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Effect of the 2020/21 season influenza vaccine on SARS-CoV-2 infection in a cohort of Italian healthcare workers

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ABSTRACT

Objectives: Healthcare workers (HCWs) are a priority group for seasonal influenza vaccination (SIV). The 2020/21 SIV campaign was conducted during the second wave of the COVID-19 pandemic. Vaccines, including SIV, may exert non-specific protective effects on other infectious diseases which may be ascribable to the concept of trained immunity. The aim of this study was to explore the association between 2020/21 SIV and SARS-CoV-2 positivity in a cohort of Italian HCWs.
Methods: In this observational study, a cohort of HCWs employed by a large (ca 5000 employees) referral tertiary acute-care university hospital was followed up retrospectively until the start of the COVID-19 vaccination campaign. The independent variable of interest was the 2020/21 SIV uptake. Both egg-based and cell culture-derived quadrivalent SIVs were available. The study outcome was the incidence of new SARS-CoV-2 infections, as determined by RT-PCR. Multivariable Cox regression was applied in order to discern the association of interest.
Results: The final cohort consisted of 2561 HCWs who underwent ≥1 RT-PCR test and accounted for a total of 94,445 person-days of observation. SIV uptake was 35.6%. During the study period, a total of 290 new SARS-CoV-2 infections occurred. The incidence of new SARS-CoV-2 was 1.62 (95% CI: 1.22–2.10) and 3.91 (95% CI: 3.43–4.45) per 1000 person-days in vaccinated and non-vaccinated HCWs, respectively, with an adjusted non-proportional hazard ratio of 0.37 (95% CI: 0.22–0.62). *E*-values suggested that unmeasured confounding was unlikely to explain the association.
Conclusions: A lower risk of SARS-CoV-2 infection was observed among SIV recipients.

1. Introduction

Seasonal influenza vaccination (SIV) is a key public health measure that can reduce the socioeconomic burden of the disease, and several priority population groups for annual immunization are well recognized [1,2]. Among these, healthcare workers (HCWs) occupy a prominent place; indeed, almost all European jurisdictions recommend free-of-charge annual influenza vaccination for all HCWs [3]. The rationale behind this recommendation is to protect both HCWs themselves and their patients and may be seen as a "*core … safety practice with which noncompliance should not be tolerated*" [4]. Nevertheless, the coverage rate is below 30% in most instances [5].

In the northern hemisphere, the 2020/21 seasonal SIV campaign was carried out during an unprecedented period characterized by the circulation of SARS-CoV-2. Indeed, the number of newly diagnosed SARS-CoV-2 cases increased sharply from October 2020 onwards [6]. In the fear of the possible co-circulation of both influenza viruses and SARS-CoV-2 (with objective difficulties in making a clinical differential diagnosis) and the associated increased pressure on healthcare systems, some important policy changes were implemented. For instance, in Italy the free-of-charge influenza vaccine offer for older adults was lowered from ≥65 to

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≥60 years [7], while some Regions introduced mandatory 2020/21 influenza vaccination for HCWs and/or institutionalized subjects [8,9]. These policy changes seem to have been fruitful, with the first Italian estimates [10] indicating a significant increase in vaccine uptake among HCWs.

It has recently been observed [11,12] that several routinely administered vaccines, including SIV, may exert non-specific protective effects on SARS-CoV-2-related outcomes. The biological plausibility of such non-specific effects may be ascribable to the concept of trained immunity, whereby "*the long-term functional reprogramming of innate immune cells is evoked by exogenous or endogenous insults and leads to an altered response towards a second challenge after the return to a non-activated state*" [13].

The first ecological studies conducted in Italy [14,15] observed a significant negative relationship between regional SIV coverage rates and various SARS-CoV-2-attributable endpoints. However, such study designs are prone to the phenomenon of ecological fallacy [16] and have been criticized [17]. Subsequent retrospective studies conducted during the 2019/20 season in Italy and abroad have produced controversial results. For instance, in Reggio Emilia (Northern Italy), no significant association between SIV and hospitalizations or deaths was found. However, when the analysis was restricted to the elderly, SIV recipients showed a 34% and 30% risk reduction for hospital admission and mortality, respectively [18]. While in Brindisi (Southern Italy) no relationship between SIV uptake and hospitalization/death was established [19], in Latium (Central Italy) a significantly lower risk of death [adjusted odds ratio (OR) 0.20 (95% CI: 0.08–0.51)] in patients immunized with SIV was reported [20]. With regard to HCWs, Martínez-Baz et al. [21] did not find any association between the 2019/20 SIV uptake and positivity on molecular or rapid antibody tests for SARS-CoV-2. By contrast, Conlon et al. [22] and Wilcox et al. [23] documented a decreased risk of SARS-CoV-2-related outcomes in patients immunized with 2019/20 SIV. Finally, a recent systematic review and meta-analysis [24] showed a 14% [OR 0.86 (95% CI: 0.81–0.91)] reduction in the odds of acquiring infection, while the outcomes of hospitalization, intensive care unit admission and mortality were not statistically significant. It seems that the observed between-study heterogeneity is driven by several factors, including study design, population and COVID-19-related outcomes considered.

The objective of this study was to investigate the association between 2020/21 SIV and SARS-CoV-2 positivity in a cohort of Italian HCWs. On the basis of the available systematic evidence [24], we hypothesized some non-specific protective effect of SIV on reverse transcription polymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infection.

2. Methods

2.1. Study design and setting

This study adopted a retrospective cohort design and was conducted at San Martino Policlinico Hospital (Genoa, Italy), which is a referral tertiary acute-care university hospital that employs approximately 5000 people.

The intervention of interest was seasonal 2020/21 SIV, which started on 12th October 2020 and lasted until mid-January 2021, although most (>90%) doses were administered in October and November 2020. Both standard-dose cell culture-derived quadrivalent (QIVc; Flucelvax, Seqirus, US) and standard-dose egg-based quadrivalent (QIVe; Vaxigrip Tetra, Sanofi Pasteur, France) SIVs were used. However, in October 2020, only QIVc was available. Vaccination was actively recommended for all employees, was offered free-of-charge and was performed at the Hygiene Unit of San Martino Policlinico Hospital.

The study time window was fixed to two months (26th October–27th December 2020). The study start date on 26th October was determined *a priori* on considering both a lag of 2 weeks, which is necessary in order to achieve protective immunity [25], and the beginning of the SIV campaign on 12th October. The study finished on 27th December, since an extensive internal anti-SARS-CoV-2 vaccination campaign began the following day. In summary, for the vaccinated cohort, the index date was defined as the date of SIV receipt plus 14 days apart, while for unvaccinated counterparts the index date was 26th October 2020. The study event date was the first positive or the last negative SARS-CoV-2 RT-PCR test [22] performed within the time window (see also below).

Regarding reporting quality, the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist [26] was adopted (Appendix 1).

The study protocol was approved by the Ethics Committee of the Liguria Region (Genoa, Italy) (n° 508/2021). All HCWs provided their informed consent prior to laboratory diagnostic procedures and/or influenza vaccination.

2.2. Study outcome and eligibility criteria

The study outcome was the incidence of new SARS-CoV-2 infections, as determined by RT-PCR, which is considered the "gold standard" for COVID-19 laboratory diagnostics [27]. All RT-PCR tests were performed at the regional reference laboratory for COVID-19 diagnoses, located at the Hygiene Unit of San Martino Policlinico Hospital, within eight hours of the arrival of naso-/oropharyngeal specimens. RT-PCR was performed by means of a validated extraction-free unheated method [28] on Nimbus IVD (Seegene Inc., Republic of Korea) using the Allplex SARS-COV-2 assay kit (Seegene Inc., Republic of Korea). Briefly, a sample input volume of 5 μl was diluted 1:3 with molecular-grade water and used directly for RT-PCR. Amplification was performed at 50 °C for 20 min, followed by 95 °C for 15 min and 45 cycles at 95 °C for 10 s, 60 °C for 15 s with first acquisition, and 72 °C for 10 s with second acquisition on a CFX96 thermal cycler (Bio-Rad Laboratories, US). This multiplex assay simultaneously detects three different genes, targeting the nucleoprotein region, the RNA-dependent RNA-polymerase/spike region, and the envelope region. Specimens yielding a cycle threshold (Ct) value <40 for at least two genes were considered positive [28].

All HCWs employed by the hospital (ca 5000) during the study period were potentially eligible. To be included in the study, HCWs had to have at least one SARS-CoV-2 RT-PCR test performed during the study period. By contrast, subjects with a documented positive RT-PCR test before 26th October were excluded. Given that most HCWs in the study underwent multiple molecular tests, in positive subjects we considered the date of their first positive RT-PCR test as the event date; in negative subjects, the date of the last negative test was the event date [22].

2.3. Predictor of interest

The independent variable of interest was the 2020/21 SIV status. SIV exposure was ascertained by linking vaccination cards to signed informed consent forms. HCWs for whom vaccination records and informed consent forms were not available were considered non-vaccinated.

2.4. Potential confounders

The following variables were considered as potential confounders: age, sex, nationality (Italian vs foreign), frequency of

SARS-CoV-2 RT-PCR testing and week of the last RT-PCR test performed. The frequency of RT-PCR testing was thought to mitigate the effect of indication bias, since vaccinated subjects may be more exposed to respiratory pathogens. For each HCW, we therefore performed a count of RT-PCR tests available from the beginning of systematic testing (the first available test was performed on 7th March 2020) to the last available test performed by 27th December 2020. For positive subjects, we counted only the number of tests performed before their first positive test. This was deemed necessary since, following a positive result, an HCW undergoes follow-up testing, usually until two consecutive negative results are registered; these tests do not therefore reflect the risk of exposure. We finally adjusted for the calendar week of the last RT-PCR test, in order to account for the changing epidemiology of SARS-CoV-2.

2.5. Data analysis

Descriptive statistics were used to represent vaccinated and non-vaccinated sub-cohorts from the point of view of the independent variables considered. Specifically, categorical data were expressed as proportions with 95% confidence intervals (CIs) and compared by means of the chi-square test with Yates's correction. Continuous variables were expressed as means and standard deviations (SDs) and compared by means of the *t* test. The dataset had no missing data.

The effect size on the association between SARS-CoV-2 RT-PCR test results and SIV status was expressed as a crude risk ratio (RR) and absolute risk reduction (ARR). Crude (HR) and adjusted hazard ratios (aHR) were designed to be calculated by applying the uni- and multivariable Cox proportional hazards models, respectively. However, if the proportional hazards assumption was not met, we applied weighted Cox regression modelling, which estimates average effects of non-proportional hazards [29]. The continuous variable of age and the ordinal variable of the number of RT-PCR tests performed were treated as such, since various categorization rules applied did not improve the model fit (as measured by the Akaike information criterion). In summary, multivariable model 1 was adjusted for age, sex, nationality, and SARS-CoV-2 RT-PCR testing frequency, while model 2 was further adjusted for the calendar week of testing. Each model was tested for significant pairwise interactions.

To ascertain the robustness of the base-case results, two kinds of sensitivity analysis were performed. In the first, the effect of unmeasured confounding was quantified by means E-value for the point estimate and for the limit of the 95% CI closest to zero [30]. In the second, given that only QIVc was used at the beginning of the SIV campaign, we limited the analysis to QIVc recipients only.

All analyses were performed in R stats packages, version 4.0.3 [31].

3. Results

3.1. Characteristics of the cohort

During the study period, a total of 3231 HCWs underwent at least one RT-PCR test. Of these, 29 (0.9%) had a previously documented positive RT-PCR test and were excluded. Another 641 (19.8%) vaccinated individuals had the last available RT-PCR test within the first two weeks following SIV, and were also excluded. Therefore, the final cohort consisted of 2561 HCWs, who contributed a total of 94,445 person-day observations. These HCWs performed a total of 13,335 SARS-CoV-2 RT-PCR tests. The mean age of HCWs was 46.8 (SD: 11.5) years, 69.6% (95% CI: 67.7–71.3%) were females and 3.9% (95% CI: 3.2–4.7%) were of non-Italian origin. SIV was administered to 35.6% (95% CI: 33.7–37.5%) of HCWs; QIVc was the more prevalent [62.3% (95% CI: 59.1–65.5%)].

3.2. Association between 2020/21 influenza vaccination and SARS-CoV-2 positivity

During the study period, a total of 290 new SARS-CoV-2 infections occurred. SARS-CoV-2 positivity rate was significantly lower in vaccinated [56/911; 6.1% (95% CI: 4.7–7.9%)] HCWs than in non-vaccinated [234/1650; 14.2% (95% CI: 12.5–16.0%)] subjects with a RR of 0.43 (95% CI: 0.33–0.57)] and the ARR of 8.0% (95% CI: 5.7–10.3%). Table 1 stratifies vaccinated and non-vaccinated HCWs by the independent variables considered. Vaccinated and non-vaccinated cohorts differed from the point of view of nationality and SARS-CoV-2 testing frequency. Specifically, HCWs of foreign origin had a significantly lower vaccination coverage rate than native Italians, with an RR of 0.60 (95% CI: 0.38–0.93). Moreover, on average, vaccinated individuals underwent more SARS-CoV-2 RT-PCR tests ($p < 0.001$) than non-vaccinated HCWs (Table 1).

Overall, the incidence of new SARS-CoV-2 first positive tests was 1.62 (95% CI: 1.22–2.10) and 3.91 (95% CI: 3.43–4.45) per 1000 person-days in vaccinated and non-vaccinated HCWs, respectively, with an HR of 0.41 (95% CI: 0.30–0.55). As shown in Table 2, the adjusted weighted Cox model 1 showed a similar non-proportional HR (npHR) [0.37 (95% CI: 0.22–0.62)] of the effect of SIV on SARS-CoV-2 first positivity. The observed effect size may be explained by an unmeasured confounder that was associated with SIV and/or a SARS-CoV-2 positive test by an RR of at least 4.85-fold each, above and beyond the measured confounders. The *E*-value for the lower 95% CI limit (0.22) was 2.61. A further adjustment for the week of the last RT-PCR test (model 2) revealed an even greater effect size [npHR = 0.17 (95% CI: 0.09–0.34)]. The corresponding *E*-value was 11.24 (5.33). Moreover, each additional molecular test was associated with a 9% increase in testing positive. No significant interaction terms were found. In the second sensitivity analysis, when only QIVc was considered, no major changes occurred (Appendix 2).

4. Discussion

This study demonstrated that 2020/21 SIV was associated with a lower incidence of SARS-CoV-2 infection in a relatively large cohort of HCWs. We will now discuss our principal findings from the point of view of recent insights into the biological plausibility of this non-specific SIV effect, compare our results with the available evidence and make some policy suggestions.

Non-specific vaccine-induced protective effects have been demonstrated for BCG (Bacillus Calmette-Guérin), measles, oral polio and, more recently, SIV, and may be mediated through both innate and adaptive immune-related mechanisms [32]. The trained immunity hypothesis postulates that cells of the innate immune system may acquire memory characteristics after transient initial

Table 1
Comparison between vaccinated and non-vaccinated healthcare workers.

Variable	Vaccinated (n = 911)	Non-vaccinated (n = 1650)	p-value
Sex, % (95% CI) female	67.7 (64.6–70.8)	70.6 (68.3–72.8)	0.14
Age (years), mean (SD)	46.3 (11.5)	47.1 (11.5)	0.09
Foreign origin, % (95% CI)	2.7 (1.8–4.0)	4.6 (3.6–5.7)	0.027
SARS-CoV-2 testing frequency, mean (SD)	6.0 (3.0)	4.8 (2.9)	<0.001

CI = confidence interval.

Table 2
Multivariable weighted Cox hazard models of the association between 2020/21 influenza vaccination and SARS-CoV-2 first positive test (N = 2561).

Variable	Level	Model 1		Model 2[1]	
		npHR (95% CI)	p-value	npHR (95% CI)	p-value
Influenza vaccine	No	Ref	–	Ref	–
	Yes	0.37 (0.22–0.62)	<0.001	0.17 (0.09–0.34)	<0.001
Sex	Male	Ref	–	Ref	–
	Female	0.79 (0.55–1.16)	0.23	0.76 (0.52–1.11)	0.15
Age, years	1-year increase	1.00 (0.99–1.01)	0.91	0.99 (0.98–1.00)	0.17
Nationality	Italian	Ref	–	Ref	–
	Immigrant	1.38 (0.69–2.75)	0.37	1.39 (0.70–2.74)	0.34
SARS-CoV-2 testing frequency	1-unit increase	0.98 (0.93–1.04)	0.48	1.09 (1.02–1.16)	0.013

CI = confidence interval; npHR = non-proportional hazard ratio.
[1] Adjusted for the week of the last SARS-CoV-2 RT-PCR testing.

stimulation, which results in an enhanced response following secondary stimulus. Trained immunity is associated with non-specific increased responsiveness (including heterologous protection induced by vaccines) and is mediated through several metabolic and epigenetic reprogramming mechanisms [13,33]. Another possible explanation is that of cross-reactivity [32]. Indeed, a *post-hoc* analysis of a randomized controlled trial [34] showed that children vaccinated with 2008/09 SIV developed higher IgG response against some seasonal coronaviruses than children immunized with a hepatitis A vaccine. Murugavelu et al. [35] recently demonstrated that antibodies elicited against hemagglutinin of the influenza virus cross-reacted with the spike protein of SARS-CoV-2; however, these cross-reactive antibodies were not neutralizing.

The induction of trained immunity by QIVe was recently proved [36] in a well-established *in vitro* model. Specifically, stimulation of peripheral blood mononuclear cells with QIVe and BCG vaccines increased the production of cytokines in a dose-dependent manner. Re-stimulation of these cells with a heat-inactivated SARS-CoV-2 strain induced a higher production of interleukin-1 receptor antagonist (IL-1RA) [36]. A recent Italian study [37] conducted among HCWs (N = 710) vaccinated with 2 doses of the BNT162b2 vaccine showed that subjects previously immunized with QIVc and pneumococcal vaccines or with QIVc alone had a 58% (p = 0.01) and 42% (p = 0.07) increase in microneutralization titers to SARS-CoV-2, respectively, compared with those who did not receive any vaccine. On the other hand, no significant difference in anti-spike and interferon-γ responses were observed [37]. Finally, in a cohort of HCWs, Pallikkuth et al. [38] found that A(H1N1) antigen-specific CD4 cells were present in 92% and 76% of SARS-CoV-2-positive and -negative subjects, respectively. The A(H1N1) CD4 response also showed a strong positive correlation with SARS-CoV-2-specific CD4 T cells [38].

As mentioned earlier, a recent systematic review and meta-analysis [24] of the association between SIV and confirmed SARS-CoV-2 obtained pooled odds ratios (ORs) of 0.86 (95% CI: 0.81–0.91) and 0.86 (95% CI: 0.79–0.94) in fixed- and random-effects models, respectively. In single studies, ORs ranged from 0.41 [39] to 1.03 [21]. A study conducted by Bellingheri et al. [39] on a sample (N = 3520) of Italian HCWs reported a large adjusted OR of 0.41, which is similar to the effect size observed in our study. However, their result was imprecise, with a wide 95% CI (0.07–2.39). Moreover, as their study was cross-sectional in nature, no causality could be inferred [39]. The observed heterogeneity in terms of effect sizes may also be driven by study population (e.g., age structure), period and location (e.g., time since vaccination and influenza season) and appropriate adjustment for indication bias. Regarding the latter, we showed that the frequency of SARS-CoV-2 testing was directly associated with a higher positivity rate. The nature of this association may be at least two-fold. First, higher exposure to the virus is associated with higher positivity [40]. At the same time, a higher perceived risk of infections among HCWs themselves and/or their patients is a positive predictor of preventive and control attitudes and behaviors [41], such as vaccination or testing. Second, a higher number of tests performed may simply increase the probability of detecting new infections in asymptomatic individuals. The lack of adjustment for indication bias leads to underestimation of vaccine effectiveness [42]. This fact may also explain the relatively higher effect size observed in our study than in some previously published trials.

Despite well-recognized benefits and recommendations [1,7,43], SIV coverage in HCWs remains low in Italy and several other jurisdictions [5]. Concerns regarding adverse events, perceived low effectiveness and beliefs that influenza is not a serious illness constitute the main barriers to the uptake of SIV [44]. Acceptance of SIV and COVID-19 vaccines is interrelated. On the one hand, previous experience of SIV increases the likelihood of receiving COVID-19 vaccines; on the other hand, the uptake of SIV may increase as a consequence of the ongoing COVID-19 pandemic [45,46]. We believe that integrating messages regarding non-specific vaccine effects into a promotional communication mix could help to convince some hesitant HCWs, thereby increasing vaccine uptake.

This study may have some important limitations. First, we cannot rule out residual confounding. *In primis*, it was not possible to adjust for comorbidities that are known to affect SIV effectiveness [42]. However, we believe that this shortcoming has a limited impact on the study findings for several reasons. The *E*-values showed that, in order to refute the observed effect of SIV on SARS-CoV-2 positivity, the unmeasured confounders need to have very large effect sizes. Furthermore, as the study population was composed of non-elderly adult workers, the prevalence of serious morbidities may be assumed to be low. In addition, most chronic conditions were probably age-related, and controlling for age may therefore partly address this issue. Finally, the lack of adjustment for comorbidities usually underestimates the protective effect of SIV [42]. The second limitation is the short follow-up period. A longer observation time was not feasible, owing to the inference of the COVID-19 vaccination campaign. This choice is also justified, since SIV effectiveness is highest during the first 15–90 days following vaccination [47]. Future studies will, however, have to take into account the COVID-19 vaccination patterns and possible interaction effects between influenza and COVID-19 vaccines, since most HCWs have already been immunized. Finally, our study is among the first conducted during the 2020/21 season; it is therefore unclear whether our findings can be generalized to previous seasons and other settings.

5. Conclusion

SIV may exert non-specific protective effects against SARS-CoV-2 infection. It appears that the most biologically

plausible mechanism of this effect is the reprogramming of innate immune cells and the subsequent immune cascade (i.e., the "trained immunity" hypothesis). The non-specific effects of SIV may be used in HCW-targeted health promotion interventions in order to increase vaccination uptake.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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