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Preliminary analysis of serology results after vaccination

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Executive summary

Within Workpackage 5 of ORCHESTRA, we conducted a preliminary analysis of 26,040 HCW from public hospitals and public health authorities from three centers in Northern Italy, and of 40 HCW from one hospital in Paris. Determinants of COVID-19 vaccination and of serological response to vaccination were estimated via center-specific multivariate models and meta-analyses.

In the three Italian cohorts, the overall proportion of vaccination was 88.1%. Vaccination was associated with older age, employment as worker directly involved in health care, and previous COVID-19 infection. A serological response was elicited in 99.35% of vaccinated HCW. Female sex, young age, and previous COVID-19 infection were predictors of post-vaccination antibody level (measured on average 70.6 days after vaccination), and a positive association was also detected with pre-vaccination serology level and time between pre- and post-vaccination testing, while a decline of antibody level was suggested with time since vaccination. Results of the Paris cohort were consistent with those of the Italian cohorts.

These results stress the importance of analyzing retrospective data mainly collected via medical surveillance of HCW during the COVID-19 epidemic and following vaccination. They need to be confirmed in larger series based on prospectively collected data.

Abbreviations

AIFA, Italian Medicine Agency

CI, confidence interval

HCW, Healthcare worker

INMI, Italian National Institute of Infectious Diseases

OR, odds ratio

RR, relative risk

SD, standard deviation

SE, standard error

Introduction

The project, Connecting European Cohorts to Increase Common and Effective Response to SARS-CoV-2 Pandemic: ORCHESTRA (Orchestra) is sponsored by the European Commission and coordinated by the University of Verona. Its primary objective is to create of a new pan-European cohort built on existing and new large-scale population cohorts. Healthcare workers (HCW) represent an important population to study the characteristics of the COVID-19 epidemic. First, HCW are at higher risk of exposure compared to the population at large: this in particular was the case during the early phase of the epidemic. Second, exposure data are usually better for HCW than for other groups of the population, In particular with respect to opportunities for contacts with patients or colleagues and to use of personal protection equipment (PPE). Third, HCW were among the first population groups selected for vaccination: they would therefore provide early information on vaccine-related outcomes such as serologic response. Fourth, adherence rates and determinants of HCW adherence to vaccination are important to analyze given that adequate immunization is necessary to prevent absenteeism and protect hospitalized patients from nosocomial infections. Finally, many groups of HCW undergo regular health surveillance, which facilitates their inclusion in retrospective and prospective cohorts.

Four legacy cohorts of HCW (one from France, three from Italy) that are part of Orchestra are included in this analysis: these cohorts were assembled during 2020 before the consortium was established, and their inclusion in Orchestra allows the generation of early results on important epidemiology aspects of the COVID-19 epidemic. As part of the Orchestra collaboration, these cohorts are undergoing a process of data harmonization to enable parallel analyses among them and with newly established cohorts in other countries.

Methods

Population

Four legacy cohorts of HCW are included in this preliminary analysis: their characteristics 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 the prospective phase of the project. Comirnaty was the predominant vaccine type in all centers.

Data

Data used in this analysis were abstracted from medical surveillance records or collected using questionnaires. They were harmonized within the framework of the Orchestra project. Details are reported in **Table 2**.

Since the distributions of serology levels were skewed, they were log-transformed before the analysis. In addition, since different methods were used for serologic analyses (details in **Table 3**), they were normalized before the analysis.

We considered four outcomes

- Vaccination status;
- Reason for non-vaccination;
- Post-vaccination serology response (qualitative);
- Post-vaccination serology response (quantitative; SARS-CoV-2 spike antibody level).

Statistical analysis

In the first step of the analysis, we conducted descriptive analysis of the outcome and explanatory variables.

Subsequently, we conducted cohort-specific multivariate regression analyses on vaccination status and qualitative post-vaccination serology response (logistic regression), as well as quantitative post- vaccination serology (linear regression) to estimate odds ratios (OR) and relative risks (RR), respectively, and the corresponding 95% confidence intervals (CI). Given the small number of HCW vaccinated with Spikevax or Vaxzevria vaccine, type of vaccine was not considered in the analyses.

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

	Bologna	Brescia	Paris	Verona
Institutions	Public hospitals and public health authority of Bologna	Public hospitals and public health authority of Bologna	Bichat Claude Bernard Hospital, APHP, Paris France	University Hospital of Verona
Source of data	Health surveillance records	Health surveillance records	Prospective protocol	Health surveillance records
Time period – PCR	March 2020 – December 2020	March 2020 – December 2020	February 2020 - May 2020	February 2020 – December 2020
Time period – serology	Jan 2021- May 2021	March 2021- April 2021	February 2021 - May 2021	Jan 2021- May 2021
Type of vaccine (%)*	94.78% C 5.08% S 0.09% V 0.05% J	99.15 % C 0.22% S 0.63% V	43.75% C 6.25% S 50.00% V	100% C
Number of HCW with vaccination data	9479	8119	40	7638

* C, Comirnaty; M, Spikevax, A, Vaxzevria; J, Janssen

Table 2. Information available in the cohorts of HCW included in the analysis

Variable	Details	Bologna	Brescia	Paris	Verona
Sex		+	+	+	+
Age		+	+	+	+
Job title	Physician, nurse, technician, other HCW, administrative	+	+	+	+
Pre-vaccination COVID-19 infection (including date)	Positive PCR test at least 15 days before first vaccine dose	P	P	P	P
Pre-vaccination serology level (including date)	Serology test before first vaccine dose	QL	Q	Q	Q
Vaccination (including date and type of vaccine)		+	+	+	+
Reason for lack of vaccination	Cohort-specific categories	-	(+)	+	-
Post-vaccination serology (including date)	Serology test at least 15 days after first vaccine dose	Q	Q	Q	Q*

+, available in most cohort members

(+) available in a subset of cohort members

- not available

* measured at the day of the second vaccine dose P, PCR test; QL, qualitative; Q, quantitative

In the final step, cohort-specific results were combined using random-effects meta-analyses; heterogeneity between cohort-specific results was tested using the I^2 methods. Given the small number of subjects from the Paris cohort in the preliminary analysis, and the fact that it comprises only HCW with previous COVID-19 infection, results from this cohort were not included in the meta-analyses and are reported separately.

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.

Table 3. Methods used for serological analyses

Cohort	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
	August 2020-September 2020	ECLIA (IgG anti-N)	15.97	35.47
	October 2020-December 2020	ECLIA (IgG anti-N)	10.98	35.18
	January 2021 – February 2021	ECLIA (IgG anti-N)	9.94	31.09
	March 2021-April 2021	ECLIA (Ig anti-N)	10.59	33.77
		ECLIA-RBD (Ig anti-S)	2182.12	1836.41
Paris	February 2021_May 2021	ELISA EuroImmuno (IgG anti S)*	5.85	4.72
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 results

* ratio values above 10 were recoded as 12.5

Results

Descriptive results

A total of 26,020 HCW from the three Italian cohorts and 40 HCW from the Paris cohort were included in the analysis. The distribution of subjects in each cohort according to the outcome and the explanatory variables is provided in **Table 4**. The cohorts were similar in their distribution by sex, age, and job title.

Table 4. Distribution of cohorts by selected characteristics

	Bologna	Brescia	Paris	Verona
Total	9479	8903	40	7638
Sex+				
Men	3270 (34.5%)	2446 (27.5%)	9 (22.5%)	2352 (30.8%)
Women	6209 (65.5%)	6457 (72.5%)	31 (77.5%)	5286 (69.2%)
Age group+				
18-29	1813 (19.1%)	1386 (15.6%)	14 (35.0%)	1636 (21.4%)
30-39	2584 (27.3%)	1968 (22.1%)	11 (27.5%)	1850 (24.2%)
40-49	1898 (20.0%)	2015 (22.6%)	6 (15.0%)	1469 (19.2%)
50+	3184 (33.6%)	3534 (39.7%)	9 (22.5%)	2683 (35.1%)
Age*	42.5 (12.3)	45.0 (11.4)	37.1 (11.9)	42.8 (12.3)
Job title+				
Administration	378 (4.0%)	985 (11.1%)	1 (2.5%)	601 (7.8%)
Physician	2766 (29.2%)	2642 (29.7%)	9 (22.5%)	2642 (34.6%)
Nurse	2845 (30.0%)	2855 (32.1%)	12 (30.0%)	2564 (33.6%)
Technician	366 (3.9%)	702 (7.9%)	2 (5.0%)	660 (8.6%)
Other HCW	3124 (33.0%)	1719 (19.3%)	16 (40.0%)	1171 (15.3%)
Previous infection+				
No	8496 (86.6%)	7861 (88.3%)	0 (0.0%)	6699 (87.7%)
Yes	983 (10.4%)	1042 (11.7%)	16 (100%)	939 (12.3%)
March-June 2020	230 (23.4%)	-	-	245 (26.1%)
July-October 2020	70 (7.1%)	-	-	177 (18.8%)
After November 2020	683 (69.5%)	-	-	517 (55.1%)
Pre-vaccination Ab*	-	8.5 (18.8)	-	0.655 (2.685)
Pre-vaccination In (Ab)*	-	1.5 (0.9)	-	-1.658 (1.319)
Post-vaccination Ab*	1339.0 (938.3)	2555.4 (2520.7)	6.3 (5.3)	1985.0 (4865.5)
Post-vaccination In (Ab)*	6.9 (0.9)	7.3 (1.1)	1.3 (1.2)	6.352 (1.424)

+, Frequency and percentage for the categorical variables are reported.

*, Mean and SD for the continuous variables are reported.

The proportion of HCW with a previous COVID-19 infection was in the range 10-12% in the

Italian cohorts (the Paris cohort comprised only HCW with a previous infection). Vaccination was performed by 85-91% of Italian HCW, while this proportion was 40% in the Paris cohort.

Determinants of vaccination

A total of 22,924 HCW in the Italian cohort were vaccinated (88.1%). The results of the meta-analysis on determinants of vaccination are provided in **Table 5**. The OR of vaccination for a ten-years increase of age was 1.20 (95% CI 0.97- 1.49).

Table 5. Factors associated with COVID-19 vaccination among HCW

Characteristic*	N vaccinated (%)	OR (95% CI)
Sex [Bo, Bs, V]		
Men	7123 (88.3%)	1 (Ref)
Women	15801 (88.0%)	0.96 (0.88-1.04)
Age [Bo, Bs, V]		
10-yr increase		1.20 (0.97-1.49)
18-29	4192 (86.7%)	
30-39	5407 (84.5%)	
40-49	4885 (90.8%)	
50+	8440 (89.8%)	
Job Title [Bo, Bs, V]		
Administration	1709 (87.0%)	1 (Ref)
Physician	7128 (88.5%)	1.62 (1.16-2.26)
Nurse	7394 (89.5%)	1.59 (1.12-2.26)
Technician	1567 (90.7%)	1.72 (1.39-2.13)
Other HCW	5126 (85.2%)	0.98 (0.67-1.45)
Previous COVID-19 infection [Bo, V]		
No	20258 (87.9%)	1 (Ref)
Yes	2666 (89.9%)	0.81 (0.71-0.92)
March-June 2020	432 (90.9%)	1.50 (0.93-2.44)
July-October 2020	221 (89.5%)	1.19 (0.64-2.20)
After November 2020	971 (80.9%)	0.63 (0.51-0.77)

* Centers included in the analysis are indicated in square brackets:
 Bo, Bologna, Bs, Brescia, V, Verona
 CI, confidence interval
 OR, meta-analysis odds ratio, adjusted for sex, age, job title and previous infection, as appropriate
 Ref, reference category

Physicians, nurses and technicians were more frequently vaccinated than administrative staff. On the other hand, HCW with previous infection were less frequently vaccinated compared to HCW without infection (OR 0.81; 95% CI 0.71-0.92). The proportion of vaccinated HCW was particularly low among HCW infected after November 2020. No differences were detected according to sex.

The reason for lack of vaccination was recorded in a subset of HCW from Bologna: these data are reported in **Table 6**. Previous COVID-19 infection was reported as reason by 34%, fear of side effects by 23%, and medical or physiological conditions by 21%.

Table 6. Reason for lack of vaccination in a subset of HCW from Bologna, Italy

Self-reported reason	N	%
Previous COVID-19 infection	75	34.1
Allergies	13	5.9
Pregnancy	14	6.4
Breastfeeding	6	2.7
Immunosuppressive therapy	11	5.0
Post-surgery recovery	3	1.4
Fears of side effects	51	23.2
Other	47	21.4
Total	220	100.0

Post-vaccination serology response (qualitative)

Most vaccinated HCW elicited a serologic response (17145/17257, 99.35%); For this reason, the results of the analysis of factors associated with serologic response were only partially informative (**Table 7**). Women had a higher probability of positive immune response to vaccination, although the difference was compatible with chance (OR 1.49; 95% CI 0.97-2.30), while models did not converge in the analysis of job title. No differences were detected according to time since vaccination and pre-vaccination serology level.

Table 7. Factors associated with qualitative serologic response to vaccination in HCW

Characteristic*	N serology (%)	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)
18-29	2940 (99.9%)	
30-39	3825 (99.8%)	
40-49	3814 (98.3%)	
50+	6500 (98.7%)	
Job Title [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)
Time since vaccination [Bo, Bs]		
10-14-day interval		0.48 (0.12-1.85)
Pre-vaccination serology level		
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 CI, confidence interval
 OR, meta-analysis odds ratio of serologic response, adjusted for sex, age, job title and previous infection, as appropriate Ref, reference category

Post-vaccination serologic response (quantitative)

The results of the meta-analysis on determinants of post-vaccination serology response are reported in **Table 8**. These results are expressed as OR of having a one-SD higher level of antibody level. Women had higher antibody titer than men (OR 1.07 (1.04-1.11), and there is a decrease in antibody response with age (OR 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 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 (OR; 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 Comirnaty.

Table 8. Results of analysis on quantitative serologic response

Characteristic*	Mean (SD) standardized antibody level (ln(Ab)/SD)	OR (95% CI)
Sex [Bo, Bs, V]		
Men	6.63 (1.15)	1 (Ref)
Women	6.68 (1.10)	1.07 (1.04-1.11)
Age [Bo, Bs, V]		
18-29	7.05 (0.93)	
30-39	6.83 (0.95)	
40-49	6.63 (1.07)	
50+	6.43 (1.22)	
10-yr increase		0.83 (0.78-0.88)
Job Title [Bo, Bs, V]		
Administration	6.44 (1.17)	1 (Ref)
Physician	6.71 (1.07)	0.98 (0.87-1.09)
Nurse	6.70 (1.14)	0.98 (0.91-1.06)
Technician	6.57 (1.07)	1.03 (0.97-1.10)
Other HCW	6.68 (1.12)	0.98 (0.91-1.06)
Previous COVID-19 infection [Bo, Bs, V]		
No	6.45 (0.95)	1 (Ref)
Yes	7.99 (0.90)	4.10 (2.38-7.07)
Time since vaccination [Bo, Bs]	-	
10-14-day interval	-	0.96 (0.94-0.98)
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)

* Centers included in the analysis are indicated in square brackets: Bo, Bologna, Bs, Brescia, V, Verona CI, confidence interval

CI, confidence interval OR, meta-analysis odds ratio for one standard deviation increase in antibody level, adjusted for sex, age, job title and previous infection, as appropriate; Ref, reference category; SD, standard deviation

† Additionally adjusted for pre-vaccination serology level and time between serology tests, as appropriate

Results of the Paris cohort

Results of the analysis of HCW from the Paris cohorts are reported in **Table 9** (determinants of vaccination) and **Table 10** (determinants of serologic response). Although they were based on small number of subjects, they were broadly consistent with those of the other centers.

Table 9. Determinants of vaccination – Paris cohort

Characteristics	N Vaccinated (%)	OR (95% CI)
Sex		
Men	6 (66.7%)	1 (Ref)
Women	10 (32.3%)	0.38 (0.03 4.23)
Age		
10-yr increase	-	1.71 (0.83 3.52)
Job Title		
Administration and technician	2 (66.7%)	1 (Ref)
Nurse	5 (41.7%)	0.33 (0.02 5.39)
Physician	8 (88.9%)	2.85 (0.09 89.8)
Other HCW	1 (40.0%)	0.02 (0.001 0.56)

CI, confidence interval

OR, odds ratio, adjusted for sex, age, job title Ref, reference category

Table 10. Determinants of serologic response – Paris cohort

Characteristic	N (%)	OR (95% CI)
Sex		
Male	6 (37.5%)	1 (Ref)
Female	10 (62.5%)	1.45 (0.44- 4.74)
Age (10-yr increase)		0.61 (0.34- 1.08)
Job title		
Administration and Technician	2 (12.5%)	1 (Ref)
Physician	8 (50%)	1.81 (0.37- 8.71)
Nurse	5 (31.3%)	1.41 (0.31- 6.42)
Other HCW	1 (6.3%)	1.07 (0.10- 11.56)
Time since last dose (continues)		1.03 (0.98- 1.08)
Vaccine type		
Comirnaty and Spikevax	8 (50%)	1 (Ref)
Vaxzevria	8 (50%)	0.31 (0.08- 1.25)

CI, confidence interval

OR, odds ratio, adjusted for sex, age, job title, time since last dose and vaccine type. Ref, reference category

Conclusions

Older age, employment as physician, nurse or technician and lack of previous SARS-CoV-2 infection were factors associated with vaccination. Specifically, HCW over 40 years appear to be more frequently vaccinated than younger subjects, and workers directly involved in healthcare are more frequently vaccinated than administrative. These differences may be attributable to perceived higher risk of infection in these categories, in fact older people have a higher risk of complications due to COVID-19 and workers dedicated to patient care are more exposed to infection than administrative staff, who have been more frequently employed remotely since the beginning of the pandemic. Perception of risk associated to infection could also explain why subjects who developed the infection, especially shortly before vaccination started, were less likely to be vaccinated.

The overwhelming majority of HCW who underwent vaccination experienced a serologic response. Both qualitative and quantitative analysis showed that women have a better immunological response following vaccination than men. Furthermore, these analyses showed higher serological titres among younger population and in previously infected individuals. Previous COVID 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.

Recommendations

Follow-up analyses of cohorts of HCW included in ORCHESTRA will be important to confirm these results and identify determinants of long-term response to vaccination.