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Evaluation of antibody titer kinetics and SARS-CoV-2 infections in a large cohort of healthcare professionals ten months after administration of the BNT162b2 vaccine

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ABSTRACT

Background: Real-world population studies have shown waning immunity, over time, after receiving the two doses of the BNT162b2 COVID-19 vaccine. Studies reporting the long-term humoral response are important to drive future vaccination strategies like the introduction of the booster dose. Yet, available literature on long follow-up periods is scarce. Covidiagnostix is a multicenter study aiming to assess the antibody response in >1000 healthcare professionals (HCPs) who received the BNT162b2 vaccine.
Methods: Serum was tested at time-0 (T0), before the first dose and then at T1, T2, T3 and T4, respectively, 21, 42, 177 and 302 days after T0. Antibodies against the SARS-CoV-2 nucleocapsid-protein were measured to assess SARS-CoV-2 infections, whereas antibodies against the receptor-binding domain of the spike protein were measured to assess vaccine response.
Results: The antibody titer observed 10 months post-vaccination showed a decrease of approximately 80% from the peak measured at T2, yet the median titer of the seronegatives HCPs was still higher than seropositives before vaccination. We identified 12 post-vaccination infected HCPs within 6 months after receiving the first dose and another 12 post-vaccination infected HCPs between 6 and 10 months post-vaccination.
Conclusion: Vaccination induced a humoral response which is well detectable even 10 months post-vaccination. Yet a high anti-spike serum antibody titer does not guarantee protection from infection. Differences in symptomatology between SARS-CoV-2 infections occurred within the first 6 months post-vaccination and the following 4 months, and differences in COVID-19 prevalence and vaccination coverage observed in these two time intervals were consistent with a decrease in vaccine efficacy 6 months after receiving the first dose.

1. Introduction

The administration of messenger RNA vaccine, and other vaccines, against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to the general population resulted in a significant decrease in coronavirus disease 2019 (Covid-19) incidence and mortality (Lopez Bernal et al., 2021; Dagan et al., 2021; Bernal et al., 2021; Pritchard et al., 2021; Harris et al., 2021; Arbel et al., 2021). Yet, as of March 14th 2022, SARS-CoV-2 is responsible for almost 500 million infected people and more than 6 million deaths (Worldometers, 2021). The BNT162b2 mRNA COVID-19 vaccine (Comirnaty®, Pfizer BioNTech) was the first vaccine formulations to be developed and approved (under an Emergency Use Authorization (EUA) on December 11th 2020 by the FDA, and on December 21st by the European Union; on August 23rd it received full approval by the FDA (FDA, 2021)) and is currently the most used worldwide (NIKKEI Asia, n.d.). Its administration protocol has been based on a double dose approach which proved to be effective in preventing 95% of COVID-19 cases (Polack et al., 2020; Mulligan et al., 2020). The Comirnaty vaccine promotes the production of anti-Spike protein (anti-S) receptor binding domain (RBD) neutralizing

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antibodies (Wrapp et al., 2020; Pallesen et al., 2017) which can be detected with quantitative serological tests (Ferrari et al., 2021a). Several studies monitored the antibody response after administration of the two Comirnaty vaccine doses observing a higher anti-S antibody response in persons previously infected by SARS-CoV-2 compared to subjects never infected by SARS-CoV-2 (Ferrari et al., 2021a; Saadat et al., 2021; Krammer et al., 2021; Prendecki et al., 2021; Manisty et al., 2021; Ferrari et al., 2021b) and a decrease in anti-S antibody titer by, approximately, 70% after 6 months (Bayart et al., 2021; Ferrari et al., 2021c).

Real-world population studies have shown waning immunity, over time, after receiving the two vaccine doses (Pritchard et al., 2021) and a third vaccine dose, approximately 6 months after the administration of the first one, has been implemented in most countries worldwide. Noteworthy, on July 30th 2021, the Israeli Ministry of Health was the first to approve the use of a third (booster) dose despite the lack of robust scientific evidence and the absence of regulatory approval by the International Agencies (Arbel et al., 2021).

Due to the third dose implementation, long follow-ups evaluating the vaccine response after the second administration are scarce. To the best of our knowledge, only one study showed the anti-S antibody titer after 8 months, yet the number of participants was limited to one hundred.

We took advantage of our ongoing multicenter longitudinal study (*Covidiagnostix*), funded by the Italian Ministry of Health, to investigate the antibody responses after the second dose, in a 10 months period, in a cohort of approximately 1000 health professionals (HCPs) (Tomaiuolo et al., 2021; Di Resta et al., 2021). The objective of the study was the evaluation of the antibody response, and vaccine efficacy, induced by the two doses administration protocol in different sex/age groups as well as in subjects seropositives/seronegative for anti-SARS-CoV-2 antibodies before vaccine administration. We also analyzed in detail those HCPs who were SARS-CoV-2 infected despite vaccination.

2. Methods

2.1. Covidiagnostix study

The *Covidiagnostix* is a multicenter study, approved by the Institutional Ethical Review Boards (CE:199/INT/2020), which aims to monitor the antibody response of a population of HCPs who were offered the BNT162b2 mRNA COVID-19 (Comirnaty®) vaccine.

2.2. Inclusion criteria and methodology

This study included 1172 HCPs (766 females aged 49.0 ± 16.7 years old and 406 males aged 52.0 ± 20.0 years old) from the San Raffaele Hospital, Milan, Italy, who received both the first and second dose (21 ± 1 days interval between the two doses) during January and February 2021.

Blood samples were withdrawn for serological evaluation, as previously described (Ferrari et al., 2019) at: time 0 (T0), 1–2 min before receiving the first vaccination dose; Time 1 (T1), 21 ± 0 days after T0 right before (1–2 min) the injection of the second dose; Time 2 (T2), 42 ± 0 days after T0; Time 3 (T3), 177 ± 8 days after T0; Time 4 (T4), 302 ± 7 days after T0.

To discriminate between SARS-CoV-2 previously infected and non-previously infected individuals, at T0, samples were tested for the presence of pan-immunoglobulins (Pan-Ig: IgA, IgG and IgM) against the SARS-CoV-2 viral nucleocapsid protein (N-protein) using the Roche electrochemiluminescence immunoassay (ECLIA) "Anti-SARS-CoV-2" on a COBAS 601 platform (Roche, Basel, Switzerland). Results above the manufacturer's suggested cutoff (1 U/mL) were associated to a previous SARS-CoV-2 whereas results below 0.2 U/mL were considered as negative. Test results between 0.2 and 1 U/mL were considered dubious (Favresse et al., 2020), when available, previous diagnostic tests (Real-Time Reverse-Transcription PCR (RT-PCR) tests) were used to identify HCPs previously infected by SARS-CoV-2. Only at T0, thanks to an instrument query, samples >1 U/mL were further tested on the same platform with the Roche ECLIA "anti-SARS-CoV-2-S" test (Roche, Basel, Switzerland) detecting anti-S Pan-Ig against the receptor binding domain (RBD). The standard quantification range (0.4–250.0 U/mL) was further extended to 2500.0 U/mL by a 1:10 sample dilution automatically performed by the instrument. The manufacturer's suggested cutoff was 0.8 U/mL. The "anti-SARS-CoV-2-S" test was used at T1, T2, T3 and T4 to quantify the vaccine elicited anti-S-RBD antibodies. At T3 and T4, 50.5% and 44.6% of the samples, respectively, were also tested for the presence of antibody against the N-protein (Roche Anti-SARS-CoV-2).

Roche declared that the conversion factor between "anti-SARS-CoV-2-S" test results expressed in U/mL and Binding antibody units per milliliter (BAU/mL) proposed by the WHO is 1. Thus, test results expressed in U/mL throughout the paper correspond to BAU/mL.

2.3. COVID-19 diagnostic data

From the beginning of the COVID-19 pandemic, as part of a follow up institutional program, RT-PCR swab tests were performed both routinely and whenever a HCP showed symptoms consistent with COVID-19. Nasopharyngeal swabs were analyzed using the Tib-Molbiol's 2019-nCoV RT-PCR Kit (cat# 61011896) on a Roche Cobas Z480 thermocycler (Roche Diagnostic, Basel, Switzerland). RNA purification was performed using the Roche Magna pure system (cat# A42352) (Ferrari et al., 2020).

2.4. Statistical analysis

Observed categorical measures were summarized by means of frequencies or percentages. Numeric observed measures were summarized with median and Inter Quartile Range (IQR). Differences in antibody titer between genders was assessed by Mann-Whitney U statistic test. Relationship between antibody titer and age classes was analyzed by means of the exact Jonckheere trend test (Jonckheere, 1954).

3. Results

3.1. Serological evaluation at T0

Of the 1172 HCPs tested, 95 showed anti-N antibody titer above the manufacturers' suggested cutoff limit, thus consistent with previous SARS-CoV-2 infection, and were listed in the COV+ group. Twenty HCPs showed dubious results (0.2–1 U/mL). Among them, 3 HCPs had previous positive RT-PCR tests bringing the number of subjects belonging to the COV+ group to 98 (8.4%) (Table 1).

Their anti-RBD-S antibody titers median value was 68.6 (IQR: 154.6) U/mL (Table 1). The remaining 1074 HCPs showing no evidence of previous SARS-CoV-2 infection were listed in the COV- group (Table 1).

3.2. COV+ group

As previously described (11,12,14,16,31), at T1, upon receiving the first vaccine dose, the 98 HCPs showed an exceptional anti-RBD-S antibody titers response. Eighty-six (87.8%) HCPs showed titers above the 2500 U/mL instrument limit (Table 1). The remaining 12 HCPs showed a median titer equal to 648 (IQR: 1034) U/mL. At T2 the number of HCPs exhibiting anti-RBD-S titers above the 2500 U/mL instrument limit increased to 94 (95.9%). The remaining 4 HCPs showed a median titer of 1045 (IQR: 1247.3) U/mL. At T3, six months after the first vaccination dose, the adherence to the study was 92.8% (Table 1) and the median anti-RBD-S titer value, was still above the instrument limit (Table 1). Yet, the number of HCPs showing test results within the instrument reading range (0.8–2500 U/mL) increased to 38 (41.7%) with a median value of 1288 (IQR: 780) U/mL. At T4, the adherence to the

Table 1
Serological evaluation at 0 (T0), 21 (T1), 42 (T2), 177 (T3) and 302 (T4) days post first vaccination dose. Test results are expressed as median (IQR). Results above the 2500 U/mL instrument limit were rounded to 2500 U/mL.

	T0		T1		T2		T3		T4	
	Subjects (adherence)	Test result (U/mL)	Subjects (adherence)	Test result (U/mL)	Subjects (adherence)	Test result (U/mL)	Subjects (adherence)	Test result (U/mL)	Subjects (adherence)	Test result (U/mL)
COV+	98 (100%)	68.6 (154.6)	98 (100%)	2500	98 (100%)	2500	91 (92.8%)	2500	69 (70.4%)	2308.0 (1345.5)
Females	59 (100%)	79.15 (162.6)	59 (100%)	2500	59 (100%)	2500	55 (93.2%)	2500	39 (66.1%)	2305.0 (1416.5)
Males	39 (100%)	55.2 (90.7)	39 (100%)	2500	39 (100%)	2500	36 (92.3%)	2500	30 (76.9%)	2363.0 (1363.0)
COV-	1074 (100%)	N.A.	1074 (100%)	32.3 (74.0)	1074 (100%)	1659.0 (1611.5)	1037 (96.6)	584.0[a] (607.0)	753 (70.1%)	419.0[b] (526.5)
Females	707 (100%)	N.A.	707 (100%)	36.8 (75.8)	707 (100%)	1804.0 (1753.0)	687 (97.2%)	635.5[a] (658.5)	514 (72.7%)	442.5[b] (591.8)
Males	367 (100%)	N.A.	367 (100%)	25.5 (64.1)	367 (100%)	1472 (1608.5)	350 (95.4%)	499.5 [a] (502.3)	239 (65.1%)	388.0[b] (380.0)

[a] 12 subjects (8 females and 4 males) who were post-vaccination infected were omitted from calculation (median and IQR are based on 1025, 679 and 346 data for total, female and male calculation, respectively).
[b] a total of 24 subjects (19 females and 5 males) who were post-vaccination infected were omitted from calculation (median and IQR are based on 725, 493 and 232 data for total, female and male calculation, respectively).

study dropped to 70.4%. More than 50% of the HCPs (36 subjects, 52.2%) showed titers within the instrument reading range, bringing the median anti-RBD-S titer to 2308 (IQR: 1345.5) U/mL.

3.3. Seronegative group

At T1, out of the 1074 HCPs seronegative at T0, 39 (3.6%) showed anti-RBD-S titers below the instrument cutoff limit, thus consistent with no humoral response upon receiving the first vaccination dose. On the other hand, 4 HCPs exhibited anti-RBD-S titers above the 2500 U/mL instrument limit. The median value of the whole group was 32.3 (IQR: 74) U/mL, thus the same order of magnitude as that observed, at T0 (before vaccination), in the COV+ group (Table 1). At T2, the median antibody titers of the COV- group, increased by approximately two-orders of magnitude (1659.0 (IQR: 1611.5) U/mL, Table 1). Only 3 HCPs (0.3%) showed anti-RBD-S titers below the cutoff limit, thus not responding even to the second vaccine dose. In contrast, 327 (30.4%) HCPs showed anti-RBD-S titers above the 2500 U/mL instrument limit. At T3 the adherence to the study decreased to 96.6%. The median antibody titers dropped to 584.0 (IQR: 607.0) U/ml consistent with a decrease that was previously quantified in a 65–70% loss from T2 (Ferrari et al., 2021c). At T3 the number of HCPs showing antibody titers >2500 U/mL dropped to 28 (2.7%). Interestingly, out of the 3 HCPs that didn't respond to the second vaccination dose (T2), 2 of them showed detectable, albeit low, antibody titers at T3. It must be noted that the singular non-responder HCP was an oncological patient under treatment. The 1172 HCPs were all actively working within the hospital and we can assume that the vast majority of them were represented by healthy people. Stratification of the 1172 subjects for possible pathological situations was beyond the scope of our paper and, yet, to the best of our knowledge, this was the only immunocompromised HCP. At T4 the adherence to the study dropped to 70.1%. Only 8 HCPs (1.1%) showed antibody titers above the instrument limit and the median value decreased to 419 (IQR: 526.5) U/mL, consistent with a 75–80% loss in anti-RBD-S titers compared to the estimated peak at T2 (Table 1). Only the above-mentioned non-responder showed a titer below the instrument cutoff level. Post-vaccination infected HCPs were excluded from the T2, T3 and T4 median titer calculation and were discussed in section 3.5.

3.4. Gender and age analysis

Stratifying the T4 serological tests for gender and age showed the same results observed in the shorter (6 months) follow-up period (Ferrari et al., 2021c). Females showed slightly higher, yet not statistically significant, median titers (Table 1). In contrast, median titers decreased significantly with age for both males ($p < 0.00001$) and females ($p < 0.00001$) (Fig. 1).

3.5. Post-vaccination infections

Twelve HCPs (8 females, aged 49.8 ± 6.8 years, and 4 males, aged 55.5 ± 15.3 years) were infected during the first six months post-vaccination (Table 1). One subject was infected between the first and the second vaccine dose, 9 contracted COVID-19 between 7 and 99 days after the second dose and two were oblivious to having been infected (Table 2) and found out through the serological test at T3. Eight HCPs were asymptomatic, the remaining 4 reported partial anosmia and ageusia accompanied, in 3 cases, by a mild cold and, in 1 of these 3 cases, by a generalized pain (Table 2). The 12 HCPs performed, within the hospital, different tasks (Table 2) with the exception of two nurses from the Psychiatric Department, yet they were infected 1 month apart. Notably, 8 out of 12 post-vaccination infected HCPs reported the presence of a SARS-CoV-2-positive family member (not vaccinated) as the potential source of infection (Table 2). Interestingly, 7 HCPs showed anti-RBD titers at T2 above 2000 U/mL, 3 were between 1000 and 2000 U/mL and only 2 had titers below 400 U/mL (Table 2). Because of the infection, 10 out of 12 HCPs showed in the following serological test at T3, anti-RBD titers higher than those observed at T2 (9 of them were above the 2500 U/mL instrument limit). The viral genome was sequenced for 3 HCPs and all showed the presence of the B.1.1.7 variant (alpha).

During the period from 6 to 10 months post vaccination, another 12 HCPs (11 females aged 47.8 ± 8.9 years, and 1 male aged 49 years) were SARS-CoV-2 infected (Table 2). Ten subjects contracted COVID-19 between 159 and 280 days after the second vaccine dose. The remaining 2 HCPs, oblivious to having been infected and thus asymptomatic, contracted SARS-CoV-2 at least 150 days after receiving the second vaccine dose (Table 2).

Of the 10 symptomatic HCPs, 3 complained just a light cough, cold and rhinitis (Table 2) whereas the remaining 7 exhibited more severe symptoms like fever up to 38.5, strong myalgia, asthenia, dyspnea, dysentery, ageusia and anosmia (Table 2).

As above, the 12 HCPs performed, within the hospital, different tasks (Table 2) except for subjects 13, 18 from the General Medicine Department, subjects 14, 16 from the Administration Department, subjects 15, 19 from the Psychiatric Department, subjects 17, 22 from the Intensive Care Unit and subjects 23, 24 from the Pediatrics Department.

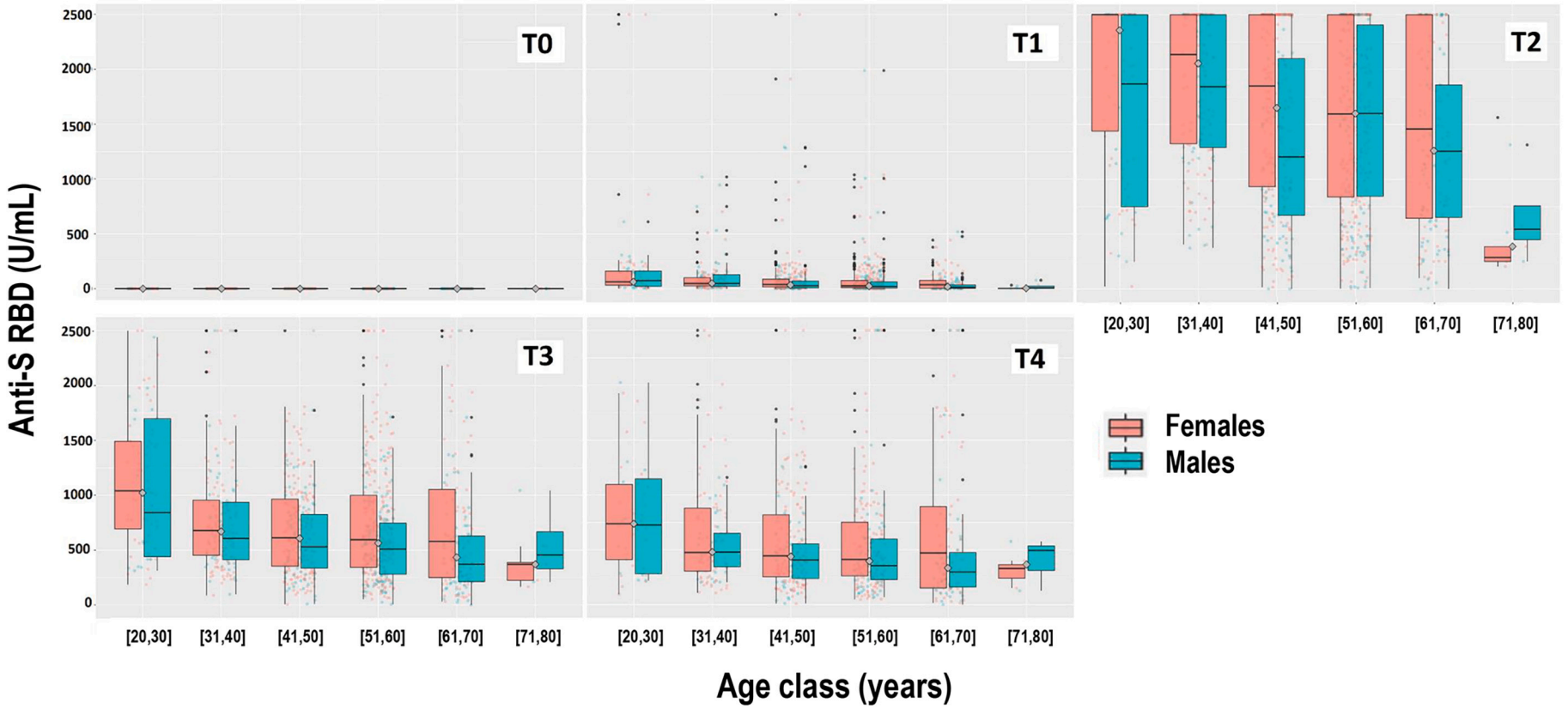

Fig. 1. Stratification by age and gender of the serological responses at T0, T1, T2, T3 and T4 observed for HCPs never infected by SARS-CoV-2 (neither before or after vaccination). The upper instrument limit was set at 2500 U/mL.

Table 2
Demographic characteristics, serological results and COVID-19 related information of the 24 HCPs post-vaccination infected by SARS-CoV-2.

Subject	Sex	Age: years	Anti-RBD expressed in BAU/mL (days from 1st dose)				PCR cycles[a]		Type of variant	2nd dose to infection[b] (days)	Symptoms	Close contacts[f]	Time length of negativization[c] (days)	HCP position
			T1	T2	T3	T4	RdRp gene	E Gene						
Subjects infected between T_0 and T_3														
1	M	76	2.93	2122	>2500	>2500	22.9	20.9	Alpha	64	Asymptomatic	Yes	21	Institutional Review Board
2	F	42	142	>2500	>2500	N/A	34.1	N/A	N/A	59	Asymptomatic	Yes	13	Psychologist
3	M	54	<0.4	196	897	355	25.6	24.2	N/A	69	Partial anosmia/ and ageusia	Yes	13	Nurse (Pediatrics)
4	M	39	1019	1866	>2500	1551	N/A	N/A	N/A	N/A[d]	Asymptomatic	No	N/A	Administrative
5	F	57	<0.4	2047	>2500	>2500	N/A	N/A	N/A	N/A[d]	Asymptomatic	No	N/A	Administrative
6	F	55	208	>2500	>2500	>2500	N/A	N/A	N/A	<0[e]	Asymptomatic	No	N/A	Nurse (Infectious Diseases)
7	F	49	77.5	>2500	>2500	>2500	22.3	22.1	Alpha	67	Partial anosmia and ageusia, cold, myalgia	Yes	13	Nurse (Psychiatry)
8	M	53	0.79	339	>2500	N/A	22.1	23.5	Alpha	84	Asymptomatic	No	16	Technician (Echography)
9	F	42	18.5	1131	>2500	2205	30.8	31.8	N/A	42	Asymptomatic	Yes	14	Nurse (Psychiatry)
10	F	55	76.3	2046	>2500	>2500	30.1	30.3	N/A	99	Partial anosmia and ageusia, cold	Yes	22	Technician (Pathological Anatomy)
11	F	42	50.1	>2500	2495	1105	21.4	20.8	N/A	14	Partial anosmia and ageusia, cold	Yes	14	Nurse (General Medicine)
12	F	56	5.7	1066	714	>2500	28.1	27.8	N/A	7	Asymptomatic	Yes	17	Nurse (Cardiology Department)
Subjects infected between T_3 and T_4														
13	F	59	22.7	1443	386	>2500	17.1	17.3	Delta	159	Asthenia, dysentery, cough	Yes	21	Medical Doctor (General Medicine)
14	F	49	43.2	>2500	984	>2500	30.5	31.1	N/A	168	Partial anosmia, myalgia, fever (38 °C), dysentery	No	33	Administrative
15	M	46	12.3	1157	839	>2500	24.5	24.7	Delta	206	Asthenia, cold, myalgia, fever (38 °C), dyspnea	Yes	15	Nurse (Psychiatry)
16	F	45	148	2344	339	>2500	17.4	17.5	Delta	216	cold	No	21	Administrative
17	F	46	45.2	1804	446	>2500	33.0	31.7	N/A	217	rhinitis	Yes	14	Nurse (Intensive Care Unit)
18	F	32	16.3	1415	526	>2500	20.2	20.3	N/A	232	Light cough	No	19	Nurse (General Medicine)
19	F	44	28.4	1483	565	232	16.7	16.7	Delta	277	Asthenia, fever (38 °C)	Yes	21	Technician (Psychiatry)
20	F	38	21.7	1040	620	>2500	21.8	20.6	Delta	274	Partial anosmia and ageusia, cold, myalgia, fever (38 °C), dyspnea, dysentery	No	18	Psychologist
21	F	54	22	799	887	>2500	19.1	18.3	Delta	280	Partial anosmia and ageusia, asthenia, fever (38.5 °C)	No	19	Nurse (Surgery)
22	F	62	57.6	2182	576	>2500	N/A	N/A	N/A	>156[d,h]	Asymptomatic	No	N/A	Medical Doctor (Intensive Care Unit)
23	F	55	112	>2500	633	>2500	N/A[i]	N/A[i]	N/A	253	Partial anosmia and ageusia, cold, myalgia, fever (38.5 °C)	No	10	Nurse (Pediatrics)
24	F	45	93.6	>2500	1071	>2500	N/A	N/A	N/A	>151[d,g]	Asymptomatic	No	N/A	Nurse (Pediatrics)

[a] Values refers to the first positive swab test. Values were considered: positive (CT between 14 and 34) slightly positive (CT between 34 and 40), negative (CT >40).
[b] Intervals are calculated from the day of the 2nd dose to the day of the 1st positive RT-PCR test.
[c] Time length of negativization was calculated from the day of the 1st positive RT-PCR test to the day of the 1st negative RT-PCR test.
[d] COVID-19 was asymptomatic, the HCPs found out about the infection through the serological test at T3 (subjects 4 and 5) or T4 (subjects 22, 23 and 24).
[e] Positivity was discovered by an occasional anti-N test performed at T1.
[f] "Close contacts" refers to the presence of a SARS-CoV-2 positive unvaccinated household at the time of infection.
[g] subject was negative for anti-N at T_3, thus infection must have occurred after T3.
[i] RT-PCR test carried out by an external laboratory. CT data not available.

However, we found no evidence of concomitant infections (Table 2). Only 4 out of the 12 HCPs infected after T3 reported the presence of a SARS-CoV-2-positive family member (not vaccinated) as the potential source of infection (Table 2). Their anti-RBD titers at T3 ranged between 339 and 1071 U/mL (Table 2). Because of the infection all, except 1, showed anti-RBD titers at T4 much higher than those observed at T3 (Table 1), above the 2500 U/mL instrument limit. The viral genome was sequenced for 6 HCPs all showing the presence of the B.1.617.2 (delta) variant.

4. Discussion

In this study we reported the antibody kinetics 10 months post-vaccination in both seronegative and seropositive individuals after receiving two doses of the BNT162b2 vaccine. As previously described, seropositive HCPs showed an exceptional increase in anti-RBD titers upon receiving the first vaccine dose (9–12,14). In contrast, seronegative subjects showed the production of limited amount of anti-RBD at T1 which was boosted by the second dose (T2). Ten months post-vaccination the median anti-RBD titers dropped (from the T2 peak) by approximately 75–80%. Such decrease is less pronounced in young individuals whereas no statistically significant differences were observed between genders. Despite such decrease, the titer of the seropositive subjects 10 months post-vaccination was still 4-folds higher than that of the seronegative ones which, in turn, showed titers approximately one order of magnitude higher than that of seropositive individuals before vaccination (T0). Thus, even 10 months post-vaccination, seronegative individuals still showed the presence of a considerable amount of anti-RBD antibodies if compared to COVID-19 recovered individuals.

Within the first 6 months post-vaccination 12 HCPs were SARS-CoV-2 infected. The only 3 sequencing results showed the presence of the B.1.1.7 variant. None of the 12 infected HCPs showed severe symptoms, thus confirming the vaccines' efficacy previously described in clinical trials (3). Closer investigation showed that 8 of them had been in close contact with a non-vaccinated SARS-CoV-2 positive family member. It must be noted that in Italy, as well as worldwide, HCPs were the first category to be vaccinated, whereas vaccination of the general population started a few months later. Thus, in the first months of 2021 the presence of unvaccinated non-HCPs was quite common. The serological test results at T2 of these 12 post-vaccination infected HCPs showed that a high anti-RBD-S serum antibody titer does not guarantee protection from infection. The possibility of an in-hospital outbreak was ruled out since the 12 HCPs perform, within the hospital, different tasks.

Between 6 and 10 months post vaccination, we observed another 12 infected HCPs. The main differences between them and those infected during the first 6 months were a more serious symptomatology (although none required hospitalization), the unique presence of the B.1.617.2 variant (among the 6 sequenced genomes) and fewer non-vaccinated SARS-CoV-2 positive family member close contacts as the likely origin of infection. The latter might be easily explained by the advancement in the Italian vaccination campaign: by July 15th only 42.3% of the Italian population received the second vaccine dose whereas by November 15th such percentage increased to 73.5% (Our World in Data, 2022). In contrast, the same number of post-vaccination infected HCPs observed in the two periods, and the different symptomatology must be discussed at the light of the different circumstances taking place within the first 6 months post-vaccination and the following 4 months. During the first 6 months post-vaccination (between January 15th 2021 and July 15th 2021), Italy experienced approximately 7000 SARS-CoV-2 infections per day, whereas between July 15st and November 15th the daily cases were only 3000. At the light of the above data, if vaccine efficacy remained constant, we would have expected, during the shorter 6 to 10 months post-vaccination period, less than half the number of infected HCPs observed in the first 6 months. Thus, our data are consistent with a vaccine efficacy decrease after six months. One might argue that the number of infected HCPs and the more severe symptomatology observed during the second observational period might be associated to the more contagious delta variant (Shiehzadegan et al., 2021). Yet, recent study showed that vaccine efficacy against the alpha and delta variant are essentially the same (Nasreen et al., 2022). It must be noted, however, that although the HCPs cohort was rather large, the number of post-vaccination infected HCPs is relatively small and might be susceptible of statistical criticisms.

To conclude, we observed an adherence to the study which decreased with time. The 100% adherence at T0 and T1 can be explained by the fact that blood withdrawal was concurrent with the first and second vaccination doses, respectively. T2 also showed a 100% adherence demonstrating that, at the beginning of the vaccination campaign, the HCPs were very concerned on the effect of the novel COVID-19 vaccine and interested in knowing their immune response. The adherence decreased at T3 and, even more, at T4. We might speculate that such decrease reflects the fact that serological tests have gradually lost their importance over time as scientific studies have gradually assigned more importance to immunological memory (Turner et al., 2021).

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Data availability

Data will be made available on request.

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