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Quantitative serological evaluation as a valuable tool in the COVID-19 vaccination campaign

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Abstract

Objectives: After exceptional research efforts, several vaccines were developed against SARS-CoV-2 which sustains the pandemic COVID-19. The Comirnaty vaccine showed high efficacy in clinical trials and was the first to be approved for its distribution to the general population. We evaluated the immune response induced by the first vaccine dose in different sex/age groups and subjects with or without naturally present anti-SARS-CoV-2 antibodies.
Methods: As part of an Italian multicenter project (*Covidiagnostix*), serum samples from 4,290 health-professionals were serologically tested the day of the first vaccination dose, and 21 days later, using two different instrumentations (Siemens-Healthineers and Roche).
Results: In total, 97% of samples showed the presence of specific antibodies 21 days after the vaccination dose; the percentage of non-responders increased with age in both genders. Remarkably, naturally seropositive individuals showed antibody persistence up to 11 months and an exceptionally higher vaccination response compared to subjects never infected by SARS-CoV-2.
Conclusions: This study highlighted the importance of the serological test i) to identify naturally SARS-CoV-2 seropositive individuals and ii) to evaluate the antibody level elicited by the first vaccination dose. Both tests, highlighted differences in the immune response, when subjects were stratified by sex and age, and between naturally seropositive and seronegative subjects.

The data obtained show how serological tests could play a crucial role in the triage of the population subjected to the vaccination campaign for COVID-19. The definition of suitable instrumentation-specific thresholds is needed to correctly follow eventually acquired post-vaccination immunity in the general population.

Keywords: COVID-19; mRNA vaccine; Roche Anti-SARS-CoV-2-S; serological test; Siemens SARS-CoV-2 IgG.

Introduction

Few months following the appearance of the coronavirus disease in China, known as COVID-19, and caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the viral infection has threatened the health of the world's population, leading to significant social and economic impacts. The virus has spread globally, becoming a public health emergency of international concern on January 30th, 2020, as declared by the World Health Organization (WHO), and a pandemic officially on March, 7th, 2020 [1]. As of March 26th, 125 million people have been infected and more than 2.7 million have died as a result [2].

Although distancing, masks, and strict lockdowns implemented by most countries have slowed the spread of SARS-CoV-2, there is a strong consensus globally that COVID-19 vaccines can protect individuals and will enable the development of immunity [3]. Consequently, if administered to a large proportion of the population, high efficacy vaccines may provide a degree of herd immunity, which in turn, will prevent the healthcare system from

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becoming overwhelmed and will favor the return to a degree of normality, preventing further economic damage and social hardships.

For the above reasons, there have been exceptional research efforts and global coordination which led to the rapid development of vaccines, some of which are undergoing large-scale production.

The Comirnaty COVID-19 mRNA BNT162b2 vaccine was the first vaccine to be approved, initially in the United Kingdom on December 2nd, 2020, then in the US on December 11th, 2020, and Europe on December 21st, 2020, showing a remarkable 95% efficacy [4, 5]. Unlike conventional vaccines, such as live attenuated and inactivated pathogens, and recombinant protein subunits vaccines, the Pfizer vaccine is a lipid nanoparticle-formulated, nucleoside-modified RNA (modRNA) encoding the SARS-CoV-2 full-length spike [4], modified by two proline mutations to lock it in the pre-fusion conformation [6]. RNA vaccines represent a promising alternative to conventional vaccines because of their high potency, rapid development, safe administration, and low-cost manufacture [7, 8]. Being the first time that an RNA-based vaccine is administered to the general population, most of the currently available scientific data comes from a few clinical trials [4, 5]. However, immune responses following previous natural infection have not been assessed in clinical studies and the only information available are from a few studies, often enrolling a limited number (<160) of participants [9–11]. These studies observed a stronger immune response in seropositive subjects when compared to subjects never infected by SARS-CoV-2, raising doubts about the opportunity of vaccinating naturally seropositive individuals with the standard two vaccination doses [9–12]. Therefore we took advantage of our ongoing multicenter longitudinal study (*Covidiagnostix*), funded by the Italian Ministry of Health, to investigate the antibody responses of over 4,200 health professionals injected with the Comirnaty vaccine.

The objective of the study was the evaluation of the immune response induced by the first dose of the Comirnaty vaccine in different sex/age groups as well as in subjects with or without a natural presence of anti-SARS-CoV-2 antibodies.

Materials and methods

Participants and procedures

Laboratory testing and available clinical/diagnostic data were collected and analyzed according to the *COVIDIAGNOSTIX* (CE:199/INT/2020) protocol, approved by the Institutional Ethical Review Board of the centers involved in the study: the Institutional Ethical Review Board of the IRCCS San Raffaele Hospital (OSR), Milan, Italy (which has jurisdiction also on the IRCCS Orthopedic Institute Galeazzi (IOG), Milan, Italy) and the Institutional Ethical Review Board of the IRCCS Casa Sollievo della Sofferenza Hospital (CSS), San Giovanni Rotondo, Italy.

Between January 4th and February 12th, 2021, a total of 4,290 health professionals, from OSR, IOG and CSS, were offered the Comirnaty vaccine and included in the study.

Blood samples were withdrawn for serological evaluation, as previously described [13] at:

1) Time 0 (T_0), 1–2 min before receiving the first vaccination dose. The T_0 serological results were used to discriminate subjects with (COV+) or without (COV–) natural presence of anti-SARS-CoV-2 antibodies.
2) Time 1 (T_1), 21 days after T_0, before (1–2 min) the injection of the second dose. The T_1 serological results were used to determine the antibody response to the first vaccine dose.

No exclusion criteria were applied.

For a limited number of patients (n=100) a third blood sample was withdrawn 21 days after the second vaccination dose (T_2).

IRCCS San Raffaele Hospital: population and methods: At the OSR, 3,340 health professionals were included in the study: 2,140 females (44.5 ± 11.3 years) and 1,200 males (44.6 ± 12.7 years). Blood samples were collected, as described elsewhere [9], into clot activator BD vacutainer tubes (cat. 369032) without a separator (Becton, Dickinson and Company, NJ, US). At T_0, serum samples were tested for the presence of SARS-CoV-2 antibodies using the Elecsys Anti-SARS-CoV-2, an electrochemiluminescence immunoassay (ECLIA) (Sensitivity: 100%; Specificity: 99.8%, as for manufacturer's specification), on a COBAS 601 platform (Roche, Basel, Switzerland), targeted on total Immunoglobulins (IgT) against the viral nucleocapsid protein (N-protein) [14, 15]. In case of a positive result, thanks to an instrumental query, the same sample was further tested with the Roche Anti-SARS-CoV-2-S test, on the same platform, targeted on IgT against the receptor-binding domain (RBD) of the viral spike protein (S-protein). Subjects showing N-protein titers between 0.165 and 1 U/mL were considered dubious thus, when available, previous diagnostic tests were exploited to discriminate between SARS-CoV-2 previously infected and non-previously infected individuals.

T_1 samples were screened with the Roche Anti-SARS-CoV-2-S test, for the detection of IgT against the S-protein RBD. As reported in the manufacturer's datasheet (ref: 09289267190), the Roche Anti-SARS-CoV-2-S test has a signal range that spans from 0.4 to 250 U/mL that is further extended to 2,500 U/mL thanks to a 1:10 dilution automatically performed by the instrument. A fraction of the samples whose signal exceeded the 2,500 U/mL upper instrumental limit were diluted with pre-pandemic serum to bring the signal back into the reading range of the instrument. The manufactured indicated positivity cutoff is 0.8 U/mL. (Sensitivity: 96.6%; Specificity: 100%; sensitivity refers to sample >14 days after disease diagnosis). No information about test linearity was provided by the manufacturer.

During the COVID-19 period health professionals from the OSR were included in a follow-up program which consisted of routine swab tests and a serological evaluation, during May 2020. RT-PCR swab tests were performed using the Tib-Molbiol's 2019-nCoV Real-Time Reverse

Transcription PCR Kit on a Roche Cobas Z480 thermocycler (Roche Diagnostic, Basel, Switzerland). RNA purification was performed using the Roche Magna pure system [13]. The May 2020 serological tests was performed, using the LIAISON SARS-CoV-2 S1/S2 IgG chemiluminescence immunoassay (CLIA) which exploit the viral S-protein as antibody target. Both, the results from the May 2020 serological evaluation and the swab tests, were used to complement the serological result at T_0 in order to discriminate between individuals previously infected and those never infected by SARS-CoV-2. Additionally, a brief questionnaire was sent to the subjects belonging to the OSR COV+ group asking: 1) whether they knew about a previous SARS-CoV-2 infection; in case of a positive answer we further asked: 2) about the symptomatology and 3) the approximate date of symptoms onset. The symptomatology options were: asymptomatic, moderate (if the disease was treated at home), severe (if the subject needed hospitalization).

IRCCS Orthopedic Institute Galeazzi and IRCCS Casa Sollievo della Sofferenza Hospital: population and methods: At the IOG and CSS, a total of 950 healthcare workers were included in the study: 522 females (46.8 ± 14.4 years) and 428 males (48.5 ± 14.5 years). Blood samples were collected, into clot activator BD vacutainer tubes (cat. 367955) with a gel separator (Becton, Dickinson and Company, NJ, US).

In the two centers, equipped with the same instrumentation, serum from healthcare professionals were screened at T_0 and T_1 for the presence of anti-SARS-CoV-2 antibodies using the SARS-CoV-2 IgG (COV2G), an immunoassay (CLIA), on the Atellica IM Analyzer (Siemens Healthineers, Erlangen, Germany), targeted on IgG against the RBD of the S-protein. As reported in the manufacturer's datasheet (ref: 11206997), the test has a signal interval that range from 0.05 to 150 U/mL that is further extended to 750 U/mL thanks to a 1:5 dilution automatically performed by the instrument. The manufactured indicated positivity cutoff is 1.0 U/mL (Sensitivity: 100%; Specificity: 99,9%). No information about test linearity was provided by the manufacturer. Since both IOG and CSS performed the same assay, the 773 subjects from IOG and the 177 subjects from CSS were combined in a single group called "IOG-CSS".

Statistical analysis

The analyses were performed using R Software v4.0.3 (R Core Team, Wien, Austria). Shapiro Wilk test was used to assess data distribution. For continuous variables, median and interquartile ranges (IQR) are reported if not differently specified. Correlations between continuous variables were assessed using Spearman's method. Two-way ANOVA with Tukey post hoc test was used to evaluate the contemporary effect of age class and gender on the outcome. Log-transformation of data was applied in order to respect the test assumptions. p-Values <0.05 were considered statistically significant.

Results

Serological evaluation at T_0

The analysis performed at T_0 revealed the presence of antibodies against SARS-CoV-2 in 305 (69 had a previous positive RT-PCR test) of the 3,340 serum analyzed at the OSR by the Elecsys Anti-SARS-CoV-2 (Roche) and in 175 serum analyzed by at the IOG-CSS by the SARS-CoV-2 IgG (COV2G) (Siemens Healthineers). However, a recent paper [16] proposed to use a cutoff of 0.165 U/mL for the Elecsys Anti-SARS-CoV-2 (Roche), in place of the manufacturers' recommended ≥1 U/mL, to discriminate between seropositive and seronegative individuals. We found 76 subjects showing titers in the 0.165–1 signal range and, thanks to the available diagnostic information available at the OSR database, we could identify 10 health professional with a documented diagnostic history for COVID-19 (previous positive swab test and/or previous positive serological evaluation) which were then included in the COV+ group.

The OSR COV+ and COV− groups were composed, respectively, by 315 and 3,025 individuals whereas for the "IOG-CSS", the COV+ and COV− groups were composed, respectively, by 201 and 749 individuals (Table 1). The median value of the 305 subjects belonging to the OSR COV+ group for which an anti-S-RBD titer was obtained at T_0 (with the Roche instrumentation), was 89.3 U/mL (IQR 23.1–210 U/mL) (Figure 1) whereas for the 201 subjects belonging to the IOG-CSS COV+ group the titer at T_0 (obtained with the Siemens instrumentation) was 2.8 U/mL (IQR 1.38–5.24 U/mL) (Figure 1).

Serological evaluation at T_1: COVID-positive group (COV+)

Of the 315 COV+ subjects (62.8% females) belonging to the OSR, 294 (93.3%) showed antibody titers at T_1 that were above the 2,500 U/mL high detection limit of the Roche Elecsys Anti-SARS-CoV-2-S (Table 1, Figure 1) and will be hereafter called the "high-responding group" (HRG). The remaining 21 subjects (76.1% females) showed antibody titers at T_1 within the instrumental range and always above the 0.8 U/mL cutoff limit.

To better inquire into the T_1 values of the HRG, we diluted 36 randomly chosen samples with human pre-pandemic serum to bring the instrumental response within the instrumental range. The 36 samples (representing the 12.2% of the total values above the limit) showed a median value equal to 18,000 U/mL (IQR 9,600–34,000 U/mL), thus approximately 200-fold higher than the corresponding antibodies level naturally present at T_0 (89.3 U/mL).

For the IOG-CSS group, 201 subjects (55.7% females) representing the 21.2% of the participants were included in the COV+ group (Table 1). As observed in the OSR group, 100% showed the presence of Anti-SARS-CoV-2 antibodies at T_1 (Table 1). The median response was 225.8 U/mL (IQR

Table 1: Serological evaluation at T_1 (21 days post first vaccination dose) of the immunity response in the COV− and COV+ groups.

		OSR (Roche SARS-CoV-2-S)					IOG-CSS (Siemens SCOV2G)				
		Subjects		Test results, U/mL[a]			Subjects		Test results, U/mL[b]		
		n	Age, years[c]	Median	IQR	n >cutoff (%)	n	Age, years	Median	IQR	n >cutoff (%)
COV+	Total	315	44.2 ± 15.7	>2,500[b]	N.D.[d]	315 (100)	201	48.3 ± 14.2	225.8	150.0–584.8	201 (100%)
	M	117	41.5 ± 13.1	>2,500[b]	N.D.[d]	117 (100)	89	51.1 ± 14.6	217.2	139.9–532.9	89 (100%)
	F	198	45.8 ± 16.9	>2,500[b]	N.D.[d]	198 (100)	112	45.6 ± 14.1	233.1	150–595.6	112 (100%)
COV−	Total	3,025	44.6 ± 11.8	43.3	15.9–110.0	2,966 (98.0)	749	46.7 ± 13.8	7.4	2.4–18.1	703 (93.9%)
	M	1,083	44.9 ± 12.6	38.2	12.0–108.5	1,055 (97.4)	339	47.8 ± 14.5	6.2	2.7–16.5	310 (91.4%)
	F	1,942	44.4 ± 11.3	46.0	18.2–110.8	1,911 (98.4)	410	47.2 ± 14.5	8.8	4.1–19.2	393 (95.9%)
	M 20–30	182	27.9 ± 1.8	96.3	36.2–161.0	182 (100)	59	28.7 ± 2.1	11.1	5.9–23.1	58 (98.3%)
	M 31–40	267	34.9 ± 2.8	54.2	21.5–133.0	265 (99.3)	80	36.2 ± 2.8	7.4	3.2–18.7	78 (97.5%)
	M 41–50	231	46.0 ± 2.8	36.7	10.7–90.5	225 (97.4)	68	46.4 ± 3.0	4.8	2.8–12.6	64 (94.1%)
	M 51–60	273	55.5 ± 2.8	25.1	9.0–75.1	262 (96.0)	76	56.5 ± 2.8	5.8	1.9–14.8	66 (86.8%)
	M 61–70	111	64.4 ± 2.4	15.8	4.6–38.2	103 (92.8)	56	65.6 ± 3.1	3.7	1.1–5.7	44 (78.6%)
	M 71–80	19	73.8 ± 2.6	4.9	2.1–6.8	18 (94.7)					
	F 20–30	287	27.2 ± 2.3	71.3	35.4–149.5	287 (100)	73	27.9 ± 2.1	17.5	9.3–42.4	73 (100%)
	F 31–40	462	35.5 ± 3.0	52.7	24.1–128.0	459 (99.4)	76	36.0 ± 3.2	13.9	6.4–34.4	75 (98.7%)
	F 41–50	522	45.9 ± 2.9	45.2	19.4–103.7	517 (99.0)	129	46.7 ± 2.7	7.5	3.5–15.4	123 (95.3%)
	F 51–60	544	55.0 ± 2.8	33.5	12.5–88.5	528 (97.1)	102	55.6 ± 2.7	5.3	2.7–11.1	94 (92.2%)
	F 61–70	111	63.3 ± 2.3	32.9	6.5–72.5	108 (97.3)	30	64.9 ± 3.7	5.9	4.1–5.9	28 (93.3%)
	F 71–80	16	73.1 ± 2.0	10.6	4.5–32.8	12 (81.3)					

[a]OSR subjects were tested with the Roche Elecsys SARS-CoV-2-S assay targeting the S-protein RBD. Signal range: 0.4–2,500 U/mL. Positivity cutoff: 0.8 U/mL. [b]IOG-CSS subjects were tested with the Siemens Atellica SCOV2G test targeting the S-protein RBD. Signal range: 0.05–750 U/mL. Positivity cutoff: 1 U/mL. n>cutoff" represents the number of samples with an antibody titer higher than the instrumental cutoff. [c]Age is expressed as average ± STD. [d]Median and IQR were not calculated because of the many results above the 2,500 U/mL high instrumental limit.

150.0–584.8 U/mL) (Table 1, Figure 1), about one-order of magnitude higher than the corresponding measurement at T_0 (2.8 U/mL; IQR 1.38–5.24 U/mL) (Figure 1).

Serological evaluation at T_1: COVID-negative group (COV−)

Of the 3,025 COV− subjects from the OSR (64.2% females) 2,966 (98.0%) showed detectable antibody titers at T_1 whereas 59 (2.0%) were below the instrumental cutoff limit and were considered as "non-respondents" (NR). Because the COV− group inclusion criteria was the T_0 negativity to the anti-N protein antibodies, we assumed that their S-protein RBD antibody level at T_0 was also null. Fifty T_0 samples were actually tested with the Elecsys Anti-SARS-CoV-2 (Roche) and all of the measurements resulted below the lower instrumental limit (<0.4 U/mL). Figure 1 shows the comparison between the COV− group antibody response at T_0 and T_1 for both COV− and COV+ group. It must be noted that, in contrast to the COV+ group, only 10 subjects (0.3%) showed, at T_1, titers >2,500 U/mL.

Stratifying the male and female groups by age showed that the median value of the Roche Anti-SARS-CoV-2 test results decreased with increasing age (Table 1, Figure 2). A multivariate analysis showed significance differences between the age groups ($p<0.0001$) and, although to a less extent, between genders ($p<0.001$). In other words, the signal decline with age is more pronounced in the male group as shown by a significant interaction ($p=0.014$) between the age and gender variables (Figure 2).

The Siemens instrument used by IOG-CSS showed that 703 samples (93.9%) had detectable antibody titers 21 days post first vaccination dose whereas 46 samples (6.1%) were below the instrumental cutoff limit and were considered as NR (Table 1). Stratifying for age and gender showed that the median of the Siemens SARS-CoV-2 IgG test results decreased with increasing age (Table 1, Figure 2) in both males and females. As observed in the OSR cohort, the effect of age was significant ($p<0.001$), as well as the effect of gender ($p=0.014$), with males decreasing more rapidly than female as age increased (significant interaction, $p=0.028$) (Figure 2).

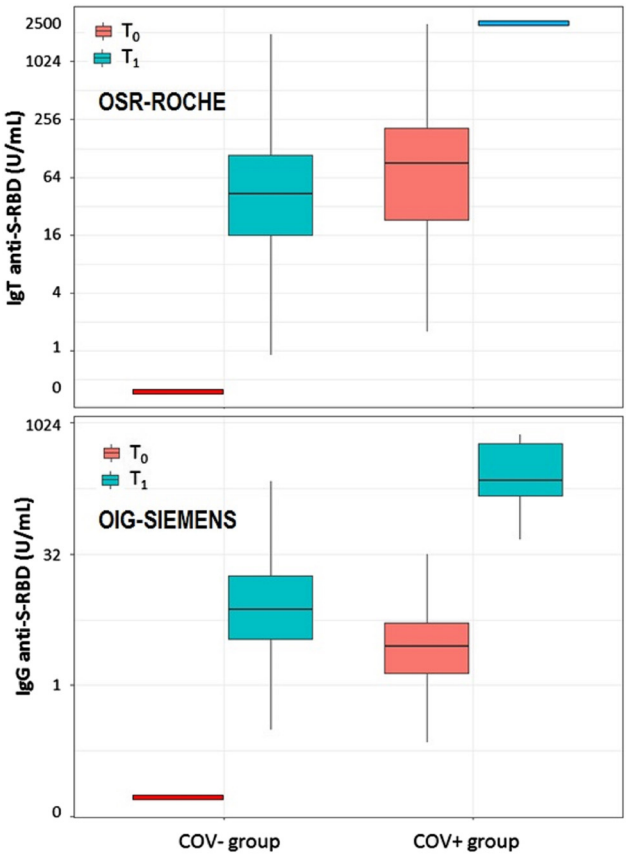

Figure 1: Serological response 21 days after the first dose of the Comirnaty mRNA vaccine in healthcare professionals with (COV+ group) and without (COV– group) laboratory confirmed SARS-CoV-2 previous infection.

Serological evaluation at T_2: COVID-negative group (COV–)

For a limited number of patients (100) belonging to the OSR group, we also measured the antibody titer 21 days after receiving the second vaccination dose. For 25 of them (25.0%) the values were above the instrumental limit (>2,500 U/mL) whereas the remaining 75 patients showed a median value of 957 U/mL. Twelve of the 25 sample exceeding the upper instrumental limit were diluted with human pre-pandemic serum to bring the instrumental response within the instrumental range. The median value was 3,280 U/mL (IQR 2,840–4,140 U/mL).

Diagnostic laboratory data and questionnaire

From both the OSR health professionals follow-up program and the post-vaccination questionnaire, sent to the 315 subjects belonging to the COV+ group, we obtained information about the symptomatology of the disease as well as the time interval between the disease onset and the first vaccination dose. Of the 315 questionnaire sent we obtained answers from 267 subjects whereas laboratory information allowed to trace the timing of the disease for 277 health professionals. Table 2 shows that 50.9% of the subjects were asymptomatic and only a small fraction (6 subjects, 2.2%) needed hospitalization. It must be noted that none of them needed intensive care unit therapy. Table 2 shows that most of the health professionals got infected either during the first wave, approximately framed between March 1st and April 30th 2020, or the second wave (approximately between October 1st and November 30th, 2020), whereas only 2 subjects were infected between the beginning of May and the end of September 2020. Thirty-five health professionals, with neither a positive swab test nor symptoms of COVID-19, resulted positive at the May 2020 serological screening. Thus, their time interval between the disease and the first vaccination dose

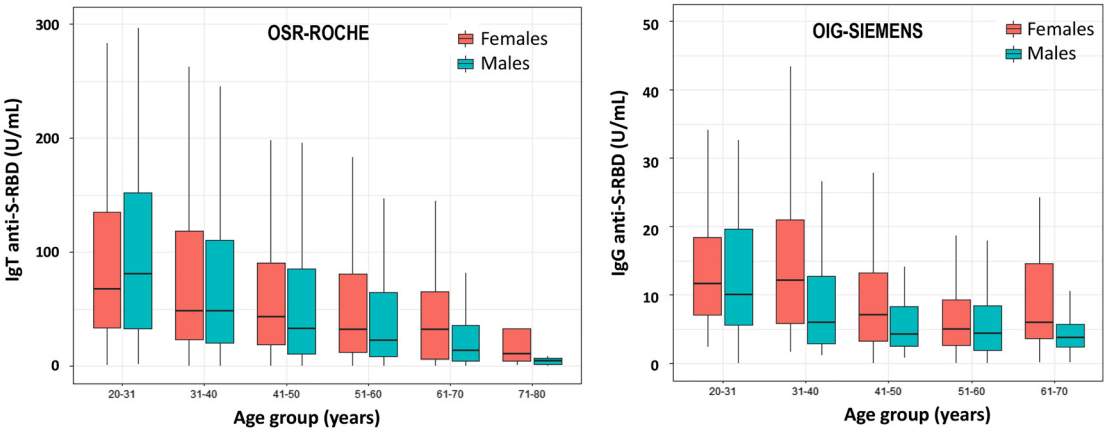

Figure 2: Stratification by age and gender of the serological responses 21 days after the first dose of the Comirnaty mRNA vaccine in healthcare professional without previous SARS-CoV-2 infection.

Table 2: Clinical information obtained from the OSR database and from the questionnaire sent to the health professionals with a laboratory confirmed previous SARS-CoV-2 infection.

Items	Answers	n	Males	Females
Symptoms	Asymptomatic	136	52	84
	Moderate	125	46	79
	Hospitalized[a]	6	4	2
	Total	267	102	165
Time from disease to vaccination (month of the disease)	1 month (December)	5	2	3
	2 months (November)	24	7	17
	3 months (October)	42	13	29
	7 months (June)	2	2	0
	>8 months	35	16	19
	9 months (April)	10	5	5
	10 months (March)	60	28	32
	11 months (February)	3	1	2
	?[b]	96	32	64
	Total	277	106	171

[a]None of the subjected was hospitalized in ICU. [b]The question mark refers to subjects unaware of having being infected by SARS-CoV-2 until the T_0 serological test.

was set at >8 months. Interestingly, Table 2 shows that 96 subjects (34.6%) were not aware of being infected in the past by SARS-CoV-2 before performing the T_0 serological evaluation. It must be noted that of the 73 subjects with the longest disease to vaccination intervals (9, 10 and 11 months), 71 (97.3%) showed values at T_1 above the 2,500 U/mL upper instrumental limit.

Discussion

Overall, of the 4,290 individuals tested, 4,185 (97.5%) showed the presence of S-protein specific antibody 21 days after the first vaccination dose. When considering naturally seropositive and seronegative individuals, we observed that 21 days after receiving the first dose, 98.2% of the participants belonging to the COV– group of the OSR, and 95.2% of the participants belonging to the COV– of the IOG-CSS showed the presence of antibodies specific for the RBD of the viral S-protein, elicited by vaccination. Such percentages are similar to the 95% vaccine efficacy declared by Pfizer which was based on the number of individuals who got infected in the vaccine (8 participants) and the placebo (162 participants) groups [4].

Our data suggest a vaccine efficacy near 100%, for people younger than 40 years old, that decreases with increasing age, especially within the male population. This was expected considering that elderly individuals are known to be less responsive to vaccination due to immunosenescence [17, 18]. Although the presence of antibodies does not guarantee protection against virus infection, a recent paper showed a good correlation between antibody titers measured with both the Roche and Siemens instrumentation and pseudo-neutralization assays [19, 20]. Thus, we might speculate that, among vaccinated individuals, those who did not respond to the first vaccination dose might be more at risk, in the future, of being infected by SARS-CoV-2. Monitoring these subjects a few weeks/months after the second dose, will reveal whether the lack of vaccination response persists. This is a critically important information for the NR subjects themselves, which will act consequently, but also for the healthcare system that can track NR in order to improve the general population vaccine protection. Factors responsible for vaccine failures are either vaccine-related (failures in vaccine attenuation, vaccination regimes or administration) or host-related (genetics, immune status, age, health nutritional status) [17]. Changing the type of vaccine (i.e. from mRNA-based vaccine to recombinant-protein-based vaccine) or simply a change in the immunization route (e.g. from intramuscular to intradermal), as shown in the elderly with intradermal influenza vaccine [17] might be sufficient to ensure protection of this not negligible risk population. Thus, identification of subjects failing to mount appropriate antibody levels, especially in the older population, represents a strategic procedure needed to reach a general population vaccine protection close to 100%.

A different issue concerns the COV+ group. For both HRS and IOG-CSS datasets we observed an exceptionally high vaccine response for those individuals previously infected with SARS-CoV-2. The IOG-CSSs' dataset showed a median value at T_1 for the COV+ group approximately 35-fold higher than the COV– group whereas the majority of the individuals belonging to the OSRs' COV+ group were above the instrumental high limit of detection. Diluted samples, showed a median value (18,000 U/mL) approximately 400-fold higher than the COV– group at T_1. Such discrepancy between the OSR and IOG-CSS results might be attributed to the different instrumentations used in the two hospitals for which linearity was not declared. The 18,000 U/mL value obtained from the diluted samples, representing a 200-fold increase of the pre-vaccine levels, is consistent to what found in a previous study (including 51 participants) using the same Roche instrumentation [11], showing that the restricted group of diluted samples represents a good approximation of the whole group behavior. It must be noted that of the 100 subjects, belonging to the OSRs' COV– group and tested at T_2, 21 days after the second dose, only 25 (25%) reached a value above the detection

level whereas, 93.3% of the OSR COV+ group were above the detection limit already 21 days after the injection of a single dose. Furthermore, diluted T_2 samples, showed that the antibody titer 21 days after the second dose were approximately five-fold lower than those of the COV+ group after a single vaccine dose. Thus, one dose of the Pfizer vaccine elicits a strong and rapid immune response in seropositive individuals and their titers largely exceed those found in seronegative subjects 21 days after injection of the second dose. Data from the questionnaires showed that, of the overall OSR health professionals tested, more than 2.5% had experienced COVID-19 unawarely. Furthermore, available diagnostic laboratory data from the OSR follow-up program showed that even subjects with the longer disease-to-vaccination time-intervals (9, 10 and 11 months) mount a strong immune response after the first vaccination dose. Noteworthy, the first Italian autochthonous case was on February 21st, 2020, meaning that 11 months represents the largest time-interval possibly available for this study. Thus, immunological memory seems to last for at least 11 months, in line with recent studies showing antibody persistence up to 10 months after infection [21].

These findings raise questions about the large portion of population previously infected by SARS-CoV-2: should they receive the two doses of the Pfizer vaccine? or just one dose? or no vaccine at all? From our data it appears that the first vaccine dose acts, in previously infected individuals, as a boost even more vigorously than it does the second dose in seronegative subjects. Thus, a change in vaccine recommendations indicating a single dose for seropositive individuals might be considered in order to increase vaccine doses' availability and also to avoid the high reactogenicity that might be induced by a second dose.

In conclusion we would like to point out a few technical comments. The manufacturers' cutoff for the Roche Anti-SARS-CoV-2-S test (>1 U/mL) seems to be a good compromise between specificity and sensitivity. Using a lower cutoff as suggested by Favresse et al. [16] would increase specificity but will also increase the number of false positive. Thus, rather than using a strict cutoff level we suggest to consider a "grey zone" around the manufacturers' suggested cutoff level which, for the Roche Anti-SARS-CoV-2-S test, might be set between 0.165 and 1 U/mL. A similar behavior would probably occur for the Siemens instrumentation as well. However, the lack of clinical information prevented us to perform a detailed analysis. Furthermore we showed how two well performing instrumentations, like the Roche and Siemens used in this study, provide very different numerical outputs. As an example, the high T_1 response of the previously infected individuals showed very different median values (225.8 and 18,000 U/mL for Siemens and Roche, respectively). That might create confusion not only among the vaccinated subjects but also among clinicians in case they have to evaluate the serological status/kinetic of a subject tested at different time-points with different instrumentations. In this context, a standardization/comparison of the different commercially available quantitative serological test instrumentations would be ideal in order to normalize their outputs and provide information about the immunity status of the vaccinated subjects.

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