Serological response after SARS-CoV2 vaccination in healthcare workers: a multicenter study

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ABSTRACT

Introduction: *Characterizing immunological response following COVID-19 vaccination is an important public health issue. The objectives of the present analysis were to investigate the proportion, level and the determinants of humoral response from 21 days to three months after the first dose in vaccinated healthcare workers (HCWs).* **Methods**: *We abstracted data on level of anti-SARS-CoV-2 Spike antibodies (IgG) and sociodemographic characteristics of 17,257 HCWs from public hospitals and public health authorities from three centers in Northern Italy who underwent COVID-19 vaccination (average 70.6 days after first dose). We fitted center-specific multivariate regression models and combined them using random-effects meta-analyses.* **Results**: *A humoral response was elicited in 99.3% of vaccinated HCW. Female sex, young age, and previous COVID-19 infection were predictors of post-vaccination antibody level, and a positive association was also detected with pre-vaccination serology level and with time between pre- and post-vaccination testing, while a decline of antibody level was suggested with time since vaccination.* **Conclusions:** *These results stress the importance of analyzing retrospective data collected via occupational health surveillance of HCWs during the COVID-19 epidemic and following vaccination. They need to be confirmed in larger series based on prospectively collected data.*

Introduction

The spread of the COVID-19 pandemic around the world has revealed that it is urgently important to evaluate the efficacy of vaccination in inducing immune response through rapid and inexpensive assays for antibodies in general and, specifically, anti-SARS-CoV-2 IgG antibody (anti-SARS-CoV-2 spike glycoprotein S1 antibody) in order to monitor protection conferred by vaccination [1].

Effective vaccination has begun, which could prevent continuous or repeated pandemic [2, 3]; however, methods for assessing the antibody response are still under investigation. As a result, rapid methods to detect the anti-SARS-CoV-2 antibody will be a key component of combating the pandemic, although the correlate of protection is not known, measuring antibody levels may allow protection of non-immune HCW and adaptation of vaccine regimens based on the protection durability.

Many studies are appearing in the literature on the efficacy of vaccines. It was recently demonstrated that the titer of neutralizing antibodies was markedly higher in response to the Pfizer-BioNTech vaccine than after natural infection [4].

HCWs are a group at high risk of infection in general [5] and specifically SARS-CoV-2 infection, [6]. The main aim of the present analysis was to investigate the proportion, level and determinants of serologic response at 21 days up to 3 months in HCWs who were vaccinated and were included in Orchestra, a European Multicenter project. In particular, we aimed at evaluating the presence of antibody responses (qualitative and quantitative) af-

ter the first or second dose of mRNA vaccines in a sample of HCW, and the associations for sex, age, job title, previous positive PCR test, time since vaccination and pre-vaccination serology level.

Methods

Cohorts of HCWs employed in teaching hospitals and public health administrations in three Italian centers (Bologna, Brescia and Verona) were assembled to study the prevalence of COVID-19 infection and its determinants [7]. Data on sociodemographic characteristics, PCR testing, and vaccination status, including date of vaccine doses and type, and level of anti-COVID-19 S1 IgG antibodies were abstracted from occupational health surveillance records or collected using questionnaires. The cohorts are included in the European Commission-sponsored Orchestra project, and their data have undergone extensive harmonization.

Selected characteristics of the cohorts of HCWs included in the present analysis are described in Table 1. These cohorts were mainly assembled during the first wave of the epidemic (March –May 2020) and are now included in prospective follow-up.

The outcomes of this analysis were immunologic evidence of response to vaccination and level of serum antibodies. Details on the methods to measure antibody level are reported in Table 2.

In the first step of the statistical analysis, we conducted descriptive analysis of the outcome and explanatory variables. Subsequently, we conducted cohort-specific logistic regression analyses on response to vaccination, coded as any vs. no response,

Table 1. Characteristics of the cohorts of HCW included in the analysis

	Bologna	Brescia	Verona
Institutions	Public hospitals and public health authority of Bologna	Public hospitals and public health authority of Brescia	University Hospital of Verona
Source of data	Occupational health surveillance records	Occupational health surveillance records	Occupational health surveillance records
Type of vaccine (%)*	94.78% P 5.08% M 0.09% A 0.05% J	99.15 % P 0.22% M 0.63% A	100% P
Time between vaccination and antibody test (days; mean, SD)	59.7 (16.0)	61.8 (3.0)	21.0 (0)

* P, Pfizer-BioNTech; M, Moderna; A, Astra-Zeneca; J, Johnson&Johnson

Table 2. Analytical methods used to measure SARS-CoV-2 antibody level

Center	Period	Method	Mean	SD
Bologna	April 2020 – June 2020	ELISA (IgG and IgM)	Q	–
	July 2020- December 2020	ECLIA (IgG+IgM anti-N)	Q	–
	Jan 2021- May 2021	ECLIA-RBD (IgG anti-S)	1339.04	938.27
Brescia	April 2020- July 2020	ECLIA (IgG anti-S)	7.31	20.19
	March 2021-April 2021	ECLIA-RBD (Ig anti-S)	2182.12	1836.41
Verona	May 2020 – Jan 2021	CLIA (IgM and IgG)	0.655	2.685
	Jan 2021 – May 2021	TrimericS IgG assay	1984.99	4865.50

Q, qualitative result

to estimate odds ratios (OR) and the corresponding 95% confidence intervals (CI). Antibody levels were log-transformed to take into account the skewness of the distribution. Since different methods were used across centers to measure antibodies, log-transformed results were normalized by dividing them by the center-specific standard error. Only quantitative tests were taken into account in the statistical analysis, while qualitative tests were excluded from it. Multivariate linear regression models were fitted to estimate cohort-specific relative risks (RR) and corresponding 95% CI.

In the second step, cohort-specific results were combined using standard meta-analytical techniques. To evaluate variability among studies, a test of heterogeneity was applied and the I-squared statistic was computed, which indicates the proportion of total variation among the effect estimates of different studies attributed to heterogeneity rather than sampling error. Given the heterogeneity in the underlying studies, a random effects model was used [8]. The results are displayed graphically using forest plots. Stata® software 16 (StataCorp LP, College Station, Texas, USA) was used in the statistical analysis.

The study was approved by the Italian Medicine Agency (AIFA) and the Ethics Committee of Italian National Institute of Infectious Diseases (INMI) Lazzaro Spallanzani (PARERE N.436-Registro delle Sperimentazioni 2020/2021).

Results

A total of 17,241 vaccinated HCWs from the three Italian cohorts were included in the analysis.

The distribution of subjects in each cohort according to the outcome and the explanatory variables is provided in Table 3. The cohorts were slightly different in their distribution by sex, age, and job title. The proportion of HCWs with a previous COVID-19 infection was in the range 10-12% of cohort members. Vaccination was performed on 85-91% of cohort members.

Most vaccinated HCW elicited a serologic response (17,129/17,241, 99.4%) (Table 4); results on individual cohorts are available in Supplementary Table 1. Because of the small number of non-responders, models were unstable and results were only partially informative. Women had a higher probability of positive immune response to vaccination, although the difference was not statistically significant (OR 1.49; 95% CI 0.97-2.30), while models did not converge in the analysis of job title (Table 4). Age was inversely correlated with positive immune response (OR 0.46; 95% CI 0.27-0.79). No differences were detected according to time since vaccination and to pre-vaccination serology level. Most of the results were concordant between cohorts.

The results of the meta-analysis on determinants of post-vaccination antibody level are provided in Table 5. The corresponding results of the individual cohorts are reported in Supplementary Table 2. These results are expressed as RR for an increase of one center-specific SD of antibody level. Women had higher antibody titer than men (RR 1.07; 95% CI 1.04-1.11), and there was a decrease in antibody response with age (RR for 10-yr increase of age 0.83; 95% CI 0.78-0.88). No differences were detected according to job title. The average interval between COVID-19 infection and post-vaccination

Table 3. Selected characteristics of the population included in the analysis

	Bologna	Brescia	Verona
Total N	5525	6498	5218
Sex+			
Men	1752 (31.7%)	1771 (27.3%)	1555 (29.8%)
Women	3773 (68.3%)	4727 (72.7%)	3663 (70.2%)
Age group+			
18-29	957 (17.3%)	911 (14.0%)	1074 (20.6%)
30-39	1423 (25.8%)	1257 (19.3%)	1151 (22.1%)
40-49	1182 (21.4%)	1611 (24.8%)	1087 (20.8%)
50+	1963 (35.5%)	2719 (41.8%)	1906 (36.5%)
Age*	43.16 (12.0)	44.98 (11.37)	42.8 (12.3)
Job title+			
Administration	217 (3.9%)	766 (11.8%)	378 (7.2%)
Physician	1649 (29.8%)	1644 (25.3%)	1752 (33.6%)
Nurse	1781 (32.2%)	2286 (35.2%)	1841 (35.3%)
Technician	250 (4.5%)	565 (8.7%)	462 (8.9%)
Other HCW	1628 (29.5%)	1237 (19.0%)	785 (15.0%)
Previous infection+			
No	5099 (92.3%)	4996 (76.9%)	4663 (89.4%)
Yes	426 (7.7%)	1502 (23.1%)	555 (10.6%)
Pre-vaccination ln (Ab)*	-	1.64 (0.99)	-1.66 (1.32)
Post-vaccination ln (Ab)*	6.90 (0.9)	6.52 (0.99)	6.35 (1.42)

HCW, healthcare workers; + number (%); * mean (SD)

serology, measured in 1,381 HCW, was 225.1 days (SD 116.0), that between pre- and post-vaccination serology (available in 11,101 HCW) was 215.4 days (SD 37.2). There was a more pronounced antibody response in HCW who had a previous COVID-19 infection (RR 4.10; 95% CI 2.38-7.0); pre-vaccination serology level and time between pre- and post-vaccination testing were positively associated with post-vaccination level, while time since vaccination showed an inverse association with post-vaccination serology. We did not include type of vaccine in this analysis because of the small number of HCW vaccinated with vaccines other than Pfizer-BioNTech.

Discussion

In our analysis the overwhelming majority of HCWs who underwent vaccination had a serologic response. Both qualitative and quantitative analysis showed that women have a better immunological response following vaccination than men, that is in agreement with the fact of women tending to have a more intense innate, cellular and humoral immune response to vaccinations and infections than men [9]. Furthermore, these analyses showed higher serological titers among younger population and in previously infected individuals. Overall, COVID-19 vaccination reflects instalment of specific anti-SARS-CoV-2 humoral response. Conversely, as expected, no differences were detected according to job title.

Previous COVID-19 infection was a strong predictor of post-vaccination antibody level, and a positive association was also detected with pre-vaccination serology level and time between pre- and post-vaccination testing. A decline of antibody level with time since vaccination was also suggested in these data, which needs to be confirmed in longer-term analyses.

Table 4. Determinants of qualitative serology response – Results of meta-analysis

Characteristics*	N (%) with serologic response	OR (95% CI)
Sex [Bo, Bs, V]		
Men	5037 (99.2%)	1 (Ref)
Women	12092 (99.4%)	1.49 (0.97-2.30)
Age [Bo, Bs, V]		
10-yr increase	-	0.46 (0.27-0.79)
Job Title [Bo, Bs, V]		
Administration	1343 (98.7%)	1 (Ref)
Physician	5015 (99.4%)	1.61 (0.84-3.10)
Nurse	5875 (99.4%)	1.40 (0.75-2.60)
Technician	1268 (99.4%)	1.83 (0.80-4.18)
Other HCW	3628 (99.4%)	1.16 (0.60-2.24)
Previous COVID-19 infection [Bo, Bs, V]		
No	14647 (99.2%)	-†
Yes	2482 (100%)	-†
Time since vaccination [Bo, Bs]		
14-day interval	-	0.69 (0.42-1.13)
Pre-vaccination serology level [Bs, V]		
1 SD increase	-	1.10 (0.86-1.40)

* Centers included in the analysis are indicated in square brackets: Bo, Bologna, Bs, Brescia, V, Verona; † Model did not converge; CI, confidence interval; OR, meta-analytic odds ratio, adjusted for sex, age, job title and previous infection, as appropriate; Ref, reference category

These results are in line with the available literature; several observational studies reported that the immune response to some vaccines differs between men and women. Adult women display stronger innate and adaptive immune responses than men, which can lead to a faster clearance of pathogens and greater vaccine efficacy in the former [9]. In particular, vaccinations after which higher antibody titers were detected in women compared to men include influenza, yellow fever, rubella, measles, mumps, hepatitis A and B, herpes simplex 2, rabies, smallpox, and dengue viruses [10].

Regarding the decrease of antibody level with time since vaccination, Sariol et al [11] showed a rapid decline of anti-S antibodies just 40 to 80 days after a boost with the mRNA vaccine formulations and a sustained level of neutralization ability in the same period that anti-S antibodies are declining. This means that the immune response is not fully represented by the antibody titer and there are other mechanisms that go beyond that number, such as the presence of immune memory cells.

The large sample size, the high percentages of vaccinated HCWs and the collection of sociodemographic information from occupational health surveillance records are strengths of our study. In particular, the analysis of HCW provides an estimate of the humoral response following COVID-19 vaccination in a population that is healthier and more health-conscious than the general population.

Our analysis suffers from some limitations, including the retrospective nature of the data and their origin from a single country. However, the analyses of a smaller number of HCWs from a tertiary care hospital in France show the same trends (data not shown in detail) [12].

In addition, to date, there is no certain level of cut-off protection against severe infection or disease, such as for anti-HBV vaccination; the role of T lymphocytes in the management of immunological memory and long-term immunity also appears uncertain.

Furthermore, in our study blood samples were analyzed using different kits. Despite these limita-

Table 5. Determinants of quantitative serology response – Results of meta-analysis

Characteristics*	Mean (SD) antibody level (ln(Ab)/SD)	RR (95% CI)
Sex [Bo, Bs, V]		
Men	6.57 (1.15)	1 (Ref)
Women	6.61 (1.10)	1.08 (1.04-1.11)
Age [Bo, Bs, V]		
10-yr increase	-	0.83 (0.78-0.88)
Job Title [Bo, Bs, V]		
Administration	6.33 (1.16)	1 (Ref)
Physician	6.64 (1.08)	0.98 (0.87-1.09)
Nurse	6.63 (1.13)	0.98 (0.91-1.06)
Technician	6.49 (1.09)	1.03 (0.97-1.10)
Other HCW	6.62 (1.12)	0.98 (0.91-1.06)
Previous COVID-19 infection [Bo, Bs, V]		
No	6.39 (0.94)	1 (Ref)
Yes	7.89 (0.87)	4.39 (4.24-4.54)
Time since vaccination [Bo, Bs]		
14-day interval	-	0.97 (0.95-1.00)
Pre-vaccination serology level [Bs, V]		
1 SD increase	-	1.19† (1.16-1.21)
Time between serology tests [Bs, V]		
30-day interval	-	1.03† (1.02-1.05)

CI, confidence interval; RR, meta-analytic relative risk for increase of 1 SD ln(Ab), adjusted for sex, age, job title and previous infection, as appropriate; Ref, reference category; † Additionally adjusted for pre-vaccination serology level and time between serology tests, as appropriate

tions, these preliminary results offer important insights on the experience of HCW in terms of serological response. Follow-up ongoing analyses of cohorts included in the Orchestra project will be important to confirm these results.

These findings stress the importance of analyzing retrospective data mainly collected via occupational health surveillance of HCWs during the COVID-19 epidemic and following vaccination. They need to be confirmed in subsequent analyses of these population, including occurrence of breakthrough infections and the effect of booster vaccination, as well as in larger series based on prospectively collected data.

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Supplementary Table 1. Determinants of qualitative serology response – results by center

Characteristics	Bologna		Brescia		Verona	
	N serology (%)	OR (95% CI)	N serology (%)	OR (95% CI)	N serology (%)	OR (95% CI)
Sex						
Men	1748 (99.8%)	1 (Ref)	1771 (99.8%)	1 (Ref)	1521 (97.8%)	1 (Ref)
Women	3767 (99.8%)	1.65 (0.46-5.87)	4727 (99.9%)	2.49 (0.47-13.3)	3601 (98.3%)	1.41 (0.87-2.27)
Age						
10-yr increase	-	0.31 (0.12-0.83)	-	0.85 (0.42-1.71)	-	0.38 (0.30-0.48)
Job Title						
Administration	217 (100%)	1 (Ref)	765 (99.9%)	1 (Ref)	361 (95.5%)	1 (Ref)
Physician	1649 (99.7%)	NE	1642 (99.9%)	1.02 (0.09-11.9)	1729 (98.7%)	1.67 (0.85-3.31)
Nurse	1781 (99.8%)	NE	2285 (100%)	1.02 (0.12-34.5)	1813 (98.5%)	1.42 (0.75-2.68)
Technician	250 (100%)	NE	564 (100%)	0.58 (0.04-9.61)	454 (98.3%)	2.04 (0.86-4.84)
Other HCW	1628 (99.9%)	NE	1236 (99.9%)	1.23 (0.07-20.2)	765 (97.5%)	1.16 (0.59-2.27)
Time since vaccination						
14-day interval	-	0.88 (0.53-1.48)	-	0.06 (0.01-0.26)	-	NA
Pre-vaccination serology level						
1 SD increase	-	NA	-	0.40 (0.03-5.84)	-	1.11 (0.87-1.42)

CI, confidence interval; OR, odds ratio, adjusted for sex, age, job title and previous infection, as appropriate; Ref, reference category; NA, not available; NE, not estimable

Supplementary Table 2. Determinants of quantitative serology response – results by center

Characteristics	Bologna		Brescia		Verona	
	Mean (SD) antibody level (ln(Ab)/SD)	RR (95% CI)	Mean (SD) antibody level (ln(Ab)/SD)	RR (95% CI)	Mean (SD) antibody level (ln(Ab)/SD)	RR (95% CI)
Sex						
Men	6.84 (0.91)	1 (Ref)	6.50 (1.06)	1 (Ref)	6.33 (1.46)	1 (Ref)
Women	6.94 (0.87)	1.14 (0.66-1.95)	6.54 (0.98)	1.08 (1.03-1.13)	6.36 (1.41)	1.07 (1.01-1.12)
Age						
10-yr increase	-	0.80 (0.78-0.82)		0.88 (0.87-0.90)	-	0.81 (0.79-0.82)
Job Title						
Administration	6.79 (0.73)	1 (Ref)	6.38 (0.97)	1 (Ref)	5.98 (1.62)	1 (Ref)
Physician	6.94 (0.93)	0.85 (0.75-0.98)	6.55 (0.97)	1.01 (0.94-1.08)	6.44 (1.28)	1.06 (0.96-1.17)
Nurse	6.91 (0.91)	0.90 (0.79-1.03)	6.59 (0.99)	0.97 (0.91-1.04)	6.41 (1.45)	1.05 (0.96-1.16)
Technician	6.94 (0.75)	0.97 (0.82-1.15)	6.56 (0.99)	1.05 (0.96-1.14)	6.15 (1.34)	1.04 (0.93-1.17)
Other HCW	6.87 (0.86)	0.94 (0.82-1.07)	6.47 (1.07)	0.95 (0.89-1.02)	6.33 (1.58)	1.06 (0.96-1.17)
Previous COVID-19 infection						
No	6.84 (0.86)	1 (Ref)	6.19 (0.79)	1 (Ref)	6.08 (1.18)	1 (Ref)
Yes	7.67 (0.81)	2.53 (2.31-2.78)	7.66 (0.76)	4.26 (4.07-4.45)	8.64 (1.22)	6.63 (6.17-7.13)
Time since vaccination						
10-14-day interval	-	0.97 (0.94-0.99)	-	1.01 (0.90- 1.12)	-	NA
Pre-vaccination serology level						
1 SD increase	-	NA	-	1.19 † (1.16-1.22)	-	1.18 † (1.15-1.21)
Time between serology tests						
30-day interval	-	NA	-	1.03 † (0.98-1.08)	-	1.04 † (1.02-1.05)

CI, confidence interval; RR, relative risk for increase of 1 SD ln(Ab), adjusted for sex, age, job title and previous infection, as appropriate; Ref, reference category; NA, not available; † Additionally adjusted for pre-vaccination serology level and time between serology tests, as appropriate