RASSEGNE E ARTICOLI

Immunogenicity and effectiveness of BNT162b2 COVID-19 vaccine in a cohort of healthcare workers in Milan (Lombardy Region, Northern Italy)

Immunogenicità ed efficacia del vaccino anti-COVID-19 BNT162b2 in una coorte di operatori sanitari a Milano

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ABSTRACT

OBJECTIVES: to evaluate immunogenicity and effectiveness of BNT162b2 COVID-19 vaccine in a cohort of healthcare workers (HCWs).

DESIGN: cohort study.

SETTING AND PARTICIPANTS: in a hospital in Milan (Lombardy Region, Northern Italy) HCWs without ("negative cohort") and with ("positive cohort") history of SARS-CoV-2 infection or elevated serum antibody before the vaccination campaign (27.12.2020) were included. Data collection and follow-up covered the period 27.12.2020-13.05.2022.

MAIN OUTCOMES MEASURES: 1. serum anti-spike-1 (anti-S1) antibody levels after vaccination; **2.** vaccine effectiveness (VE) against SARS-CoV-2 infections (either symptomatic or not) in the negative cohort. Data on infections were extracted from multiple sources (laboratory, accident reports, questionnaires). Vaccination was treated as a time-dependent variable. Using unvaccinated person-time as reference, hazard ratios (HR) of infections and 95% confidence intervals (95%CI) were calculated with a Cox regression model adjusted for gender, age, and occupation. VE was calculated as (1 – HR)×100.

RESULTS: 5,596 HCWs were included, 4,771 in the negative and 825 in the positive cohort. In both cohorts, serum anti-S1 antibodies were high one months after the second dose, halved after six months, and returned to high levels after the third dose. In the negative cohort, 1,401 SARS-CoV-2 infections were identified. VE was 70% (95%CI 54-80; 46 infected) in the first four months after the second dose and later declined to 16% (95%CI 0-43; 97 infected). After the third dose, VE increased to 57% (95%CI 35-71; 61 infected) in the first month but rapidly declined over time, particularly after three months (24% in the fourth month and 1% afterwards). The number of infections avoided by vaccination was estimated to be 643 (95%CI 236-1,237).

CONCLUSIONS: in spite of rapidly declining effectiveness, vaccination helped to avoid several hundred infections in the considered hospital.

WHAT IS ALREADY KNOWN

- **BNT162b2 COVID-19 vaccine showed high efficacy and effectiveness in randomised controlled trials, in studies in the general population, and among healthcare workers (HCWs).**
- **Vaccine effectiveness (VE) was shown to decrease with time and against new virus variants.**
- **To date, only one study evaluated VE in HCWs during the Omicron variant epidemic.**

WHAT THIS STUDY ADDS

- **This study covers a period of more than 16 months: VE was high in the four months following the second dose and declined afterwards.**
- **VE raised after the third dose and then declined to low values during the Omicron period.**
- **COVID-19 vaccination was estimated to have prevented hundreds of infections in the considered hospital.**

Keywords: COVID-19, SARS-CoV-2, vaccination, healthcare workers

RIASSUNTO

OBIETTIVI: valutare immunogenicità ed efficacia del vaccino anti-COVID-19 BNT162b2 in una coorte di operatori sanitari (OS).

DISEGNO: studio di coorte.

SETTING E PARTECIPANTI: nell'ospedale Policlinico di Milano sono stati inclusi gli OS senza ("coorte negativa") e con ("coorte positiva") precedente infezione da SARS-CoV-2 o elevati anticorpi prima dell'inizio della campagna vaccinale (27.12.2020). Lo studio ha coperto il periodo 27.12.2020-13.05.2022.

PRINCIPALI MISURE DI OUTCOME: 1. livelli di anticorpi anti-spike-1 (anti-S1); **2.** efficacia vaccinale (EV) contro infezioni (sintomatiche e non) da SARS-CoV-2 nella coorte dei negativi. I dati sulle infezioni sono stati estratti da varie fonti (laboratorio, denunce infortuni, questionari). La vaccinazione è stata trattata come variabile dipendente dal tempo. Utilizzando il tempo-persona non vaccinato come riferimento, sono stati calcolati gli *hazard ratio* (HR) e i relativi intervalli di confidenza al 95% (IC95%) con modello di regressione multivariato di Cox, aggiustato per sesso, età e occupazione. EV è stata calcolata come: (1 – HR)×100.

RISULTATI: sono stati inclusi 5.596 operatori, 4.771 nella coorte dei negativi e 825 in quella dei positivi. in entrambe le coorti, gli anticorpi anti-S1 erano alti un mese dopo la seconda dose, si sono dimezzati sei mesi dopo la seconda dose e sono ritornati a livelli elevati un mese dopo la terza dose. Nella coorte dei negativi, sono state identificate 1.401 infezioni da SARS-CoV-2. EV era pari al 70% (IC95% 54-80; 46 infetti) nei primi quattro mesi dopo la seconda dose e poi scendeva al 16% (IC95% 0-43; 97 infetti). Dopo la terza dose, EV aumentava al 57% (IC95% 35-71, 61 infetti) nel primo mese, successivamente declinava rapidamente nel tempo, in particolare dopo tre mesi (24% nel quarto mese e 1% in seguito). È stato calcolato che il numero di infezioni evitate grazie alla vaccinazione è stato pari a 643 (IC95%CI 236-1.237).

CONCLUSIONI: nonostante l'efficacia rapidamente decrescente, la vaccinazione ha contribuito a evitare centinaia di infezioni nel nostro ospedale.

Parole chiave: COVID-19, SARS-CoV-2, vaccinazione, operatori sanitari

INTRODUCTION

Randomized controlled trials (RCTs) showed high efficacy of several vaccines against Coronavirus disease 19 (COVID-19), including the BNT162b2 mRNA vaccine (Comirnaty, Pfizer-BioNTech), which was found to be 95% protective since 7 days after the second dose.[1] It is important to continue to monitor vaccine effectiveness (VE) in real-world settings. While RCTs showed efficacy against COVID-19, field studies could contribute to verifying VE also against asymptomatic infections from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Moreover, post-marketing studies can inform about duration of VE over time.

Observational studies in several populations confirmed high VE against the original (Wuhan) virus variant, the B.1.1.7 (Alpha), and the B.1.351 (Beta), but they also showed lower VE against the B.1.671.2 (Delta) and B.1.1.529 (Omicron) variants.[2-9]

Studies among healthcare workers (HCWs) found high VE for symptomatic and asymptomatic infections against the first variants and lower VE against the Delta variant.[10-23] To the Authors' knowledge, only one study in New York City evaluated VE in HCWs during the Omicron epidemic (December 2021-January 2022).[24]

The present study aims to evaluate BNT162b2 vaccine immunogenicity and VE in a cohort of HCWs of a large hospital in Milan (Lombardy Region, Northern Italy). The study covered a period of 16.5 months period from the start of the vaccination campaign on 27.12.2020. The predominant SARS-CoV-2 strain up to June 2021 was the Alpha variant (74.9% at national level).[25] Since July 2021, the Delta variant became largely dominant (94.8% at national level, 95.3% in Lombardy).[26] Since December 2021, the prevailing variant became the Omicron.

The Authors recently documented high levels of anti-spike1 (anti-S1) antibodies about one month after the complete vaccination schedule.[27] Here, anti-S1 serum levels were assessed about one and six months after the second dose and after the third dose.

The main objective of this study was to estimate VE against SARS-CoV-2 infections (either symptomatic or asymptomatic) among HCWs never infected (i.e., negative infection history and negative antibody tests) before the start of vaccination campaign ("negative cohort"). In a secondary analysis we also evaluated VE in previously infected HCWs (i.e., positive infection history or positive serology, "positive cohort").

METHODS

A cohort study was performed among workers of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, a large tertiary referral, teaching, and research hospital in Milan by linking demographics data with data on COVID-19 vaccinations, real-time reverse transcription-polymerase chain reaction (RT-PCR) tests performed on nasopharyngeal swabs (NPS) and serology, and COVID-19 health surveillance information (positive tests performed outside the hospital, reports of infections to the Italian National Institute for Insurance against Accidents at Work – Inail). The study covered the period 27.12.2020-13.05.2022 and was approved by the local ethics committee (Milano Area 2, Prot. No. 828_2021). Participants signed an informed consent form.

STUDY POPULATION

The cohort included all the personnel performing healthcare activities (hospital and University physicians, nurses, midwives, healthcare assistants and technicians, and University residents and students) and also clerical and technical workers (hired by the hospital or by the University). All these workers will be loosely referred as HCWs. The following individuals were excluded: never tested with RT-PCR during the vaccination campaign (because

the endpoint was RT-PCR result); without a valid fiscal code (necessary to link the personal data with vaccination records); and with incomplete/inconsistent vaccination dates.

VACCINATION DATA

For HCWs vaccinated within the hospital, information was obtained from a local database. For those vaccinated elsewhere, information was completed by linking the cohort file with a regional vaccination database using fiscal code as a unique identifier. The vaccination campaign started on 27.12.2020 with the BNT162b2 vaccine.

LABORATORY DATA

Data on occurrence of SARS-CoV-2 infections were extracted from the laboratory database at the hospital. HCWs were tested with NPS for different reasons, including contact with individuals with suspected/confirmed infection or symptoms compatible with COVID-19. Only HCWs in selected units (e.g., COVID-19, emergency, pneumology, oncology, transplantation, and dialysis units) were regularly tested every two weeks.

Reverse transcription-polymerase chain reaction (RT-PCR) tests

Two different assays were used on NPS to detect SARS-CoV-2 RNA: Allplex™ SARS-CoV-2 assay (Seegene, South Korea) on the CFX96 (Bio-Rad, USA) and SARS-CoV-2 AMP Kit (Abbott, USA) on Alinity m (Abbott, USA).

SARS CoV-2 variants

SARS CoV-2 variants assessment was performed in Policlinico laboratory on selected positive samples according to national and regional guidelines.[28]

Antigenic tests

In 2021, rapid immunochromatographic assays for qualitative and specific detection of SARS-CoV-2 antigen (Nucleocapsid (N) protein) were introduced (Wondfo 2019-nCoV Antigen Test – Guangzhou Wondfo Biotech Co., Ltd.) and widely used for hospital in- and out-patients, but very rarely used for HCWs until December 2021. They have been shown to have lower sensitivity than RT-PCR, especially for Omicron variants.[29]

SARS-CoV-2 serology before vaccination

In the period 27.04.2020-08.07.2021, a seroprevalence study was performed in HCWs of Policlinico hospital (either vaccinated or not) using a chemiluminescent quantitative assay of serum anti-S1 and anti-S2 specific IgG antibodies against SARS-CoV-2 (LIAISON SARS-CoV-2 S1/S2 IgG test on LIAISON XL, DiaSorin, Italy).[30] Values ≥15 AU/mL were considered positive tests. Afterwards, another assay measuring anti-N antibodies (Elecsys Anti-SARS-COV-2, Roche, Italy) was used. Values ≥1 AU/mL were considered positive tests.

SARS-CoV-2 serology during vaccination campaign

To assess vaccine immunogenicity, anti-S1 antibodies were measured (Elecsys Anti-SARS-COV-2 S, Roche, Italy) in blood samples collected approximately one month after the second dose.[27] Anti-S1 antibodies were then measured again approximately six months after the second dose and after the third dose. The lower limit of quantification (LOQ) was 0.4 U/mL; the upper LOQ was initially 7,500 and later was increased to 12,500 U/mL. Values ≥0.8 U/mL were considered positive tests according to manufacturers instructions.

COVID-19 HEALTH SURVEILLANCE DATA

HCWs with RT-PCR documented infection had to be reported to the hospital COVID-19 health surveillance team, who followed up them until readmission to work (after a negative RT-PCR test or a 21-day isolation). As infection in HCWs was often due to exposure at work, the health surveillance team also monitored the reports to Inail.

At the time of first dose administration, one month after the second dose, and after the third dose, short questionnaires were administered to collect data on clinical history, body mass index (BMI), smoking habits, acute events following previous vaccinations, and previous SARS-CoV-2 infections.[30,31]

All these information sources were exploited to identify infections in subjects who performed NPS outside the hospital.

STATISTICAL ANALYSIS

Chi^2 test was used for comparison of proportions and non-parametric tests (Wilcoxon or Kruskal-Wallis) for continuous data. To analyse anti-S1 levels, multiple random-intercept tobit regression models were fitted on ln-transformed anti-S1 adjusted for gender, age, BMI, and smoking. Results were expressed using "sympercent" (= slope ×100).[32] To analyse the association of gender, age, and occupation with the third dose, a multiple log-binomial regression model was fitted including the three variables.

Vaccine effectiveness (VE)

Two separate analyses of VE were performed: a primary analysis, among HCWs never infected (i.e., negative infection history and negative antibody tests) before the start of vaccination campaign ("negative cohort"); and a secondary analysis in HCWs previously infected (i.e., positive infection history or positive serology, "positive cohort"). The main analysis was performed in analogy with other stud-

ies[13,14,16,18,22] and is motivated by the rarity of re-infections (at least before the advent of Omicron variant).[33-36]
In subjects never infected or infected >90 days before the start of vaccination campaign, the follow-up period started on 27.12.2020. For subjects in the positive cohort infected ≤90 days before 27.12.2020 the follow-up started 90 days after positivity. Vaccination was treated as a time-dependent variable to avoid immortal-time bias.[37,38] Individuals contributed person-days only during periods in which they were tested, i.e., until the date of the first positive RT-PCR or antigenic test or of the last negative RT-PCR or antigenic test.[18] We restricted analyses to the large majority of HCWs vaccinated with BNT162b2. To avoid selection bias,[37,38] for the few vaccinated with other vaccines the follow-up was truncated at the time of first dose. The few subjects with four doses were truncated at the date of the fourth dose.
The endpoint was defined as the first positive RT-PCR or antigenic test after 27.12.2022, either in symptomatic or asymptomatic individuals. In the last months (during the Omicron phase), the number of infections was so high that accurate collection of symptoms in infected HCWs was not feasible. Therefore, analyses of symptomatic infections, which represent the primary outcome of vaccine efficacy in RCTs, could not be performed. Infection rates per 1,000 person-years (PY), then hazard ratios (HR) and 95% confidence intervals (CI) by fitting Cox regression models (time axis: days since 27.12.2020), adjusted for gender, age category (<35, 35-44, 45-54, and 55+ years), and occupation were calculated. VE was calculated as (1 – HR)×100,[39] taking the unvaccinated person-time as reference. VE was set to 0 when HR was ≥1. When VE was ≥30%, the absolute number of avoided cases was calculated as the difference between observed and expected cases (where Expected = Cases/HR). Data management and statistical analyses were performed with Stata 17.[40]

RESULTS

Out of 8,706 workers, 2,925 were excluded, because never tested during the vaccination campaign, 67 (46 students and 15 residents) without a valid fiscal code, and 118 with inconsistent vaccination dates in the regional database, leaving 5,596 individuals in analysis, including 4,771 (85.3%) in the negative and 825 (14.7%) in the positive cohort (277 (33.6%) with a positive infection history, 217 (26.3%) with positive antibody tests, and 331 (40.1%) with both) (Table 1). Subjects in the positive cohort had older age and included a higher proportion of nurses and healthcare assistants.

VACCINATION STATUS

Overall, a total of 5,466 HCWs received a first dose of vaccine (96.5% BNT162b2), 5,425 received the second dose (96.8% BNT162b2), 5,059 received the third dose (90.9% BNT162b2), and only 4 received a fourth dose (75.0% BNT162b2). The earliest of the third dose was on 27.09.2021. In total, 15,594 doses were administered, 15,130 (94.8%) of BNT162b2, 741 (4.6%) of mRNA-1273 (Spikevax, Moderna), and 83 (0.5%) of other three vaccines.
In the negative cohort, 117 (2.5%) had no vaccination records (unvaccinated or vaccinated outside Lombardy) at the end of follow-up, while 4,346 (91.1%) received three doses (Table S1, see online Supplementary Materials). Subjects with three doses were proportionally slightly more among men (p=0.08), in those with 35 years of age (p<0.001), and in physicians and nurses (p<0.001). The findings for age and occupation were confirmed in a multiple log-binomial regression analysis.
In the positive cohort, 13 (1.6%) had no vaccination records and 709 (85.9) were vaccinated with three doses.

VACCINE IMMUNOGENICITY

In the negative cohort, the median serum level of anti-S1 measured about one month (median: 34 days, 15th-75th percentiles: 32-37 days) after the second dose was 1,359 U/mL (Table 2). Only 9 workers had values <lower LOQ and 4.8% of HCWs had values >upper LOQ. About six months (median: 195 days, 15th-75th percentiles: 182-211 days) after the second dose the anti-S1 level halved (median 658 U/mL, 2.5% of HCWs with values >upper LOQ). About three months (median: 93 days, 15th-75th percentiles: 65-110 days) after the third dose the median anti-S1 level increased to 11,737 (66.9% of HCWs with values >upper LOQ).
In the positive cohort, the pattern was similar but starting from much higher values one month after the second dose (62.3% of subjects with antibody levels >upper LOQ).
The patterns were quite similar when the analyses were restricted to subjects with all three anti-S1 determinations performed (results not shown).
In the multivariable analysis, in both cohorts anti-S1 antibodies decreased with increasing age, increased with increasing BMI, and were lower in smokers, while gender had opposite effects in the negative and positive cohorts (Table S2).

SARS-COV-2 VARIANTS

Virus genotyping was performed on 94 samples. Until June 2021, out of 20 samples, the Policlinico laboratory identified 10 Alpha variants (50%), 9 "other" (45.0%), and 1 Delta (5.0%). In the period July-November 2021, out of 36 variants 33 were Delta (91.7%) and 3 Omicron (8.3%). From December 2021 on, out of 38 samples there were 29 Omicron (76.3%), 8 Delta (21.1%), and 1 "other" (2.6%): from mid-December 2021 on, most variants were Omicron (29/31=93.5%).

CHARACTERISTICS	NEGATIVE COHORT		POSITIVE COHORT		WHOLE COHORT	
	N.	%	N.	%	N.	%
ALL SUBJECTS	4,771	100	825	100	5,596	100
GENDER						
Females	3,379	70.8	577	69.9	3,956	70.7
Males	1,392	29.2	248	30.1	1,640	29.3
AGE (YEARS)						
<35	2,324	48.7	306	37.1	2,630	47.0
35-44	774	16.2	141	17.1	915	16.4
45-54	909	19.1	229	27.8	1,138	20.3
55+	764	16.0	149	18.1	913	16.3
OCCUPATION						
Physicians	885	18.5	158	19.2	1,043	18.6
Residents	683	14.3	84	10.2	767	13.7
Nurses	1,048	22.0	271	32.8	1,319	23.6
Mid-wives	105	2.2	22	2.7	127	2.3
Healthcare assistants	293	6.1	85	10.3	378	6.8
Healthcare technicians*	594	12.5	107	13.0	701	12.5
Clerical. technicians	489	10.2	78	9.5	567	10.1
Students	674	14.1	20	2.4	694	12.4

*Includes biologists, radiology and laboratory technicians, psychologists, and other healthcare technicians. / *Include biologi, tecnici di radiologia e laboratorio, psicologi e altri tecnici sanitari.*

Table 1. Characteristics of healthcare workers included in the analysis of BNT162b2 COVID-19 vaccine effectiveness in a hospital in Milan (Lombardy Region, Northern Italy) from 27.12.2020 to 13.05.2022.
Tabella 1. Caratteristiche degli operatori sanitari inclusi nell'analisi di efficacia del vaccino anti-COVID-19 BNT162b2 in un ospedale di Milano dal 27.12.2020 al 13.05.2022.

TIME OF BLOOD SAMPLING	N.	MIN	Q1	MEDIAN	GEOMETRIC MEAN	Q3	N. (%) <LOWER LOQ*	N. (%) >UPPER LOQ*
NEGATIVE COHORT								
1 month after dose 2	3415	<0.4	755	1,359	1,301	2,335	9 (0.2)	164 (4.8)
6 months after dose 2	2644	<0.4	369	658	655	1,125	6 (0.2)	65 (2.5)
3 months after dose 3	1494	26	6,343	11,737	8,120	12,500	0 (0.0)	1,000 (66.9)
POSITIVE COHORT								
1 month after dose 2	655	56	4,200	7,500	4,745	7,500	0 (0.0)	408 (62.3)
6 months after dose 2	558	36	1,016	2,038	2,035	4,543	0 (0.0)	79 (14.2)
3 months after dose 3	316	425	6,503	10,525	8,219	12,500	0 (0.0)	208 (65.8)

LOQ: limit of quantification / *limite di quantificazione*; **Q1:** first quartile / *primo quartile*; **Q3:** third quartile / *terzo quartile*

*Laboratory lower LOQ: 0.4 U/mL; upper LOQ was 7,500 U/mL and later increased to 12,500 U/mL / *Limite inferiore di laboratorio: 0,4 U/mL; il limite superiore era 7.500 U/mL, aumentato poi a 12.5000 U/mL*

Table 2. Anti-S1 antibody levels (U/mL) in healthcare workers included in the analysis of BNT162b2 COVID-19 vaccine effectiveness in a hospital in Milan (Lombardy region, Northern Italy) from 27.12.2020 to 13.05.2022.
Tabella 2. Livelli di anticorpi anti-S1 (U/mL) negli operatori sanitari inclusi nell'analisi di efficacia del vaccino anti-COVID-19 BNT162b2 in un ospedale di Milano dal 27.12.2020 al 13.05.2022.

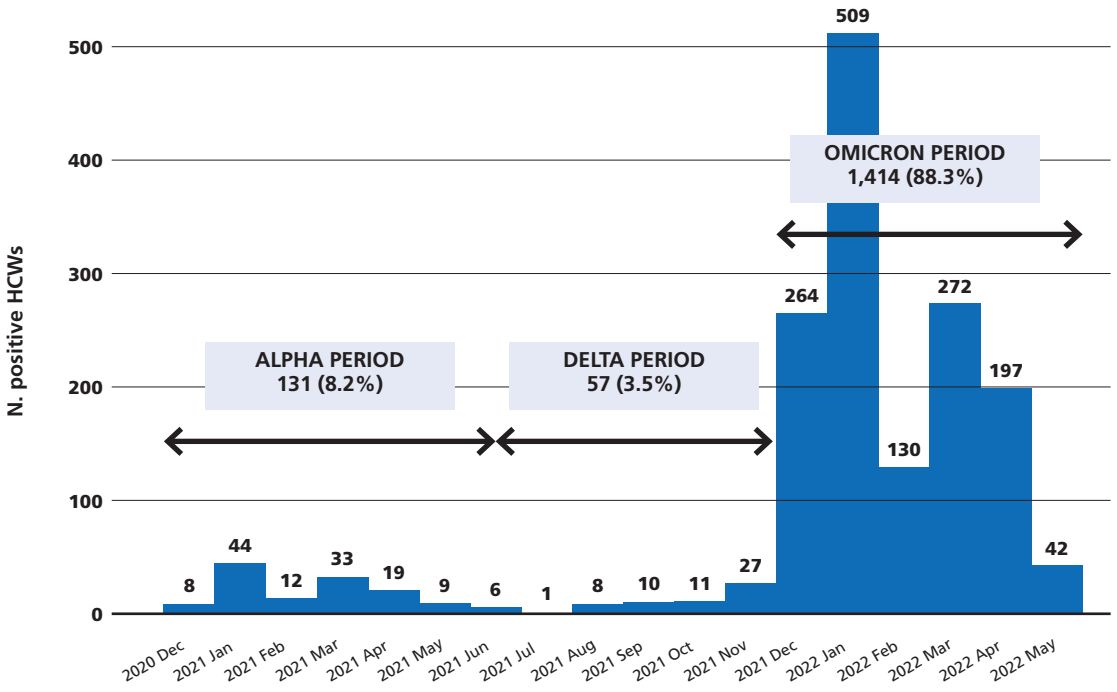

Figure 1. Number of SARS-CoV-2 infected healthcare workers (negative and positive cohorts) included in the analysis of BNT162b2 COVID-19 vaccine effectiveness in a hospital in Milan (Lombardy Region, Northern Italy) from 27.12.2020 to 13.05.2022.
Figura 1. Numero di operatori sanitari con infezione da SARS-CoV-2 (coorti negativa e positiva) inclusi nell'analisi di efficacia del vaccino anti-COVID-19 BNT162b2 in un ospedale di Milano dal 27.12.2020 al 13.05.2022.

VACCINE EFFECTIVENESS

Overall, 1,602 infected HCWs were identified, the large majority (1,414, 88.3%) in the period in which the Omicron variant was prevailing (Figure 1). As of December 2021, all infections were confirmed by RT-PCR. In the (Omicron) period January-May 2022, the proportion of infections confirmed by antigenic test was 26.1%. The proportions of positive antigenic tests were similar in unvaccinated and vaccinated HCWs (21.7% and 26.1%, respectively, P=0.63).

In the negative cohort, 1,401 SARS-CoV-2 infections (Table 3) were identified. Adjusted VE was 64% 14+ days after the first dose, increased to 70% in the first four months after the second dose, and declined to 16% afterwards. VE increased to 57% in the first month after the third dose but then declined over time, in particular after three months. The number of estimated avoided cases was 643 (95%CI 236-1,237).

In the positive cohort, 201 infections were recorded. VE was low (25%) within four months from the second dose and then very high (88%) four months after the second dose, but estimates were quite imprecise. In the first month after the third dose, VE was elevated (71%), then had an irregular pattern and finally declined to 20% after four months. The number of estimated avoided cases was 197 (95%CI 2-780).

DISCUSSION

In the present study covering a period of 16.5 months, good immunogenicity of the BNT162b2 vaccine was found in both cohorts of negative and positive HCWs. However, serum anti-S1 levels were halved after six months, making necessary a third booster dose that brought back anti-S1 to very high levels. Consistently with previous findings of the Authors,[27] antibody levels decreased with increasing age, increased with increasing BMI, and were lower in smokers.

In the large negative cohort, vaccine effectiveness against laboratory confirmed infections was 70% in the four months following the second dose, but declined to 16% afterwards. Effectiveness increased to 57% in the first month after the third dose, remained at levels between 38% and 48% in the following two months, then declined to low values. Notwithstanding the suboptimal and waning effectiveness, vaccination was estimated to have helped to avoid several hundred infections in our hospital.

COMPARISON WITH PUBLISHED RESEARCH

Studies in the general population performed in the first half of 2021 confirmed high vaccine effectiveness (VE) against the original (Wuhan) virus variant (both symptomatic and asymptomatic infections analysed separ-

VACCINATION STATUS	NUMBER OF INFECTIONS	PERSON-YEARS	RATE (PER 1,000 PY)	VE (%)*	95%CI
NEGATIVE COHORT	1,401	4,432.1	316		
Unvaccinated	98	544.5	180	Reference	
Vaccinated with 1 dose					
0-13 days	16	165.6	97	0	0-37
14+ days	7	195.7	36	64	17-84
Vaccinated with 2 doses					
7-119 days	46	1,228.9	37	70	54-80
120+ days	97	1,585.5	61	16	0-43
Vaccinated with 3 doses					
7-29 days	61	184.3	331	57	35-71
30-44 days	149	108.5	1,373	44	21-60
45-59 days	176	91.9	1,916	48	27-62
60-74 days	158	75.8	2,083	41	17-58
75-89 days	96	63.6	1,509	38	11-57
90-119 days	157	101.5	1,547	24	0-47
120+ days	340	86.3	3,939	1	0-32
POSITIVE COHORT	201	763,5	263		
Unvaccinated	8	69.1	116	Reference	
Vaccinated with 1 dose					
0-13 days	2	19.9	101	7	0-83
14+ days	1	59.2	17	91	26-99
Vaccinated with 2 doses					
7-119 days	17	207.9	82	25	0-69
120+ days	9	284.9	32	88	66-96
Vaccinated with 3 doses					
7-29 days	16	33.8	473	71	23-89
30-44 days	32	19.4	1,649	34	0-73
45-59 days	34	15.8	2,157	26	0-70
60-74 days	19	12.5	1,515	48	0-80
75-89 days	10	10.9	920	68	9-89
90-119 days	22	17.4	1,261	53	0-82
120+ days	31	12.7	2,441	20	0-72

95%CI: 95% confidence interval / *intervallo di confidenza al 95%*; **PY:** person-years / *anni-persona*; **VE:** vaccine effectiveness / *efficacia vaccinale*
*VE = (1 – HR)×100, where HR is the adjusted hazard ratio / *VE = (1 – HR)x100, dove HR è l'hazard ratio aggiustato*

Table 3. Incidence rates of SARS-COV-2 infections and BNT162b2 COVID-19 vaccine effectiveness (VE) among healthcare workers of a hospital in Milan (Lombardy Region, Northern Italy) from 27.12.2020 to 13.05.2022.
Tabella 3. Tassi di incidenza di infezioni da SARS-CoV-2 ed efficacia del vaccino anti-COVID-19 BNT162b2 negli operatori sanitari di un ospedale di Milano dal 27.12.2020 al 13.05.2022.

ately),[2] the B.1.1.7 (Alpha), and the B.1.351 (Beta) variants (symptomatic/asymptomatic infections analysed together).[3] Later on, in concomitance with the surge of the B.1.671.2 (Delta) variant, reported VE was much lower, either for symptomatic[5] or for symptomatic/asymptomatic infections analysed together.[4,6] Since in a large study in USA this low VE was observed also against non-Delta variants, the lower VE was interpreted as waning immunity 4-6 months after the second dose.[4] Recently, studies in Italy (symptomatic/asymptomatic infections analysed together),[8] Qatar (symptomatic infections),[7] and UK (symptomatic infections)[9] documented good but rapidly declining VE after the third dose while the B.1.1.529 (Omicron) variant was prevailing.

In a non-systematic literature search, 15 studies on BNT162b2 VE in HCWs (Table S3) were found. All studied found quite high VE in the first months after the second dose. Some studies found decreasing VE with time and/or in concomitance with the Delta epidemics.[11,19,20] Only one study evaluated VE of the third dose during the Omicron surge (33% compared with two doses).[24]

BNT162b2 is an innovative mRNA vaccine which acts through the inoculation of an mRNA sequence carrying the genetic information coding for the antigen, which will be produced via the ribosomal machinery of the host cells.[41] BNT162b2 has been designed to encode the SARS-CoV-2 receptor-binding domain based on the first sequenced SARS-CoV-2 virus strain (Wuhan strain). The present study covered a long period in which new variants emerged and immunity waned. In general, VE after two doses was observed to be similar to that found in studies covering long follow-up times and/or periods in which the Delta variant predominated (Table S3). The present findings on the effectiveness of the third booster dose are in line with a very recent study in the USA.[24] However, that study covered only one month and could not evaluated waning effectiveness, that the Authors documented instead.

STRENGTHS AND LIMITATIONS

To the Authors' knowledge, this is one of the first studies that evaluated VE against Omicron variant in HCWs. Also, several data sources were exploited to provide a comprehensive picture of both vaccine immunogenicity and effectiveness. However, the present study had several limitations. First, it was based on routinely collected data, making linkage errors possible, despite extensive data editing performed over time by crossing different information sources to ensure good quality. Second, demographic files for university residents and students were incomplete (because demographic data is managed by the University, not by Policlinico hospital). Although this fact reduced the size of the available sample, selection biases were not expected to arise from this administrative aspect. In fact, VE after excluding them did not change much (results not shown). Third, some positive RT-PCR tests performed outside Policlinico hospital may have been missed. However, the strict monitoring and surveillance of HCWs make unlikely the loss of information about many assays. Conversely, the number of asymptomatic infections was underestimated, because only HCWs in some departments underwent regular testing. Fourth, in the Omicron period, antigenic tests, which have lower sensitivity, were used to detect infections. However, differential use of these tests was not expected to be used in relation to vaccination status. In fact, the proportion of infections detected with antigenic tests were similar in unvaccinated and vaccinated HCWs. Fifth, it cannot be excluded that (probably a few) vaccinations performed outside Lombardy may have been missed, because the Authors do not have access to national vaccination database. Sixth, and probably the most important, in the last months (December 2021 to May 2022) the number of infections was so high that accurate collection of symptoms in infected HCWs was not feasible. Therefore, this information could not be exploited in statistical analyses. This limitation is shared with most of the other studies among HCWs (Table S3).

A final caution note in interpreting results: in this study (like in any observational study), any difference in infection rates between vaccinated and unvaccinated was attributed to the vaccination itself. However, beyond gender, age, and occupation (which were adjusted for), other relevant behavioural factors could have played a role, with possibly different impact across the various epidemic periods.

CONCLUSIONS

Good immunogenicity of the BNT162b2 vaccine in health-care workers is confirmed. However, serum anti-S1 levels decrease rapidly with time. In the large negative cohort, vaccine effectiveness was good in the four months following the second dose, but declined to very low levels afterwards. Effectiveness was raised again after the third dose, but declined to low values in a few months. In spite of the waning antibody titres and lower effectiveness, vaccination was estimated to have helped to avoid several hundred infections in Policlinico hospital and therefore significantly affected the number of individuals actively spreading the virus, thus reducing the circulation of SARS-CoV-2 and limiting the impact of the increased background incidence on the hospital staff. Since vaccine effectiveness against infections is far from optimal, it is important to maintain other preventive and protection measures.

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