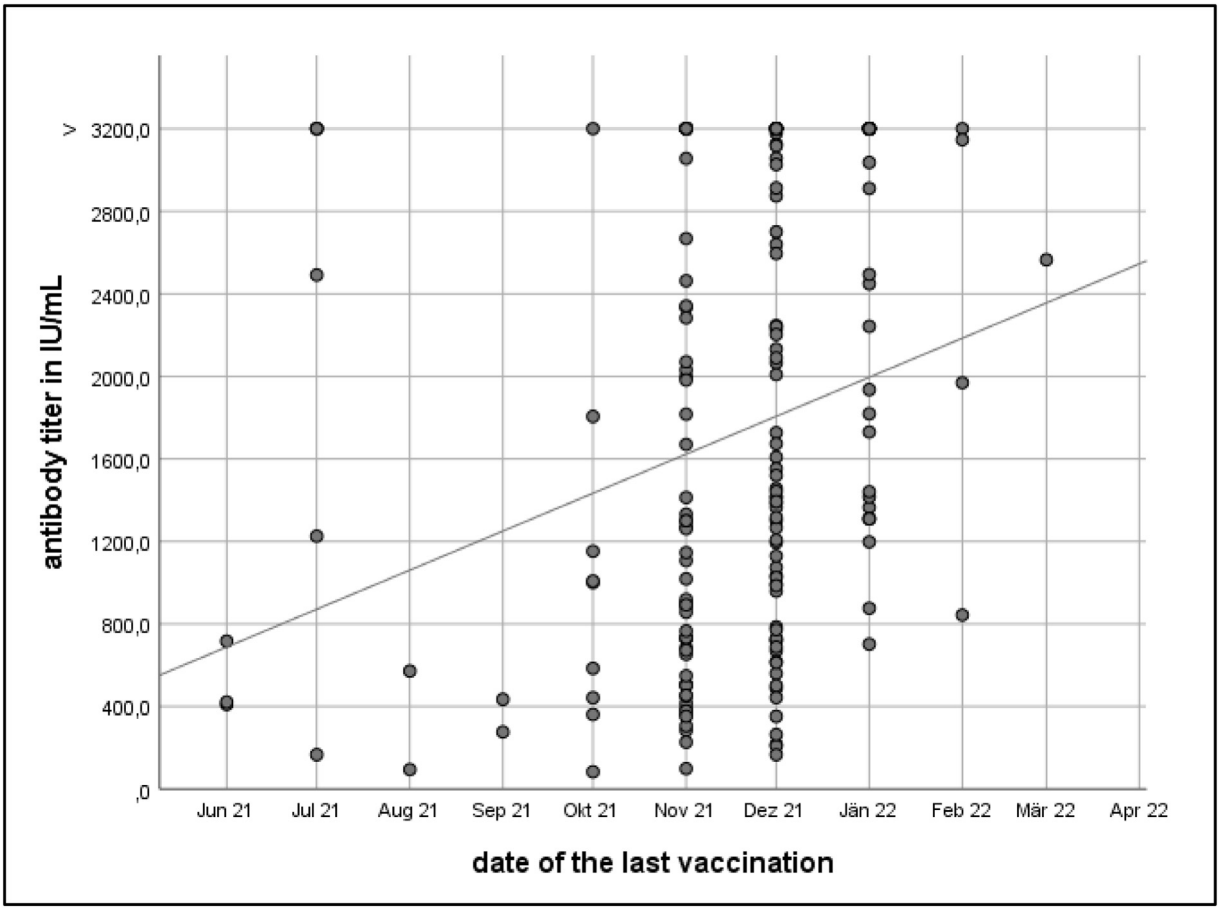

Fig. 4. Antibody titer and time point of the last vaccination

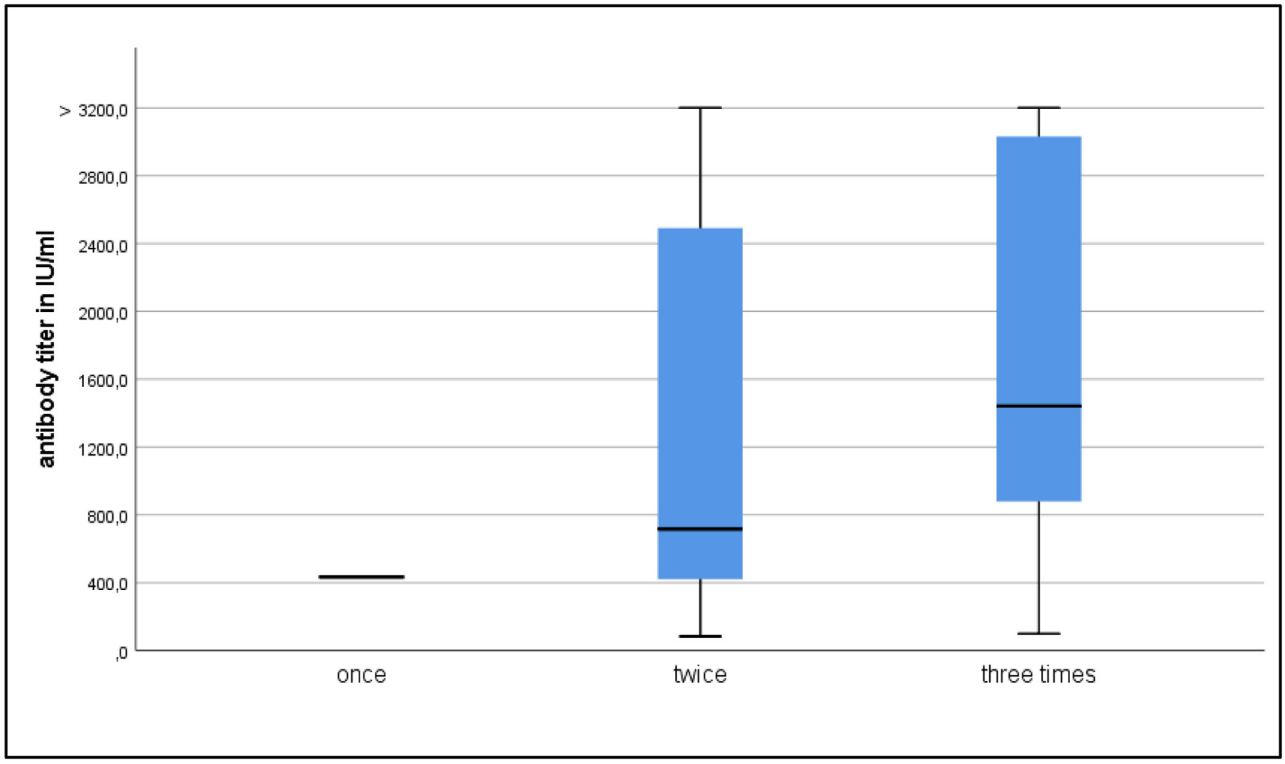

Fig. 5. Antibody titer according to the received doses

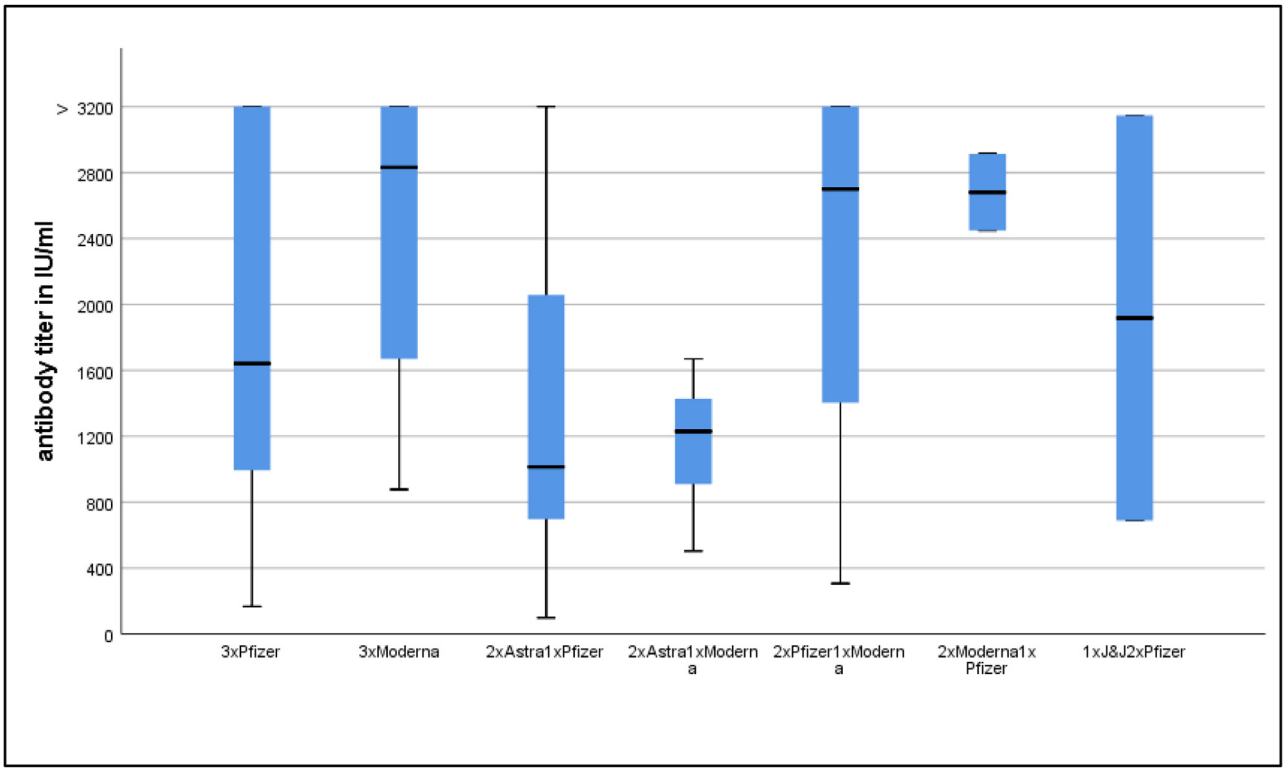

Fig. 6. Antibody titer according to the vaccine combinations

Looking only at the group of vaccinated probands, a correlation is seen between the antibody titer and the time point of the last vaccination ($p<0.001$) (Fig. 4)

A difference could be observed in the antibody titer and the number of doses given ($p=0.016$) (Fig. 5). Additionally, differences could be seen in the combination of vaccines and the antibody titer ($p=0.016$) when 3 doses are given, whereas no significant difference is seen in subjects who received only two doses ($p=0.298$) (Fig. 6).

Discussion

In this study, the antibody titer against SARS-CoV-2 was determined in 202 probands. The focus was on the detection of antibodies directed against the RBD because they are seen as 10-100-fold more potent than antibodies that recognize the NTD [10]. 95.5% of the probands were vaccinated, which does not reflect the Austrian vaccination rate of 72.36% of the Austrian vaccinable population as of 6/4/2022 [11]. The most frequently received vaccine combination was three times Pfizer (45.6%) followed by two times AstraZeneca combined with Pfizer (20.7%) and two times Pfizer with Moderna (9.8%). 66.3% reported side effects after the vaccination, whereby most side effects occurred after the first vaccine dose.

31.2% of the probands were already tested positive for SARS-CoV-2. Most positive cases were found in the proband group of 18-35 years old (38.8%). This could be due to the fact that younger persons are more socially active, leading to more social contacts and a higher risk of infection than the older population.

24.9% experienced a SARS-CoV-2 infection, even though they were vaccinated. This incidence rate is many times higher than that recently described by Ledda et al. [12]. Most of the positive-tested probands reported a mild course; only a few had an asymptomatic course. The three main symptoms were headache, tiredness and a sore throat. Mizrathi et al. could demonstrate that the risk for a breakthrough infection was significantly higher for probands vaccinated earlier, with an increased risk of hospitalization. Additionally, they could show that the antibody levels and immune system compounds decline over time following the second dose of vaccination [13]. Our study also shows a correlation between the time of the last vaccination/infection and the antibody titer. Similar to the work of Mizrathi et al., the dominant strain before/during this study was the Delta variant. It is unclear how this fact influences the effectiveness of the vaccination. Yet it could be shown that vaccine effectiveness is significantly lower in protecting against the Omnicron variant compared to the Delta variant [7].

Most of the probands of our study experienced a breakthrough infection after the third vaccination dose (58.3%), followed after the second dose (35.4%) and the first does (4.2%). Our data support findings from Andrews et al. who could show that the effectiveness of the vaccination decreases with the omicron variant [7].

The present results also show that the antibody concentration is significantly increased by the third vaccine dose. Additionally, we observed elevated antibody titers in vaccinated and/or recovered probands compared to persons who have only recovered from a SARS-CoV-2 infection. This finding also reflects our previously published results, which show a higher antibody titer after an infection recovery than after vaccination [14]. Similar results can be found in other studies [15,16]

Comparing the various vaccine combinations, it could be observed that probands who received three doses of the Moderna vaccine showed the highest antibody concentration, followed by two times Pfizer plus one time Moderna, and two times Moderna plus one time Pfizer. This leads to the conclusion that the Moderna vaccine is the most efficacious. Steensels et al. [17] showed similar results in their study when they compared Moderna and Pfizer vaccines.

One influencing factor of this study is the predominantly academic, and in many cases also health-related, background of the participants. This can be an explanation for the high vaccination rate of our proband collective since healthcare professionals must be vaccinated when they start working or complete their internship in the Austrian healthcare system.

Since there seems to be no difference between the usage of the whole S protein or only the RBD for the detection of antibodies, the ELISA using RBD as antigen which was utilized is not seen as limitation [18]

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Conflict of Interest

The authors have no conflict of interest to disclose.

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