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.

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Antibodies against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) in individuals with and without COVID-19 vaccination: A method comparison of two different commercially available serological assays from the same manufacturer

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ABSTRACT

Background: We compared two serological assays from Roche Diagnostics in individuals with and without COVID-19 vaccination, namely the Elecsys Anti-SARS-CoV-2 assay (detecting antibodies against the nucleocapsid protein of SARS-CoV-2) and the Elecsys Anti-SARS-CoV-2 S assay (detecting antibodies against the spike protein of SARS-CoV-2).

Methods: With both assays, we analyzed 3033 serum samples collected from 2496 patients without COVID-19 vaccination. In addition, we studied 34 healthcare-workers who received two injections of the BNT162b2 COVID-19 vaccine from BioNTech/Pfizer three weeks apart and who had repeatedly determined their antibody response by both assays.

Results: In our cohort of patients without COVID-19 vaccination, 62.9% of all determinations were negative with both Roche assays and 31.5% were positive with both assays. In 5.6% of our cohort, however, there were discordant results with both assays (partly because initially discordant results of the two assays became concordantly positive over time). In the healthcare-workers with the COVID-19 vaccination, the results of the Roche anti-nucleocapsid assay remained negative throughout the observation period of 5 weeks after vaccination. The initially negative antibodies against the spike protein became positive with the Roche assay in all samples two weeks after the initial injection, and the serum concentrations of anti-spike antibodies increased constantly until 4–5 weeks after the initial injection.

Conclusions: Here, we provide information on serological testing with the two Roche assays, which may be important for the application of the two assays in clinical routine. There are differences in the pattern of antibodies in individuals with and without COVID-19 vaccination.

1. Introduction

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has been on our minds worldwide for more than a year. Since the beginning of 2020, we know that SARS-CoV-2 is the cause of coronavirus disease 19 (COVID-19) [1,2]. COVID-19 can have severe courses with the occurrence of COVID-19-associated pneumonia, with the need for intensive medical treatment and with a relatively high mortality [1,2]. However, there are also mild to asymptomatic courses [2]. In January 2020, the World Health Organization (WHO) declared the outbreak of COVID-19 to be a public health emergency of international concern [1]. In March 2020, the WHO declared COVID-19 as a pandemic [1]. In the course of the last year, COVID-19 has gained more and more importance in health policy worldwide. Recently, various vaccines have become available to prevent COVID-19 [3–7]. In the member states of the European Union, for example, the vaccination campaign started at the end of December 2020.

The most important cornerstone of laboratory diagnostics is the detection of the pathogen from clinical specimens (e.g., nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage fluid) by means of

Abbreviations: COI, cut-off index; COVID-19, coronavirus disease 19; CV, coefficient of variation; IQC, internal quality control; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2.

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molecular testing of SARS-CoV-2 (nucleic acid amplification tests, mostly real-time reverse transcription polymerase chain reaction based molecular tests) [1,2,8,9]. In addition, the possibility of (rapid) antigen detection and of serological testing has become commercially available. In this context, the IFCC interim guidelines on serological testing of antibodies against SARS-CoV-2 were published recently [10]. There is consensus that serological testing can be helpful 1) in diagnosing SARS-CoV-2 infection in symptomatic hospitalized patients (especially if molecular biology testing is repeatedly negative); 2) to detect a previous infection with SARS-CoV-2 in hospitalized and non-hospitalized patients; 3) to estimate the extent of antibody production in a patient; 4) to determine the rate of individuals in certain populations who have already had contact with SARS-CoV-2 (e.g., for prevalence studies, for monitoring development of herd immunity); and possibly also 4) to detect antibody production following COVID-19 vaccination [10–13].

In this work, we wanted to compare two serological assays from Roche Diagnostics (Rotkreuz, Switzerland) in individuals with and without COVID-19 vaccination, namely the Elecsys Anti-SARS-CoV-2 assay (which can detect antibodies against the nucleocapsid protein of SARS-CoV-2) and the Elecsys Anti-SARS-CoV-2 S assay (which can detect antibodies against the spike protein of SARS-CoV-2). We were interested in how far the results of these two assays differ in vaccinated and non-vaccinated individuals. There are already publications on both Roche assays, but our two research questions have not yet been answered with previous publications [14–20].

2. Methods

2.1. Study design

This is a retrospective and exploratory study. We wanted to compare SARS-CoV-2 antibody levels in serum against the nucleocapsid protein and the spike protein in two different settings. We intended to use our data generated in routine clinical practice using two commercially available, automated, high-throughput assays. Specifically, we had two aims with this study: 1) We wanted to evaluate the extent of concordant and discordant results of the presence of antibodies against the nucleocapsid protein and spike protein in individuals without COVID-19 vaccination; and 2) we wanted to know the extent to which serum concentrations of antibodies against the nucleocapsid protein and spike protein differ over time following SARS-CoV-2 vaccination.

Because the present study is a purely retrospective data analysis, we did not consider a referral to the ethics committee necessary. For the data analysis, we used MedCalc 17.2 (MedCalc Software Ltd, Ostend, Belgium).

2.2. Measurement of SARS-CoV-2 antibody concentrations in serum

Since 29/10/2020, we have used two methods simultaneously in clinical routine when a SARS-CoV-2 serology is requested. To detect antibodies against the nucleocapsid protein, we use the Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics, Rotkreuz, Switzerland); to measure the concentration of antibodies against the spike protein, we use the Elecsys Anti-SARS-CoV-2 S assay (Roche Diagnostics, Rotkreuz, Switzerland). In our clinical routine, both assays run on two Cobas e801 systems (Roche Diagnostics, Rotkreuz, Switzerland). We follow the manufacturer's instructions when performing both tests. For blood-collection we use a serum tube from which both tests are performed (Greiner Bio-One, Kremsmuenster, Austria; CAT Serum Sep Clot Activator, Ref. 454078).

The Elecsys Anti-SARS-CoV-2 assay (Ref. # 09203079190) is a qualitative electrochemiluminescence immuno assay that detects an individual's total immunoglobulin against a recombinant nucleocapsid protein of SARS-CoV-2. This assay produces results as a cut-off index (COI; signal of sample divided by cutoff), where results $\geq$ 1.00 are reported as reactive/positive. The manufacturer's package insert does not specify an analytical coefficient of variation (CV) for the COI, but the literature indicates that the total CV of this assay is <14% at various concentration levels [14,15,17,20]. We do two different internal quality controls (IQC) from the manufacturer (PreciControl Anti SARS-CoV-2) daily on both Cobas e801 systems in clinical routine. At a mean low IQC of 0.09–0.10 COI (depending on the lot used), we have had a CV < 8% since 29/10/2020; at a mean high IQC of 2.88–3.00 COI (depending on the lot and the analyzer used), we have had a CV < 15% since 29/10/2020.

The Elecsys Anti-SARS-CoV-2 S assay (Ref. # 09289275190) is a quantitative electrochemiluminescence immuno assay that measures the concentration of an individual's total immunoglobulin against a recombinant spike protein (receptor binding domain) of SARS-CoV-2. The measurement range of the assay is from 0.40 U/mL to 250 U/mL. Values lower than the limit of quantification are reported as < 0.4 U/mL on our medical reports. For values > 250 U/mL, our analyzer automatically makes a 1:10 dilution and measures again, so that values up to 2500 U/mL can be reported. For our study, we provide measured values < 0.40 U/mL as 0.39 U/mL and values > 2500 U/mL as 2501 U/mL. Antibody concentrations of <0.80 U/mL are considered negative and of ≥0.80 positive. In the manufacturer's package insert, CV values of <3% are given for different concentrations. The literature indicates that the total CV of this assay is <4% at various concentration levels [19,20]. We do two different IQCs of the manufacturer (PreciControl Anti SARS-CoV-2 S) daily on both Cobas e801 systems in clinical routine. For the negative IQC we have consistently measured < 0.4 U/mL since 29/10/2020, with a mean value of the positive IQC of 8.8–9.6 U/mL (depending on the lot and the analyzer used) we have had a CV < 8% since 29/10/2020.

2.3. Individuals without COVID-19 vaccination

For the method comparison of the anti-nuclocapsid assay with the anti-spike assay in non-vaccinated individuals, we extracted all serological determinations made in the period from 29/10/2020 to 28/12/2020 in the Department of Clinical Pathology of Bolzano from our Laboratory Information System (LIS) and transferred them to an electronic database (including the corresponding patients' sex and age). The two dates were chosen because in our laboratory we started on 29/10/2020 to perform both assays simultaneously whenever a SARS-CoV-2 serology was requested in the clinical routine, and because in the Province of Bolzano, South Tyrol, the vaccination of healthcare workers and parts of the elderly population against COVID-19 started on 29/12/2020. We wanted to perform the method comparison in our patients without the influence of the COVID-19 vaccination. There were no exclusion criteria for this evaluation. Thus, we used all results in the mentioned period for the data evaluation, even if repeated serological determinations were made in certain patients in the course of time.

2.4. Individuals with COVID-19 vaccination

Eligible for evaluation of SARS-CoV-2 antibody concentrations in serum over time after COVID-19 vaccination were all employees of the Department of Clinical Pathology of Bolzano who received their first vaccination between 29/12/2020 (start of vaccination in the Province of Bolzano, South Tyrol) and 14/01/2021 (n = 46). Those employees who had a documented COVID-19 infection in the past were excluded from the evaluation (n = 1). Additionally, those staff members were excluded from the evaluation who did not have at least two determinations of SARS-CoV-2 antibody levels in serum by 20/02/2021 (n = 11). For each determination of SARS-CoV-2 antibodies, we always simultaneously measured the antibody concentrations against the nucleocapsid protein and the spike protein.

At the beginning of February 2021, we requested the dates of vaccinations from our laboratory staff. Subsequently, with the informed consent of our staff, we extracted the serology data from our Laboratory

Information System (LIS) and transferred it to an electronic database.

The classification of the assignment of the dates of the individual blood collections from the employees with regard to the timeline related to the date of the vaccinations of the employees was determined before data analysis as follows: As “baseline”, all blood draws for the determination of SARS-CoV-2 antibodies against the nucleocapsid protein and the spike protein in a period from a maximum of 7 days before the vaccination to a maximum of 3 days after the first COVID-19 vaccination were to be considered. The time points “1 week after baseline“, “2 weeks after baseline”, “3 weeks after baseline“, “4 weeks after baseline” and “5 weeks after baseline“ refer to 7 days after the first vaccination (allowed range, 4 to 10 days after the first vaccination), 14 days after the first vaccination (allowed range, 11 to 17 days after first vaccination), 21 days after first vaccination (allowed range, 18 to 24 days after first vaccination), 28 days after first vaccination (allowed range, 25 to 31 days after first vaccination), and 35 days after first vaccination (allowed range, 32 to 38 days after first vaccination), respectively.

3. Results

3.1. Individuals without COVID-19 vaccination

From 29/10/2020 to 28/12/2020, we received 3033 serum samples in clinical routines with the order to determine the antibodies against SARS-CoV-2. These 3033 serum samples were from 2496 patients (1273 male and 1223 female) with a median age of 61 years (25th-75th percentiles, 46–78 years; range, <1–100 years). Thus, we compared 3033 results of the anti-nucleocapsid assay with 3033 results of the anti-spike assay. In 332 patients, at least two serial determinations were made with both assays during the study period; in 2164 patients, only one determination was made with both assays.

Fig. 1 shows a scatter plot in which the COI values of the anti-nucleocapsid assay were plotted against the concentrations of the anti-spike assay (expressed as U/mL). Table 1 summarizes the results dichotomized according to the cut-off values. About 63% of all determinations were negative with both assays in our cohort and about 32% of all determinations were positive with both assays. In about 6% of our cohort, however, there were discordant results with both assays. In about 3% of all determinations, the result of the anti-nucleocapsid assay was positive and that of the anti-spike assay negative. Conversely, in about 3% of all determinations, the result of the anti-nucleocapsid assay was negative and that of the anti-spike assay was positive.

As already described, there were discordant results between the two assays in about 6% of the examinations. Of these 171 discordant results, 52 patients had at least two serial measurements over time, while 104 patients had no serial measurements. The time course of the results for the 52 patients (43 in-patients and 9 out-patients) with discordant results are shown in Table 2. Using the data from these 52 patients in Table 2, we were able to demonstrate that in 26 patients initially discordant results from the two assays became concordantly positive over time.

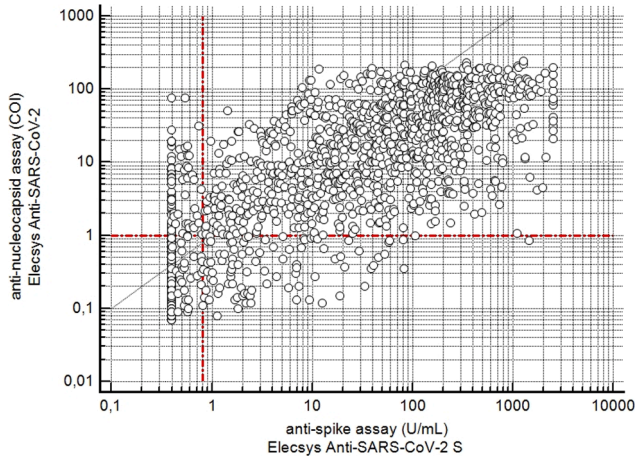

Fig. 1. Scatterplot of the values determined with the two assays from 3033 serum samples. The horizontal dotted line indicates the cut off value of the anti-nucleocapsid assay (negative, COI < 1.0; and positive, COI ≥ 1.0). The vertical dotted line indicates the cut off value of the anti-spike assay (negative, <0.80 U/mL; and positive, ≥0.80 U/mL).

Table 1
Comparison of the results of anti-nucleocapsid assay and the anti-spike assay in individuals without vaccination.

	Anti-spike assay; negative (<0.80 U/mL)	Anti-spike assay; positive (≥0.80 U/mL)	
Anti-nucleocapsid assay; negative (COI < 1.0)	n = 1907; 62.9%	n = 92; 3.0%	n = 1999; 65.9%
Anti-nucleocapsid assay; positive (COI ≥ 1.0)	n = 79; 2.6%	n = 955; 31.5%	n = 1034; 34.1%
	n = 1986; 65.5%	n = 1047; 34.5%	n = 3033; 100%

3.2. Individuals with COVID-19 vaccination

During the period 29/12/2020 to 14/01/2020, out of 77 laboratory staff members, 46 were vaccinated against COVID-19 for the first time and 31 were not vaccinated during this period. All vaccinations were administered using the BioNTech/Pfizer’s BNT162b2 COVID-19 vaccine [4] in two doses at the dose prescribed by the manufacturer. The second vaccination was administered to each staff member 21 or 22 days after the initial vaccination, i.e. between 19/01/2021 and 04/02/2021. Of the 46 vaccinated staff members, 1 individual had a documented history of COVID-19 infection, so this staff member was excluded from the study. Of the remaining 45 staff members, 5 individuals had no determination of SARS-CoV-2 antibody levels in serum between 27/12/2020 and 20/02/2021, 6 individuals had one determination of SARS-CoV-2 antibody levels in serum between 27/12/2020 and 20/02/2021, and 34 individuals had at least two determinations of SARS-CoV-2 antibody levels in serum between 27/12/2020 and 20/02/2021. For the evaluation of the courses of the SARS-CoV-2 antibody concentrations in the serum of these 34 employees, we were able to use 149 simultaneous measurements of the antibody concentrations against the nucleocapsid protein and the spike protein for the present evaluation.

The 34 participants (10 male and 24 female) had a median age of 50 years (25th-75th percentiles, 46–56 years; range, 24–62 years). The median time between blood sampling for baseline determinations and the first COVID-19 vaccination was 1 day (range, 5 days before first vaccination to 1 day after vaccination). The median time between the first COVID-19 vaccination and the blood draws for the “1 week after baseline“ determinations was 7 days (range, 5 to 9 days after the first vaccination). The median time between the first COVID-19 vaccination and blood sampling for the determinations “2 weeks after baseline” was 14 days (range, 12 to 17 days after the first vaccination). The median time between the first COVID-19 vaccination and blood sampling for the determinations “3 weeks after baseline“ was 21 days (range, 18 to 22 days after the first vaccination). The median time between the first COVID-19 vaccination and blood sampling for the determinations “4 weeks after baseline” was 28 days (range, 26 to 29 days after the first vaccination). The median time between the first COVID-19 vaccination and blood sampling for the determinations “5 weeks after baseline“ was 35 days (range, 32 to 38 days after the first vaccination).

At baseline, all 34 healthcare-workers had negative results with the anti-nucleocapsid assay and with the anti-spike assay. As shown in Table 3, the results of the anti-nucleocapsid assay remained negative throughout the observation period of 5 weeks after vaccination. The

Table 2
Time course of the results in 52 patients, with discordant serologic determinations between the two assays (if there were several time points in a patient for serial blood collections, a maximum of four are listed in the table).

Patient	Sample	Date	Anti-nucleocapsid assay		Anti-spike assay		SARS-CoV-2 RT-PCR[‡]	
	No.		COI	pos/neg	U/mL	pos/neg	Date	pos/neg
1.*	1	30 Oct	0.09	neg	0.39	neg	30 Oct	pos
	2	31. Oct	0.13	neg	0.54	neg	23 Nov	pos
	3	01 Nov	0.40	neg	1.59	pos	02 Dec	neg
	4	16 Nov	5.76	pos	224.00	pos	04 Dec	neg
2.	1	31 Oct	0.08	neg	0.39	neg	26 Apr	neg
	2	09 Nov	0.43	neg	0.96	pos	26 Oct	pos
							09 Nov	pos
							24 Nov	neg
3.	1	05 Nov	0.09	neg	0.39	neg	05 Nov	neg
	2	04 Dec	0.14	neg	1.77	pos	04 Dec	pos
							11 Dec	pos
							30 Dec	neg
4.	1	05 Nov	0.18	neg	2.47	pos	17 Aug	neg
	2	19 Nov	0.16	neg	2.24	pos	23 Oct	neg
	3	04 Dec	0.16	neg	2.10	pos	31 Oct	neg
	4	17 Dec	0.15	neg	2.03	pos	03 Nov	neg
5.*	1	06 Nov	1.27	pos	0.59	neg	04 Nov	pos
	2	07 Nov	1.48	pos	1.53	pos	10 Nov	pos
6.*	1	06 Nov	0.13	neg	9.32	pos	28 Aug	neg
	2	12 Nov	5.05	pos	454.00	pos	04 Nov	pos
							05 Nov	pos
							12 Nov	pos
7.*	1	06 Nov	0.26	neg	0.96	neg	03 Jun	neg
	2	16 Nov	35.80	pos	661.00	pos	06 Nov	pos
							16 Nov	pos
8.*	1	07 Nov	6.91	pos	0.39	neg	07 Nov	neg
	2	09 Nov	14.50	pos	3.41	pos	09 Nov	neg
9.	1	07 Nov	0.74	neg	0.39	neg	03 Aug	neg
	2	10 Nov	12.20	pos	0.49	neg	14 Sept	neg
							26 Oct	pos
							10 Nov	pos
10.*	1	08 Nov	12.30	pos	0.56	neg	08 Nov	pos
	2	09 Nov	19.60	pos	0.96	pos	15 Nov	pos
							22 Nov	pos
							15 Dec	neg
11.	1	09 Nov	0.10	neg	0.39	neg	08 Nov	pos
	2	16 Nov	0.48	neg	16.60	pos	21 Nov	pos
	3	23 Nov	0.56	neg	52.40	pos		
12.	1	09 Nov	0.36	neg	6.98	pos	20 Apr	neg
	2	04 Dec	0.30	neg	6.78	pos	30 Apr	neg
							26 May	neg
13.	1	09 Nov	0.09	neg	0.39	neg	01 Nov	neg
	2	15 Dec	0.13	neg	0.93	pos	03 Nov	neg
							09 Nov	pos
							10 Nov	pos
14.	1	11 Nov	0.08	neg	0.39	neg	11 Nov	pos
	2	16 Nov	0.18	neg	0.87	pos	20 Nov	pos
							27 Nov	pos
							16 Dec	neg
15.	1	11 Nov	0.09	neg	0.39	neg	11 Nov	pos
	2	18 Nov	6.28	pos	0.39	neg	18 Nov	pos
							30 Nov	pos
							07 Dec	pos
16.*	1	11 Nov	0.08	neg	0.39	neg	14 Oct	neg
	2	27 Nov	12.30	pos	0.59	neg	11 Nov	pos
	3	03 Dec	17.10	pos	43.90	pos	18 Nov	pos
							03 Dec	neg
17.	1	13 Nov	27.60	pos	0.39	neg	21 Mar	neg
	2	16 Nov	75.90	pos	0.39	neg	13 Nov	pos
							20 Nov	pos
							28 Nov	neg
18.	1	13 Nov	1.02	pos	1.42	pos	09 Sep	neg
	2	16 Nov	1.06	pos	25.10	pos	21 Oct	neg
	3	18 Nov	0.86	neg	1458.00	pos	13 Nov	pos
							21 Nov	pos
19.	1	13 Nov	0.09	neg	0.39	neg	13 Nov	pos
	2	20 Nov	1.20	pos	0.39	neg	21 Nov	pos
							27 Nov	pos
							04 Dec	neg
20.	1	14 Nov	1.00	pos	0.39	neg	13 Nov	pos
	2	16 Nov	0.91	neg	0.39	neg	14 Nov	pos
							21 Nov	pos

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Table 2 (*continued*)

Patient	Sample	Date	Anti-nucleocapsid assay		Anti-spike assay		SARS-CoV-2 RT-PCR‡	
	No.		COI	pos/neg	U/mL	pos/neg	Date	pos/neg
							12 Dec	neg
21.*	1	14 Nov	0.10	neg	0.39	neg	11 Nov	pos
	2	16 Nov	0.22	neg	1.45	pos	23 Nov	pos
	3	19 Nov	0.62	neg	29.10	pos	02 Dec	pos
	4	30 Nov	3.79	pos	400.00	pos	17 Dec	neg
22.*	1	15 Nov	1.99	pos	0.41	neg	03 Oct	neg
	2	16 Nov	3.30	pos	0.95	pos	09 Nov	pos
							13 Nov	pos
							20 Nov	pos
23.*	1	15 Nov	0.09	neg	0.39	neg	27 Oct	neg
	2	19 Nov	0.32	neg	2.22	pos	09 Nov	pos
	3	23 Nov	17.40	pos	60.70	pos	15 Nov	pos
							22 Nov	pos
24.*	1	15 Nov	15.00	pos	0.47	neg	11 Aug	neg
	2	15 Nov	15.80	pos	0.53	neg	12 Nov	pos
	3	16 Nov	26.30	pos	1.82	pos	15 Nov	pos
	4	23 Nov	69.80	pos	343.00	pos	23 Nov	pos
25.	1	16 Nov	4.33	pos	598.00	pos	15 Oct	pos
	2	07 Dec	1.55	pos	167.00	pos	23 Oct	pos
	3	14 Dec	0.88	neg	80.20	pos	27 Oct	pos
	4	21 Dec	0.98	neg	106.00	pos	09 Nov	pos
26.*	1	17 Nov	5.76	pos	0.65	neg	17 Nov	pos
	2	19 Nov	10.50	pos	1.55	pos	26 Nov	pos
							07 Dec	neg
27.*	1	18 Nov	1.48	pos	0.73	neg	30 Jul	neg
	2	04 Dec	4.10	pos	2.55	pos	23 Oct	neg
							31 Oct	pos
							12 Nov	pos
28.	1	18 Nov	0.12	neg	12.50	pos	13 Mar	neg
	2	14 Dec	0.50	neg	45.70	pos	26 Oct	pos
							03 Nov	pos
							16 Nov	neg
29.*	1	19 Nov	0.88	neg	8.25	pos	19 Oct	pos
	2	09 Dec	0.90	neg	11.10	pos	28 Oct	pos
	3	17 Dec	1.02	pos	12.80	pos	11 Nov	pos
							25 Nov	neg
30.	1	20 Nov	0.83	neg	0.89	pos	20 Nov	pos
	2	22 Nov	0.98	neg	1.51	pos	27 Nov	pos
							28 Nov	pos
							08 Dec	neg
31.	1	20 Nov	0.15	neg	0.39	neg	20 Nov	pos
	2	23 Nov	16.80	pos	0.39	neg	20 Nov	pos
							26 Nov	pos
							07 Dec	neg
32.*	1	20 Nov	0.67	neg	7.70	pos	20 Nov	neg
	2	24 Nov	7.27	pos	61.50	pos	22 Nov	pos
							29 Nov	pos
							30 Nov	pos
33.*	1	22 Nov	0.55	neg	1.96	pos	13 May	neg
	2	23 Nov	1.29	pos	7.29	pos	17 Nov	pos
	3	26 Nov	25.60	pos	197.00	pos	24 Nov	pos
							17 Dec	neg
34.*	1	23 Nov	0.34	neg	1.57	pos	17 Nov	pos
	2	28 Nov	15.20	pos	244.00	pos	23 Nov	pos
							27 Nov	pos
							10 Dec	pos
35.	1	24 Nov	0.09	neg	0.39	neg	19 Nov	pos
	2	24 Nov	1.03	pos	0.79	neg	24 Nov	pos
							04 Dec	pos
36.	1	25 Nov	0.14	neg	0.83	pos	28 Mar	neg
	2	26 Nov	0.19	neg	1.19	pos	12 Oct	neg
							20 Nov	pos
							27 Nov	pos
37.	1	25 Nov	0.28	neg	0.39	neg	25 Nov	neg
	2	28 Nov	8.50	pos	0.58	neg	28 Nov	pos
							04 Dec	pos
38.	1	27 Nov	0.10	neg	0.39	neg	14 Apr	neg
	2	30 Nov	0.92	neg	8.20	pos	10 Nov	neg
							16 Nov	pos
							28 Nov	pos
39.	1	27 Nov	0.10	neg	0.39	neg	27 Nov	pos
	2	30 Nov	3.95	pos	0.39	neg	04 Dec	pos
							11 Dec	pos
							25 Dec	pos

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Table 2 (*continued*)

Patient	Sample	Date	Anti-nucleocapsid assay		Anti-spike assay		SARS-CoV-2 RT-PCR‡	
	No.		COI	pos/neg	U/mL	pos/neg	Date	pos/neg
40.*	1	27 Nov	0.08	neg	1.12	pos	27 Nov	pos
	2	02 Dec	15.50	pos	50.90	pos	02 Dec	pos
	3	07 Dec	41.90	pos	461.00	pos	14 Dec	pos
	4	14 Dec	51.30	pos	453.00	pos	21 Dec	neg
41.*	1	02 Dec	0.08	neg	0.39	neg	28 Nov	pos
	2	07 Dec	0.32	neg	0.90	pos	03 Dec	pos
	3	14 Dec	2.36	pos	96.60	pos	14 Dec	pos
							15 Dec	neg
42.*	1	04 Dec	0.20	neg	7.26	pos	07 May	neg
	2	07 Dec	1.90	pos	59.90	pos	23 Nov	pos
							03 Dec	pos
43.*	1	04 Dec	0.09	neg	0.39	neg	11 Aug	neg
	2	12 Dec	0.40	neg	6.94	pos	24 Nov	neg
	3	14 Dec	2.17	pos	115.00	pos	04 Dec	pos
	4	21 Dec	30.80	pos	1409.00	pos	21 Dec	pos
44.*	1	07 Dec	0.27	neg	1.53	pos	07 Dec	pos
	2	09 Dec	2.61	pos	13.30	pos		
45.*	1	08 Dec	1.19	pos	0.39	neg	28 Aug	neg
	2	21. Dec	15.30	pos	135.00	pos	07 Dec	pos
							15 Dec	pos
							24 Dec	pos
46.	1	09 Dec	0.09	neg	0.39	neg	08 Dec	pos
	2	11 Dec	0.24	neg	1.01	pos	15 Dec	pos
							22 Dec	pos
							29 Dec	neg
47.	1	09 Dec	0.09	neg	0.39	neg	09 Dec	pos
	3	14 Dec	0.70	neg	4.09	pos	12 Dec	pos
	3	21 Dec	9.78	pos	312.00	pos	21 Dec	pos
							20 Dec	pos
48.*	1	11 Dec	1.45	pos	0.43	neg	24 Apr	neg
	2	14 Dec	7.22	pos	7.76	pos	18 Nov	neg
							30 Nov	pos
							11 Dec	pos
49.	1	13 Dec	0.15	neg	18.70	pos	07 Dec	pos
	2	14 Dec	0.35	neg	38.30	pos	13 Dec	pos
							20 Dec	pos
							29 Dec	pos
50.*	1	15 Dec	0.09	neg	0.39	neg	15 Dec	pos
	2	18 Dec	0.10	neg	1.80	pos	18 Dec	pos
	3	21 Dec	1.20	pos	26.70	pos	21 Dec	pos
	4	28 Dec	5.70	pos	658.00	pos	28 Dec	pos
51.	1	19 Dec	0.11	neg	0.39	neg	28 Nov	neg
	2	21 Dec	3.15	pos	0.39	neg	03 Dec	neg
							10 Dec	pos
							19 Dec	pos
52.*	1	23 Dec	31.10	pos	0.73	neg	20 Dec	pos
	2	24 Dec	32.80	pos	2.33	pos	27 Dec	pos

* Initially discordant results of the two assays became concordantly positive over time.

‡ In each patient, all medical records of SARS-CoV-2 real-time reverse transcription polymerase chain reaction (RT-PCT) from 01/01/2020 to 31/12/2020 were retrieved from our laboratory information system (LIS). The result of the first SARS-CoV-2 RT-PCR performed in 2020 is given for each patient. If there were several SARS-CoV-2 RT-PCRs performed in a certain patient in 2020, a maximum of four PCR results are listed in the table.

concentrations of antibodies against the spike protein remained negative in all workers during the first two weeks after the first vaccination. From week 3 onwards, the antibody concentrations against the spike protein rose into the positive range of the assay, and then continued to rise steadily until 4–5 weeks after vaccination. The results of the anti-spike assay in vaccinated individuals are summarized in Table 3 and Fig. 2.

4. Discussion

We were able to show in the patient cohort without vaccination that, using the two Roche assays, a large proportion of the determinations of SARS-CoV-2 antibodies against the nucleocapsid protein and the spike protein showed concordant results. In our cohort, however, there were discordant results in about 6% of the cases.

In the patients with discordant results and serial measurements over time, we observed in about half of all cases that initially discordant results of the two assays became concordantly positive over time. We therefore speculate that after contact with SARS-CoV-2, in a certain percentage of cases either the antibodies against the nucleocapsid protein or the antibodies against the spike protein (measured with the Roche assays) may initially become positive, but then both antibodies become detectable with the Roche assays over time. The other cases in our cohort, in which the results of the antibody determination also remained discordant over time, or became discordant, remain obscure with the data from our study.

It would be necessary to conduct prospectively designed studies that systematically clarify the course of the antibodies over a longer period, and which reasons could be responsible for any persistent discordant antibody measurements. Nevertheless, based on our results, we believe that the simultaneous use of the two Roche assays for the detection of antibodies against the nucleocapsid protein and against the spike protein in clinical routine might make sense. The detection of only one of the two antibodies in distinct patients early in time course should result in a higher sensitivity for the detection of previous contact with SARS-CoV-2. However, even this assumption must first be proven by a suitable study.

Table 3
Time course of the results of both assays in 34 individuals with COVID-19 vaccinations (a total of 149 simultaneous measurements of antibody concentrations against the nucleocapsid protein and the spike protein were available).

	Number of serum samples	anti-nucleocapsid assay results positive/ negative	anti-spike assay results median U/mL (range)	positive/ negative
At baseline (time of first vaccination)	n = 25	positive: n = 0 negative: n = 25	0.39 U/mL (0.39–0.39)	positive: n = 0 negative: n = 25
1 week after baseline	n = 21	positive: n = 0 negative: n = 21	0.39 U/mL (0.39–0.39)	positive: n = 0 negative: n = 21
2 weeks after baseline	n = 30	positive: n = 0 negative: n = 30	9.90 U/mL (0.90–247)	positive: n = 30 negative: n = 0
3 weeks after baseline (time of second vaccination)	n = 27	positive: n = 0 negative: n = 27	57.7 U/mL (5.35–2049)	positive: n = 27 negative: n = 0
4 weeks after baseline	n = 24	positive: n = 0 negative: n = 24	2384 U/mL (106–2501)	positive: n = 24 negative: n = 0
5 weeks after baseline	n = 22	positive: n = 0 negative: n = 22	2120 U/mL (789–2501)	positive: n = 22 negative: n = 0

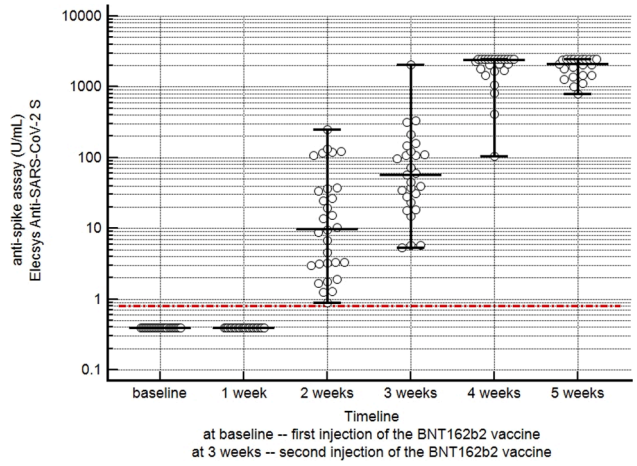

Fig. 2. Time course of the results of the anti-spike assay in 34 individuals with COVID-19 vaccinations. The horizontal solid lines indicate the median and the whiskers indicate the range of antibody concentrations against the spike protein at the different time points. The horizontal dotted line indicates the cut-off value of the anti-spike assay (negative, <0.80 U/mL; and positive, ≥0.80 U/mL).

In the second part of our work, we addressed the question of how the concentrations of antibodies against the nucleocapsid protein and against the spike protein measured with the Roche assays behave after COVID-19 vaccination. We observed that after injection of the BNT162b2 COVID-19 vaccine from BioNTech/Pfizer [4], the antibodies against the nucleocapsid protein remained consistently negative, but the antibodies against the spike protein became positive in all vaccinated individuals.

The initially negative antibodies against the spike protein became positive with the Roche assay in all samples two weeks after the initial injection, and the serum concentrations of anti-spike antibodies increased constantly until 4–5 weeks after the initial injection. We would like to emphasize, however, that our observation can only be related to the Roche assay and the BioNTech/Pfizer vaccine [4]. Other assays or a different vaccine may show different results. Nevertheless, this will certainly be the subject of other future studies (even with a longer follow up period than ours).

We excluded individuals with a history of SARS-CoV-2 infection from our analysis. In this context, we had only one employee who had a mildly symptomatic SARS-CoV-2 infection several months before vaccination. In this employee, at the time of the initial injection of the BNT162b2 COVID-19 mRNA vaccine from BioNTech/Pfizer [4], both the antibodies against the nucleocapsid protein and the antibodies against the spike protein were positive (data not shown). Approximately one to two weeks after the first vaccination, the serum concentration of antibodies against the spike protein increased rapidly and markedly (data not shown). This phenomenon has also been reported by a recent study demonstrating a robust spike antibody response and an increased reactogenicity in seropositive individuals after a single dose of a SARS-CoV-2 mRNA vaccine [21]. It would therefore be interesting for a future study to systematically investigate this phenomenon. In addition, it is still unclear how the antibody concentrations behave in the case of SARS-CoV-2 infection after vaccination but before the onset of full vaccination protection.

In summary, our study has provided information on serological testing with the two Roche assays, which may be important for the application of the two assays in clinical routine. There are differences in the pattern of antibodies in individuals with and without COVID-19 vaccination. The limitation of our study is the retrospective design and the relatively small number of cases in vaccinated individuals. In addition, we do not have any information on neutralizing antibodies in our two cohorts. Nevertheless, a recent study demonstrated that the concentrations of antibodies against the spike protein as measured with the Roche assay correlate well with SARS-CoV-2 neutralization activities [20]. Further studies are needed to systematically investigate the open questions discussed above in the unvaccinated individuals and to confirm our findings in the vaccinated individuals in a larger cohort.

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Thomas Mueller: Conceptualization, Formal analysis, Writing - original draft.

Declaration of Competing Interest

None declared.

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