UNIVERSITY OF GENOA

School of Pharmaceutical Sciences
Department of Health Sciences (DiSSaL)

FACULTY OF MEDICINE AND SURGERY

An in-depth epidemiological analysis of the association between seasonal influenza vaccination and COVID-19 related outcomes

Rapporteur: Prof. *Giancarlo Icardi*
Co-rapporteur: Dr. *Alexander Domnich*

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ABSTRACT

In the context of the ongoing COVID-19 pandemic, whit many countries still struggling because of SARS-CoV-2 vaccine shortage, it is imperative to provide individuals with all preventive measures available, able to reduce the pressure on healthcare systems. An increasing number of epidemiological studies suggest that some routinely administered vaccines (e.g., influenza) may exercise non-specific effects on COVID-19-related outcomes. The aim of this thesis was to conduct an in-depth analysis of the association between seasonal influenza vaccination and outcomes attributable to SARS-CoV-2. This objective was achieved in two consecutive steps: (i) a systematic review and meta-analysis of the previously published reports on this topic and (ii) a retrospective observational study on a cohort of healthcare workers (HCWs).

The systematic review identified a total of 33 primary research reports. The meta-analysis of 8 studies (167,579 subjects) showed that compared with non-vaccinated individuals, those immunized against seasonal influenza had a 19% [pooled adjusted odds ratio 0.81 (95% CI: 0.70–0.94)] risk reduction of testing positive for SARS-CoV-2. Influenza vaccination was also protective against different clinical outcomes (e.g., COVID-19 related hospitalization, intensive care unit admission, mortality), although the pooled effect size did not always reach an alpha <0.05.

We then conducted a retrospective cohort study composed of HCWs of the San Martino Policlinico Hospital (Genoa). In particular, we analyzed the incidence of newly diagnosed RT-PCR-confirmed SARS-CoV-2 infections in healthcare workers with regards to 2020/21 influenza vaccinal status. Following the application of inclusion and exclusion criteria, a total of 2,561 individuals that contributed to 94,438 person-day observations were analyzed. A total of 290 new positive cases were identified. The incidence of SARS-CoV-2 was 1.62 (95% CI: 1.22–2.10) and 3.91 (95% CI: 3.43–4.45) per 1,000 person-days in vaccinated and non-vaccinated HCWs (P<0.001). The multivariable Cox's proportional hazard model adjusted for potential confounders showed generally a significant protective effect of influenza vaccination.

In conclusion, our findings suggests that influenza vaccination is associated with a lower risk of COVID-19 related outcomes and underline, particularly while the global response to COVID-19 pandemic is still suboptimal, the importance of promote and carry out effective influenza vaccination campaigns, in order to reduce the clinical and socioeconomic burden of respiratory infections.

RIASSUNTO

La pandemia da COVID-19 è ancora in corso e molti sono i paesi in difficoltà a causa della carenza di vaccini contro SARS-CoV-2. In questo contesto, è imperativo fornire alla popolazione generale tutti gli strumenti di prevenzione a disposizione in grado di ridurre la pressione sui sistemi sanitari. Un crescente numero di studi epidemiologici suggerisce che alcuni vaccini somministrati di routine (ad esempio, il vaccino antinfluenzale) possano esercitare effetti protettivi non specifici sugli esiti clinici associati alla patologia da COVID-19. L'obiettivo principale della presente tesi è quello di condurre un'approfondita analisi epidemiologica dell'associazione tra la vaccinazione contro l'influenza stagionale e gli esiti da SARS-CoV-2. Questo obiettivo è stato raggiunto in due fasi successive: (i) una revisione sistematica e meta-analisi degli studi precedentemente pubblicati su questo argomento; (ii) uno studio osservazionale e retrospettivo condotto su una coorte di operatori sanitari.

La revisione sistematica ha identificato un totale di 33 studi di ricerca primaria. La meta-analisi di 8 di questi studi (167.579 soggetti) ha mostrato che, rispetto agli individui non vaccinati, quelli immunizzati contro l'influenza stagionale presentavano una riduzione del rischio di positività per SARS-CoV-2 del 19% [odds ratio aggiustato aggregato 0,81 (95% CI: 0,70-0,94)]. Inoltre, sebbene la dimensione dell'effetto aggregata non abbia sempre raggiunto un alfa <0,05, la vaccinazione antinfluenzale è risulta protettiva anche rispetto a diversi esiti clinici (ad esempio, ospedalizzazione correlata a COVID-19, ricovero in unità di terapia intensiva e mortalità).

Abbiamo quindi condotto uno studio retrospettivo su una coorte di operatori sanitari italiani operanti presso l'Ospedale Policlinico San Martino (Genova, Italia). In particolare, abbiamo analizzato l'incidenza delle infezioni da SARS-CoV-2, confermate da RT-PCR, in relazione allo stato di immunizzazione contro l'influenzale stagionale 2020/21 negli operatori sanitari. A seguito dell'applicazione dei criteri di inclusione ed esclusione, sono stati analizzati un totale di 2.561 individui che hanno contribuito a 94.438 osservazioni giorno-persona e sono stati identificati un totale di 290 nuovi casi positivi. L'incidenza di SARS-CoV-2 è risultata essere, rispettivamente, 1,62 (95% CI: 1,22-2,10) e 3,91 (95% CI: 3,43-4,45) per 1.000 giorni-persona negli operatori sanitari vaccinati e non vaccinati (P<0,001). In generale, il modello di rischio proporzionale multivariabile di Cox, aggiustato per potenziali fattori confondenti, ha mostrato un significativo effetto protettivo della vaccinazione antinfluenzale.

In conclusione, i risultati suggeriscono che la vaccinazione antinfluenzale è associata a un minor rischio di esiti clinici da COVID-19 e sottolineano, in particolare, mentre la risposta globale alla pandemia di COVID-19 è ancora insufficiente, l'importanza di condurre e implementare efficaci campagne di vaccinazione antinfluenzale, al fine di ridurre il peso sanitario e socioeconomico delle infezioni respiratorie.

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CHAPTER 1

BACKGROUND

1.1. **Influenza: A historical companion**

Ancient Greek texts dating back as far as the fifth century B.C. [Potter et al. 2001] are evidence of the fact that influenza viruses have long accompanied mankind. Similar writings dating back to 1510 [Morens et al. 2010] serve as the earliest records of what we can now assume to be the first influenza pandemic, arisen in Asia and then spread to North Africa and Europe. To date, influenza virus represents a major infectious agent that causes a significant socio-economic burden worldwide [CDC 2019].

Influenza virus is an airborne, highly contagious, enveloped and single-stranded ribonucleic acid (RNA) virus, that belongs to the family *Orthomyxoviridae*. The antigenic specificity of the ribonucleotide nucleoproteins (RNPs), of which the viral RNA is composed, distinguishes four types of influenza viruses: A, B, C and D. Particularly, there are eight gene segments encased in 10 or 11 proteins respectively in influenza virus types A and B. Each gene segment codes for some key proteins: nucleoprotein (NP), polymerase A (PA), polymerase B1 (PB1), polymerase B2 (PB2), hemagglutinin (HA), neuraminidase (NA), matrix proteins 1 (M1), matrix proteins 2 (M2) and non-structural (NS) proteins [Wright et al. 2001; Su et al. 2017; Cox et al. 2004; Bouvier and Palese 2008; Wang J et al. 2016].

While the role of type D influenza virus, which has been identified only a few years ago, is unclear in the context of human pathology, types A, B and C of influenza virus can affect humans [Wright et al. 2001; Su et al. 2017; Trombetta et al. 2020]. Influenza virus types A and B are the major protagonists of human disease and can cause both epidemics (both types A and B) and pandemics (only type A). By contrast, the disease caused by virus type C usually causes mild cold-like symptoms, thus it is considered to be of limited public health importance [Wright et al. 2001; Gubareva et al. 2000;].

Of critical importance for influenza virulence are the glycoproteins hemagglutinin (HA or H) and neuraminidase (NA or N). In particular, HA binds sialic acid on the surface of the sialylated respiratory cells causing membrane fusion and allowing the virus to enter the host cell; NA cleaves the bonds between newly replicated virions to

sialic acid allowing for movement of the virus and for the infection to spread [Gubareva et al. 2000; Petrova and Russell 2018].

Different HA and NA subtypes are used to identify influenza A virus. In particular, at least 18 HA and 11 NA subtypes have been identified to date, although the subtypes H1N1 and H3N2 represents the most common ones detected nowadays [Petrova and Russell 2018; Tong et al. 2013]. By contrast, influenza B viruses are classified into two antigenically distinct lineages instead of subtypes. These are called Victoria and Yamagata and have both been circulating for about last 40 years. Moreover, both influenza A and B can be classified into specific clades and sub-clades (also called groups and sub-groups) [Rota et al. 1990; Biere et al. 2010; CDC 2018].

As briefly mentioned, influenza also infects a variety of animals. Influenza A is able to infect different species of poultry, pigs, bats, dogs, seals and horses. Influenza B has also been found in seals, and influenza C has been found in pigs [CDC 2018]. Although these influenza strains are usually species-specific (due to the ability of HA to bind to different sialic acid receptors on respiratory tract epithelial cells), mutations of the HA could create new strains with the potential to spread from animals to humans. This is, for example, the case of the avian subtype A/H5N1, for which the vast majority of cases have been acquired from direct contact with live poultry. While it is known that A/H5N1 can occasionally infect humans but it is not normally transmitted from human to human, A/H1N1pdm09 influenza subtype (usually referred to as swine flu), that during the 2009-2010 outbreak killed around 3900 people only in the US, can be spread from an infected person to other humans [CDC 2009; WHO 2016a].

From an epidemiological point of view, it is important to underline that both influenza types A and B mutate continuously. These mutations occur through the phenomena of antigenic drift – which consists in minor point mutations that tend to affect the surface glycoproteins HA and/or NA, enabling the virus to evade the annually acquired immunity in humans, by changing its antigenicity – and shift – a less frequent but major change in which two or more strains of the virus combine to form a novel virus subtype [Drake et al. 1993; Wright et al. 2001; Petrova and Russell 2018].

The antigenic drift phenomenon, that cause seasonal influenza epidemics, is present in both A and B influenza types. Antigenic shift is instead pathognomonic to the virus type A that has, in fact, a rate of spontaneous mutations 300-times higher than other microbes and may be at the root of a pandemic [Wright et al. 2001; Paules and Subbarao 2017]. Three deadly influenza pandemics have taken place during the last century: the 1918 H1N1 influenza pandemic (Spanish flu), which is estimated to have caused 30-40 million deaths worldwide, mostly among people of 15-35 years of age; the 1957 H2N2 influenza pandemic (Asian flu) with an estimated number of deaths of 1-2 million deaths worldwide, and the 1968 H3N2 influenza pandemic (Hong Kong flu) that presumably caused about 0.7-1 million deaths worldwide [Nguyen 2021].

The World Health Organization (WHO) estimates that influenza affects 5–10% of adults and 20–30% of children causing 3-5 million cases of severe disease and 250-500,000 deaths each year [WHO 2012]. The US Centers for Disease Control and Prevention (CDC) estimates of annual influenza-associated deaths, for the period between 1976 and 2007, ranged from 3,000 to 49,000 [CDC 2020a]. In Europe, seasonal influenza causes on average a burden of 81.8/100,000 disability-adjusted life years (DALYs) [Cassini et al. 2018]. Moreover, in Italy only, seasonal influenza causes up to 25,000 deaths, with an estimated economic burden of € 1,356,000,000 on average per year [Lai et al. 2011; Rosano et al. 2019].

International guidelines have identified several target groups for annual influenza vaccination. For instance, in 2009 the European Council, following the recommendations of the European Centre for Disease Prevention and Control (ECDC) and the WHO, recommended that influenza vaccination coverage in all risk groups (older adults; people ≥ 6 months of age with chronic medical conditions; pregnant women; and children < 5 years, particularly children < 2 years of age) should reach at least 75% vaccine uptake in all EU countries [Council of the European Union 2009; ECDC 2015; Nicoli et al. 2008; Resolution WHA56 2019]. European influenza vaccination programs, however, present marked variability between countries due to the differences in the EU recommendation adoption and reimbursement policies. In Italy, for instance, influenza vaccination is

recommended, and its fully reimbursed, for the following principal risk groups: (i) older adults aged ≥ 60/65 years; (ii) people ≥ 6 months up to 64 years of age affected by a predefined list of medical conditions including, for example, chronic respiratory and cardiovascular pathologies, immunodeficiency, etc.; (iii) professionals employed in public services of primary interest, such as healthcare professionals, police officers, firefighters, etc.; (iv) professionals that may have close contact with animals that may represent sources of influenza viruses such as farmers, butchers, veterinarians, and (v) other risk categories [Italian Ministry of Health 2020]. In the US, the CDC guidelines recommend, by contrast, the universal vaccination for all subjects aged ≥6 months who do not have contraindications [Grohskopf et al. 2020].

Even if the routine annual vaccination strategy is the most effective public health tool available that can reduce the burden of influenza, the efficacy/effectiveness of the currently available influenza vaccines is considered suboptimal and fluctuates significantly from year to year. This observation has been supported by three Cochrane reviews conducted with the aim of establishing the efficacy/effectiveness of influenza vaccines in children, adults and the elderly. In particular, it has been found that the pooled absolute efficacy of the inactivated vaccine versus placebo was 64% [95% confidence interval (CI): 52–72%] in children [Jefferson et al. 2018], 59% (95% CI: 53–64%) in adults [Demicheli et al. 2018a] and 58% (95% CI: 34–73%) in the elderly [Demicheli et al. 2018b].

Even if the efficacy of the currently available influenza vaccines is still suboptimal, it should be considered that influenza is a highly contagious annually occurring disease that affects up to 20% of the population each season, often requires hospitalization and it is responsible for a large number of deaths every year. For these reasons, the public health and economic benefits of influenza vaccination campaigns are noteworthy [Gasparini et al. 2002; de Waure et al. 2012]. Despite this, influenza vaccination coverage rates in Europe are still far below the Council of the European Union established goals of 75% for all risk groups [Council of the European Union 2009; ECDC 2015].

1.2. **A brief overview of the COVID-19 pandemic**

COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become pandemic since its first outbreak as a cluster of pneumonia cases in Wuhan, China, at the end of 2019. From the time of its identification, globally, over 160 million confirmed cases of COVID-19 and 3 million related deaths have been reported [WHO 2021a; WHO 2021b].

The cumulative incidence of COVID-19 is constantly increasing. An up-to-date interactive map of confirmed cases throughout the world, created by Johns Hopkins University & Medicine, can be found at https://coronavirus.jhu.edu/map.html [Coronavirus resource center 2020]. In addition, it is likely that the official reports underestimate the overall burden of COVID-19. European and American surveys on seroprevalence have in fact suggested that the rate of prior exposure to SARS-CoV-2, reflected by seropositivity, is about 10 times the reported incidence [Stringhini et al. 2020; CDC 2020b; Havers et al. 2020].

SARS-CoV-2 is the seventh coronavirus known to infect humans [Andersen, 2020]. These are enveloped positive-stranded RNA viruses whose name originates from their characteristic crown-like aspect in electron micrographs (Figure 1.2.2) [Masters 2013].

They are further classified into four genera: alpha, beta, gamma, and delta coronaviruses, among which the human coronaviruses (HCoVs) are in the genera alpha (HCoV-229E and HCoV-NL63) and beta (HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV and SARS-CoV-2) [ICTV 2015]. Hence, SARS-CoV-2 is a betacoronavirus of the same subgenus as the virus causing the Severe Acute Respiratory Syndrome (SARS) and it is related - although more distantly - to the Middle East respiratory syndrome (MERS) virus [Gorbalenya et al. 2020; Lu R et al. 2020].

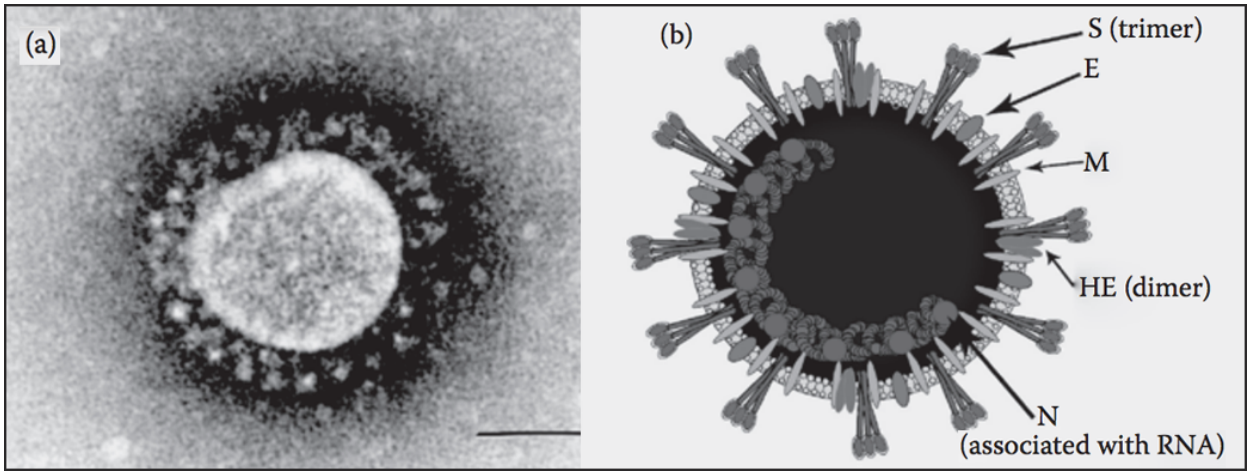


Figure 1.2 - Overview of the coronaviruses: electron microscopic appearance (a; bar = 100 nm) and schematic diagram of the viral particle (b).
Adapted by: Desforges M, Favreau DJ, Brison É, Desjardins J, Meessen-Pinard M, JacomyH, et al. Human Coronaviruses: Respiratory pathogens revisited as infectiousneuroinvasive, neurotropic, and neurovirulent agents. 2013

Coronaviruses have a genome with a length of 27-32 kilobases (kb). This is, in fact, the largest known viral RNA and it encodes for four or five structural proteins: the spike (S) protein, the membrane (M) protein, the nucleocapsid protein (N), the hemagglutinin-esterase glycoprotein (HE) - which is only found in the betacoronaviruses HCoV-OC43 and HKU1 -, and the small envelope (E) protein.

The characteristic spikes that form the coronavirus "crown" consists of the S proteins that protrude through the viral envelope. The S protein possesses the major antigens able to stimulate neutralizing antibodies and it represents an important target for cytotoxic lymphocytes. It also carries out a crucial role by facilitating receptor binding and fusion with the host cell membrane [Enjuanes et al. 1995]. The host receptor that mediates SARS-CoV-2 cell entry is the angiotensin-converting enzyme 2 (ACE2) (the same as for SARS-CoV), alongside the cellular protease TMPRSS2, that also play a role [Hoffmann et al. 2020].

Results from the SARS-CoV-2 viral genome sequencing showed that it shares the 79.5% of sequences with SARS-CoV and it is even more closely related (96% identical sequences) to bat coronavirus genome. Since the virus was first identified in Wuhan,

China, in persons exposed to seafood or wet markets, bats appear likely to be the primary source. However, whether the virus is transmitted directly from bats or through an intermediate host is unknown [Perlman et al. 2020; Zhou P et al. 2020].

Direct person-to-person respiratory transmission represents the main mode of SARS-CoV-2 transmission. It mainly occurs through close contact (within approximately two meters) via respiratory particles, secretions (through speaking, coughing, or sneezing), through handshakes (if a person's hand is contaminated by these secretions) and by touching contaminated surfaces. This last modality, however, is not considered to be a major route of transmission, in fact studies aiming to find the virus in specimens collected in health care facilities have led to contradictory results [Meyerowitz et al. 2021; Zhou J et al. 2020; Santarpia et al. 2020].

Reports of outbreaks in restaurants and public transport have pointed out that airborne transmission in closed and poorly ventilated spaces can also occur at longer distances [Lu J et al. 2002; Hamner et al. 2020; Shen et al. 2020]. These findings are consistent with studies that, employing specific imaging methodologies, have found aerosolized respiratory droplets to reach distances beyond two meters [Bahl et al. 2020; Bourouiba et al. 2020; Stadnytskyi et al. 2020]. SARS-CoV-2 RNA has also been found in ventilation systems and in air samples of hospital rooms of COVID-19 patients, endorsing this hypothesis [Zhou J et al. 2020; Santarpia et al. 2020; Liu et al. 2020]. This evidence explains why households [Madewell et al. 2020], hospitals, long-term care facilities [Wang D et al. 2020]; McMichael, 2020], homeless shelters [Baggett et al. 2020], penitentiaries [Barnert et al. 2020], and student dorms [Wilson et al. 2020], in which prolonged exposure in an enclosed space is likely, are the places in which most of the secondary infections have been reported.

In summary, the risk of transmission depends on various factors: the type and duration of contact, the use of personal protective equipment (PPE) and other preventive measures (physical distancing, hand/surface hygiene), individual factors such as the amount of virus particles present in respiratory secretions [Cevik et al. 2020a].

In regards to this last point, studies have found that viral RNA levels in respiratory specimens are the highest during early stage disease which, as a consequence, is when the infected individuals are more likely to be contagious [He et al. 2020].

In particular, He *et al.* found that infectiousness starts ≃2.3 days prior to symptom onset, peaks ≃0.7 days before symptom onset, and tends to decline after 7 days [He et al. 2020]. The duration of viral RNA shedding is variable as well: detection in respiratory specimens is possible, in regard to the median, up to ≃18 days after the onset of symptoms. However, in some cases, viral RNA is present in the respiratory tract even after several months following the initial infection, especially in immunocompromised patients [Fontana et al. 2020].

In addition, transmission from asymptomatic or pre-symptomatic individuals has been ascertained as well: a CDC study using a decision analytical model estimated that 59% of transmission could be attributed to individuals without symptoms (35% pre-symptomatic, 24% asymptomatic) [Johansson et al. 2021]. On the other hand, it must also be considered that the detection of viral RNA does not necessarily mean that infectious virus is present, and there is a threshold of viral RNA level (reverse-transcription polymerase chain reaction (RT-PCR) cycle threshold (Ct), ranging between <24 and ≤32) below which transmission is unlikely [Bullard et al. 2020; Basile et al. 2020].

Once inhaled, SARS-CoV-2 virus binds to epithelial cells in the nasal cavity and starts replicating. At this stage, there is local propagation of the virus and a limited innate immune response. Although the viral load may be low, these individuals are infectious and the virus can be detected by nasal swabs. Subsequently, the virus propagates and migrates down the respiratory tract along the conducting airways, and a more robust innate immune response is triggered. At this time, the disease clinically manifests itself [Mason et al. 2020; Cevik et al. 2020a].

After viral entry, the initial inflammatory response attracts virus-specific T cells to the site of infection where, for about 80% of the infected patients - which will develop a mild disease, mostly restricted to the upper and conducting airways - the infected cells are eliminated before the virus spreads. On the other hand, about 20% of the

infected patients will progress and develop pulmonary infiltrates and some of them will develop very severe respiratory disease. Evidences show that, in patients who develop severe disease, an aberrant host immune response leads to the development of bilateral diffuse alveolar damage. These pathological findings, characterized by hyaline-membrane formation and interstitial mononuclear inflammatory infiltrates, are consistent with the lung pathology seen in MERS and SARS disease [Mason et al. 2020; Cevik et al. 2020b]

In Italy, during the first wave of the pandemic, 12% of all detected COVID-19 cases were admitted to the intensive care unit, with an estimated case fatality rate reaching 7.2% during March 2020 [Grasselli et al. 2020; Onder et al. 2020]. By contrast, during the same period, the estimated case fatality rate in South Korea was 0.9% [KCDA 2020]. However, these differences narrow considerably after age standardization [Sudharsanan et al. 2020] and the current case fatality rate is, worldwide, about 2.3% [McIntosh 2021]. Noticeably, the proportion of critical cases is higher among hospitalized patients [McIntosh 2021]. In this regards, a study conducted at the New York City Langone Health, reported that, of 2741 patients who were hospitalized for COVID-19, 24% died or were discharged to hospice and, of the 647 patients who received invasive mechanical ventilation, 60% died by the end of the study [Petrilli et al. 2020]. Nonetheless, over the course of the pandemic, also the in-hospital case fatality rates have declined [Horwitz et al. 2021].

Following the infection with SARS-CoV-2, the majority of patients who have recovered develop specific antibodies and cell-mediated responses, that generally last for several month. The antibody neutralizing activity, associated with protection from SARS-CoV-2 reinfection, is maintained for up to 6-8 months following the infection [Dan et al. 2021]. Although the overall short-term risk of infection is lower among previously infected individuals and evidences show that those who present repeated positive PCR tests are more likely to have ongoing viral RNA shedding rather than a reinfection [KDCA 2020], reinfection is a possible occurrence, also because the ability of these protective effects to last over time is still unknown. For this reason, to mitigate the effects

of COVID-19 pandemic on public health, economy and society, vaccines are considered the most promising approach for flattening the contagion curve.

Being urgently needed, COVID-19 vaccine development has worldwide accelerated the traditional steps and most of the currently (as of June 2021) have been only "provisionally" authorized on the condition of emergency. By the end of 2020 over 40 candidate vaccines were in human trials. A regularly updated list of candidate vaccines under evaluation can be found at:
https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines
[WHO 2021c].

The spike protein, already studied in previous trials, not fully pursued, for SARS-CoV and MERS-CoV vaccines development [Graham et al. 2013], is the main target for COVID-19 vaccine development [Krammer et al. 2020].

Various approaches have been employed to develop the existing COVID-19 vaccines: microRNA (mRNA), inactivated or live attenuated viruses, recombinant proteins, vectors, vaccines, etc.

mRNA vaccines, that represent an entirely new technology, were among the first vaccines authorized against SARS-CoV-2. These vaccines are created *in vitro* and once administered trigger an immune response due to the translation of the contained mRNA into a protein with antigenic power. Also because of the fact that this is a new approach, mRNA vaccines may present some shortcomings: for example, vaccines must be maintained at very low temperatures inside of cold and ultracold freezers. A feature that also have an impact on the distribution process [Edwards 2020]. BNT162b2 (Comirnaty®, Pfizer/BioNTech) and mRNA-1273 (Moderna COVOD-19 vaccine) mRNA vaccines are now available in Italy.

Currently in use are also Ad26.COV2.S (Johnson&Johnson vaccine) and ChAdOx1 nCoV-19/AZD1222 (Vaxzevria®, AstraZeneca). These are called viral vector vaccines because they employ a modified version of a different virus (a replication-incompetent adenovirus 26 and a replication-incompetent chimpanzee adenovirus, respectively) that

expresses the spike protein and, acting as a vector, trigger an immune response [CDC 2021a; Edwards 2020].

Among the vast amount of candidate vaccines, Comirnaty, Moderna, Vaxzevria and Janssen are authorized for use in the European Union and CVnCoV, NVX-CoV2373 and Sputnik V (Gam-COVID-Vac) are currently under rolling review. Their main features are described in **Table 1.2.**

Table 1.2. - COVID-19 vaccines currently in use / under review by EMA.

Name	Company/ developer	Platform	Efficacy against symptomatic COVID-19	Doses and intended interval	Storage requirements
Comirnaty BNT162b2	Pfizer/BioNTech	mRNA	95% (Phase III); 89,5% against B1.1.7 (UK) variant (Phase III); 75% against B1.351 (South Africa) variant (Phase III);	2 doses 21 days apart	-80°C: 6 months; +2-8°C: 5 days; 25°C: 2 hours
Moderna mRNA-1273	Moderna	mRNA	94% (Phase III)	2 doses 28 days apart	-20°C: 7 months; +2-8°C: 30 days; +25°C: 12 hours
Vaxzevria	Oxford, AstraZeneca	Viral vector	76% (Phase III); 10% against B1.351 (South Africa) variant;	2 doses 12 weeks apart	+2-8°C: 6 months; +25°C: 6 hours
Janssen JNJ-78436735 /Ad26.COV2.5	Johnson&Johnson	Viral vector	67% (Phase III); 64% against B1.351 (South Africa) variant; 68% against P.1 (Brazil) variant;	1 dose	-20°C: 2 years +2-8°C: 3 months
NVX-CoV2373	Novavax	Recombinant protein	89,7% (Phase III); 86,3% against B1.1.7 (UK) variant (Phase III); 48,6% against B1.351 (South Africa) variant (Phase IIb);	2 doses 21 days apart	-20°C: 2 years +2-8°C: 6 months
Gam-COVID-Vac (Sputnik V)	Gamaleya Institute	Viral vector	91% (Phase III)	2 doses 21 days apart	20°C: 2 years +2-8°C: 6 months
CVnCoV	Curevac	mRNA	Data not available (Ongoing Phase III)	2 doses 28 days apart	Data not available

Source: Frusone F., H3_Surgical_Team, h3-surgical-team.com (Accessed on 15 May 2021).

The approved vaccines are recommended for every individual older than 16/18 years of age and agreements for pediatric investigation plans have been settled [EMA 2021].

Following EMA authorizations, the Italian Medicines Agency (AIFA) recommended the same vaccines for every individual older than 18 (16 for Comirnaty) years of age, with the exception of Vaxzevria and Janssen for which, as a consequence of "*the finding of an association between the vaccines and very rare cases of thromboembolism, even severe, in unusual locations and associated with thrombocytopenia*", their preferential use was recommended in people over the age of 60, "*in which the association with the thrombotic events described has not been found*" [Italian Ministry of Health 2021 a and b]. These recommendations may, however, change after the publication of this thesis.

The percentage of people who need to be immune in order to achieve herd immunity, which is achieved when a large part of the population of an area is immune to a specific disease, stopping its transmission, varies with each disease [WHO 2020a].

The proportion of the population that must be vaccinated against COVID-19 to begin inducing herd immunity is yet not known. However, because the R0 for COVID-19, also called basic reproduction number (which is the expected number of cases directly generated by one case in a population where all individuals are susceptible to infection), is between 2 and 3, it is estimated that at least 50%-70% of the population would need to be resistant before infection rates start to go down [Baylis et al. 2020; Aschwanden et al. 2021]. This is the reason for which the continuation of worldwide vaccination campaigns is crucial in the effort towards ending the pandemic.

1.3. **COVID-19 and influenza: A clinical comparison.**

As SARS-CoV-2 is continuing to spread around the world, comparisons have been drawn to other respiratory diseases, such as influenza. Both diseases are caused by respiratory viruses, yet there are important differences between them and how they spread [WHO 2020b].

SARS-CoV-2 and influenza share a similar disease presentation with a spectrum of infection that ranges from asymptomatic to mild/severe symptomatic and death. Moreover, both viruses are transmitted by contact, droplets and fomites (objects or materials which are likely to carry infection) and are, as a result, preventable trough the same public health measures such as hand hygiene and social distancing [WHO 2020d].

An important difference between the two viruses is in the speed of transmission. The median incubation period (number of days between the infection and the appearance of symptoms) after infection is 1 to 4 days for influenza and 2 to 14 days for SARS-CoV-2. This means that it can take longer for the infection to become symptomatic and people can be contagious, without realizing it, for a longer time. [WHO 2020b; CDC 2021b]. The basic reproduction number (the expected number of cases directly generated by one case in a population where all individuals are susceptible to infection) – is about 2,6 (1,5-3,5) for COVID-19 virus, and 1,3 for Flu. Furthermore, COVID-19 is more contagious among certain populations and age groups than flu [Cricelli et al. 2020].

Pregnant women, elderly, those with underlying chronic medical conditions and those who are immunosuppressed are at higher risk for both severe influenza and COVID-19 [CDC 2021b]. However, the in-hospital fatality rate associated with COVID-19 is higher than that for influenza. The data we have so far has indicated that the case fatality rate (the number of reported deaths divided by the reported cases) is about 2.3%. For seasonal influenza, mortality is usually well below 0.1%. However, it must be considered that the COVID-19 mortality rate (the estimated mortality rate among all individuals with infection) is estimated to be considerably lower (0.5-1%). Moreover, "*mortality is to a large extent determined by access to and quality of health care*" [WHO 2020b; McIntosh 2021]. **Table 1.3.1** reports an overview over the major differences between the two viral infection clinical presentations.

Table 1.3 - Comparison between clinical features of COVID-19 and influenza.

Clinical findings	**Influenza**	COVID-19
Epidemic or large outbreak	Usually	Often
The patient is a child	Often	Rarely
Less than two years old	Often	Rarely
Fever (>38°C)	Rarely	Often
Severe illness	Rarely	Often
Illness is recurrent or >= 3 weeks	Never	Rarely
Compromised host	Rarely	Often
Skin and soft tissue – rash, wound, lesion, IV device	Never	Rarely
Diffuse or multifocal rash	Never	Rarely
Localized or unifocal rash	Never	Rarely
Lesion(s) limited to lower extremity(ies)	Never	Rarely
Neurological – headache, meningitis, etc	Usually	Rarely
Ophthalmological	Often	Rarely
Ears, nose, throat and oral cavity	Often	Rarely
Musculoskeletal – muscle, bone and joint	Usually	Often
Exposure – animal, food, sex, blood products	Often	Rarely
Conjunctivitis	Often	Rarely
Keratitis	Never	Rarely
Uveitis or retinitis	Never	Rarely
Loss of vision	Never	Rarely
Photophobia	Rarely	Never
Sore or inflamed pharynx or larynx	Often	Rarely
Stomatitis, gingivitis, glossitis, caries, oral ulcer(s)	Never	Rarely
Rhinitis, rhinorrhea or sneezing	Often	Rarely
Epistaxis	Rarely	Never
Cough	Usually	Often
Pneumonia or lung infiltrate	Rarely	Usually
Lung abscess, cavity, mass, nodule, cyst or granuloma	Never	Rarely
Pericarditis (established or suspected)	Never	Rarely
Pancreatitis	Never	Rarely
Macules and/or papules	Never	Rarely
Vesicles or bullae	Never	Rarely
Urticaria	Never	Rarely
Hemorrhagic or purpuric rash	Never	Rarely

Erythema multiforme	Rarely	Never
Headache	Usually	Rarely
Coma	Rarely	Never
Seizures	Rarely	Never
Paresthesia or neuropathy	Never	Rarely
Back pain	Often	Rarely
Myalgia; or muscular mass or swelling	Usually	Rarely
Neutrophilia	Often	Rarely
Lymphocytosis	Rarely	Never
Thrombocytopenia	Rarely	Often
Eosinophilia	Never	Rarely
CSF pleocytosis: neutrophiles predominate	Never	Rarely
Hepatic dysfunction	Never	Often
Renal dysfunction	Never	Rarely
Proteinuria	Never	Rarely
Bird contact	Rarely	Never
Other mammal contact	Never	Rarely
Diabetes mellitus	Rarely	Often
Never - not reported; **Rarely** - reported in 0.01 - 19% of cases; **Often** - reported in 20 - 79% of cases; **Usually** - reported in 80 - 99% of cases; **Always** - reported in all cases.		

Source: GIDEON Informatics, Inc. www.gideononline.com.

WHO recommendations for testing suspected active SARS-CoV-2 infections include, wherever possible, nucleic acid amplification tests (NAATs) employing real-time reverse-transcription polymerase chain reaction (rRT-PCR) or alternative amplification/detection methods, such as transcription loop-mediated isothermal amplification (RT-LAMP). Rapid diagnostic tests that detect the presence of SARS CoV-2 viral proteins (antigens) in respiratory tract specimens, can also be employed [WHO 2020c].

For a patient with Acute Respiratory Illness (ARI) symptoms, with or without fever, the CDC recommends influenza and SARS-CoV-2 testing by employing a panel of multiplex nucleic acid assays for Influenza A/B/SARS-CoV-2. This tool is also helpful in the suspicion of co-infections [CDC 2020c].

In sum, the current approach for the management of the COVID-19 pandemic evolves as rapidly as clinical data emerge and, while scientific research worldwide struggles to find effective treatments and vaccines for COVID-19, public health measures (quarantine, social distancing, use of personal protective equipment (PPE) remain of critical importance in order to reduce the morbidity and mortality of this disease.

1.4. **A possible cross-protective immunity.**

Among public health measures able to reduce COVID-19 related complications and deaths, some are taken in order to reduce the rate of respiratory comorbidities in high-risk populations [Zanettini 2021]. Particularly, since seasonal respiratory viral co-infections, such as influenza A and B, have been reported in COVID-19 patients [Xing, Quansheng and Li 2020], influenza vaccination campaigns have been enforced worldwide.

Notably, preliminary studies have suggested some protection against SARS-CoV-2 to be conferred from vaccination to other pathogens such as *M. tuberculosis* and influenza [Mosaddeghi et al. 2020]. However, first studies on the association between seasonal influenza vaccination and SARS-CoV-2-attributable endpoints, conducted in different settings, have produced contrasting results. Ecological studies conducted in Italy [Amato et al. 2020; Marín-Hernández et al. 2021] have reported a significant negative relationship between regional influenza vaccination coverage rates and various SARS-CoV-2 related outcomes. Martínez-Baz *et al.* [Martínez-Baz et al. 2020] have not found any association between the 2019/20 Influenza vaccination and SARS-CoV-2 positivity rate in a cohort of HCWs. By contrast, Conlon *et al.* [Conlon et al. 2021] and Wilcox *et al.* [Wilcox et al. 2021] have found a decreased risk of SARS-CoV-2-related outcomes in patients immunized with 2019/20 Influenza vaccine.

The biological plausibility of an association between influenza vaccine and SARS-CoV-2 susceptibility, has also been explored. Some evidences, in fact, underline a

possible cross-reactivity between influenza and coronaviruses. In order to understand this concept, it is helpful to bear in mind their structures. Both viruses possess well-distinguished surface proteins. Indeed, SARS-CoV-2 is a beta-coronavirus covered in spike protein that, binding to cell receptor angiotensin-converting enzyme 2 (ACE2), facilitate invasion of host cells. Influenza is an *Orthomyxovirus* that relies on the collaborative functions of 2 viral surface proteins, HA and NA to enter and exit the host cells, for which the receptor is sialic acid [American Society for Microbiology, 2020].

Two coronaviruses of the same genus (beta) as SARS-CoV-2 (HCoV-OC43 and HKU1) share a similar surface protein with influenza viruses. This is called Hemagglutinin esterase (HE) [McIntosh, 2009]. HEs are a family of viral envelope glycoproteins that mediate reversible attachment to O-acetylated sialic acids. Prior studies that found that HE genes of coronaviruses present sequence homology with influenza C HE glycoprotein [Luytjes 1988]. More recently, it has been demonstrated that that their fusion and R domains are similar in structure and influenza C HE fusion protein HEF1 and coronaviruses HE share 30% identity [Zeng 2008]. These evidences suggest that that coronaviruses HE may have arisen from an influenza C-like HE fusion protein (HEF) [Zeng 2008]. As a consequence of a possible early recombination between the two viruses, it is plausible that influenza infection or vaccination may generate a certain level of immunity against SARS-CoV-2 as well.

An alternative hypothesis backing up the plausibility of influenza vaccine-induced immunity against SARS-CoV-2 involves the phenomena of the so-called "Bystander effect" of trained immunity. According to this concept, following an exogenous or endogenous insult, a "*long-term functional reprogramming of innate immune cells*" would onset causing, after the return to a non-activated state, an altered response towards a second insult [Netea et al., 2020]. In a nutshell, influenza infection or vaccination would generate sustained immunity, that overall enhances the local lung immune system response, as the result of cellular interactions occurring without antigen recognition [Salem 2020]. This would also explain why the rate of SARS-CoV-2 in the pediatric population, that usually catches flu more than adults, is low [Salem 2020, Kumar 2017].

1.5. **Objectives**

Motivated by the above-described observations on the biological plausibility of the influenza vaccine-induced immunity against SARS-CoV-2, we have performed an in-depth analysis of the available scientific literature, on this propose.

In order to substantiate this hypothesis with the available local data, we then carried out a retrospective cohort study at San Martino Policlinic Hospital (Genoa, Italy), investigating the association between 2020/21 season influenza vaccination and SARS-CoV-2 positivity rate in a cohort of healthcare workers (HCWs).

CHAPTER 2

PROTECTIVE EFFECT OF SEASONAL INFLUENZA VACCINATION ON COVID-19 RELATED OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS

2.1 **Background and Rationale**

As a consequence of the second wave of the COVID-19 pandemic taking place in the middle of influenza season, there is a growing interest in exploring the association between seasonal influenza vaccination and SARS-CoV-2-attributable endpoints. However, first studies on this regard, conducted in different settings, have produced controversial results [Amato et al., 2020; Marín-Hernández et al., 2021, Martínez-Baz et al., 2020, Conlon et al., 2021, Wilcox et al., 2021].

These, together with the biological plausibility of influenza vaccine-induced immunity against SARS-CoV-2 [Zeng et al., 2008, Netea et al., 2020, Salem et al., 2020], led us to perform a systematic review and meta-analysis of the previously published literature on this topic.

2.2. **Methods**

2.2.1 Eligibility criteria

This systematic review has adopted the guidelines for reporting systematic reviews and meta-analysis PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) [The PRISMA 2020 statement].

The methodology of the systematic research was conceived, using the PICOS model (P - Population, I - Intervention, C - Comparators, O - Outcome; S - Study design):

P: Any

I: Seasonal influenza vaccination with any available vaccine

C: Non-vaccination

O: Any SARS-CoV2 related outcome

S: Randomized controlled clinical trials (RCTs) and observational studies of any design (cross sectional, case-control, retrospective and prospective cohort, including ecological studies).

2.2.2. Search strategy

A comprehensive search was carried out in OVID on 9/03/2021 and the following databases were used:

- Ovid MEDLINE® ALL;
- Biological Abstracts;
- CAB Abstracts (including Global Health).

The bibliographic search, illustrated in **Table 2.2.1**, was conducted using the following string: (Influenza Vaccines OR influenza vaccin* OR ((influenza OR flu*) adj5 (vaccin* OR immune* OR inoculat*))) OR ((Influenza OR Influenza,Human) AND (Vaccines OR vaccin* OR Viral Vaccines OR immuni* OR Vaccines, Subunit OR Vaccines, Synthetic)) AND ((sars-cov2 OR covid-19 or 2019-ncov or 2019nconv) OR (sars-cov2 or covid-19)). In order to increase the sensitivity, no other filter (e.g., language, time) has been applied.

The systematic search was then updated in PubMed Central® (PMC) on 12/04/2021 using the following string: ("influenza"[Title/Abstract] AND vaccin*[Title/Abstract]) AND ("covid 19"[Title/Abstract] OR "sars cov 2"[Title/Abstract]).

On the same day, the so-called “grey literature” was also searched via OpenGrey.

2.2.3. Selection of studies and data extraction

The automatic search identified 1,072 manuscripts. Of these 354 were excluded through automatic duplicate removal. Once duplicates had been removed, titles and abstracts obtained from the automatic search were screened.

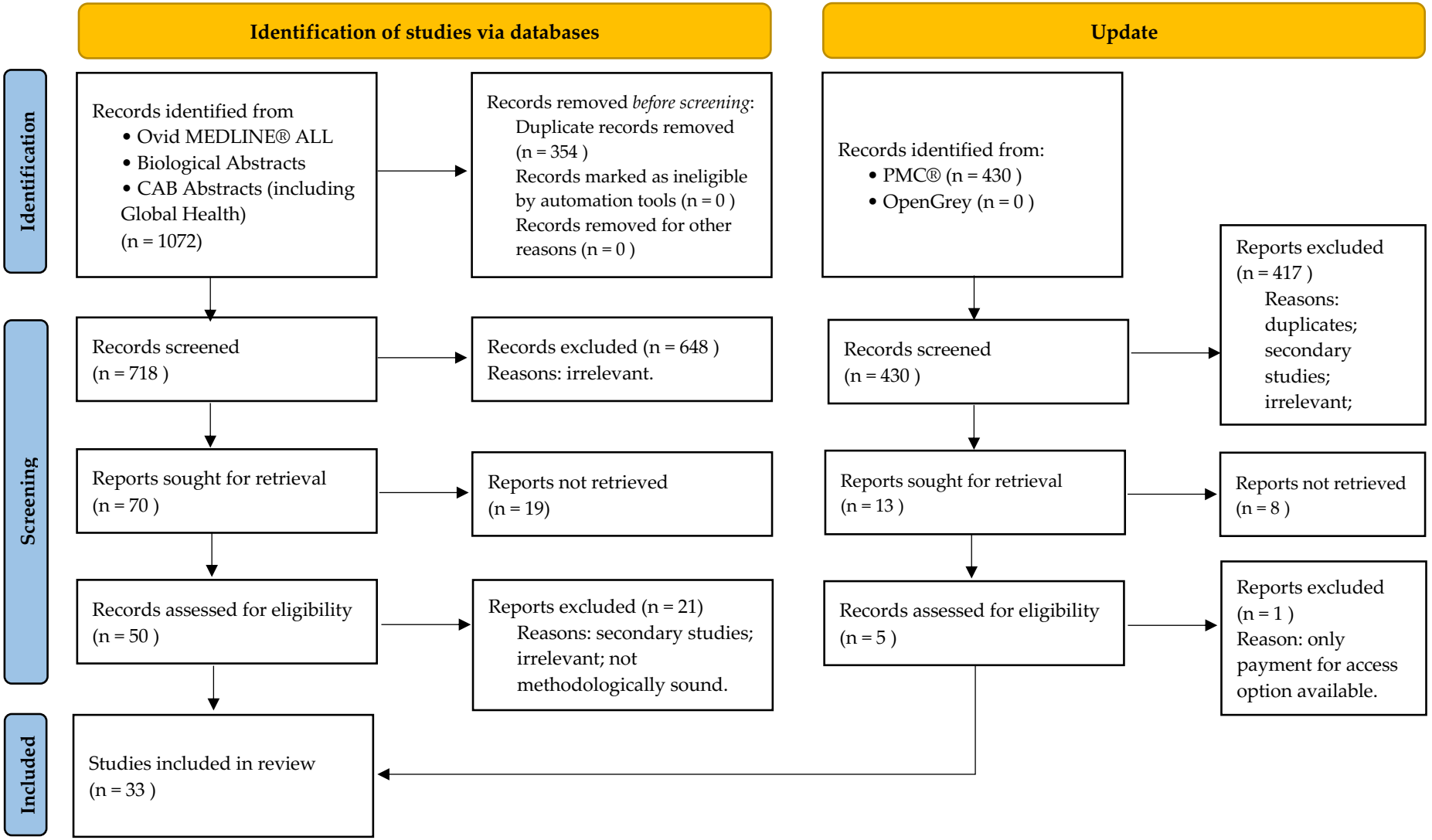
Of 718 titles/abstracts revised, 667 records, both secondary studies (e.g., reviews, viewpoints, etc and those works considered clearly irrelevant, were removed. Subsequently, full texts of potentially eligible studies were assessed by applying a set of inclusion and exclusion criteria. These conformed to the PICOS model specified above. Of 50 full-text papers evaluated, 29 were included.

During the subsequent update, the automatic search identified 430 articles among which 13 were selected for full text reading. Of these, 4 studies were further identified. The whole selection and screening process in shown in **Figure 2.2.1**

Table 2.2.1 - Bibliographic search **strategy**

#	Ovid Search History - 9/03/2021	Results (n)
1	exp Influenza Vaccines/ or influenza vaccin*.mp. or ((influenza or flu*) adj5 (vaccin* or immuni* or innoculat*)).mp.	65099
2	influenza.mp. or exp Influenza, Human/	194678
3	exp Vaccines/ or vaccin*.mp. or exp Viral Vaccines/ or immuni*.mp. or Vaccines, Subunit/ or Vaccines, Synthetic/	1249749
4	2 and 3	75903
5	1 or 4	84725
6	(sars-cov-2 or covid-19 or 2019-ncov or 2019ncov).tw.	147699
7	exp sars-cov2/ or exp covid-19/	62844
8	6 or 7	153091
9	5 and 8	1072
10	Remove duplicates from 9	718

Figure 2.2. - Systematic Review selection and screening process.



Adapted From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n7

The data from the selected studies were extracted and entered into an Excel sheet.

The following information was collected (if relevant):

- Authors and year of publication;
- Study location;
- Influenza season(s) in which the study was conducted;
- Study design;
- Study population;
- Sample size;
- COVID-19-related outcome (e.g. infection, hospitalization, need for mechanical ventilation or intensive care, mortality etc..);
- Any statistical measure related to relevant outcomes (OR, HR, RR, %, regression coefficients) and associated dispersion measures;
- Main results;
- Other potentially relevant information.

2.2.4. **Summary of results and statistical analysis**

Study populations were classified on the base of their age groups (pediatric and adultand/or elderly population). In accordance with the study setting, HCWs were also differentiated. Characteristics of the studies included in the systematic review are summarized in **Table 2.2.2**.

Meta-analyses were conducted where possible/reasonable. In fact, following the first screening phase, further inclusion and exclusion criteria were applied in order to make a distinction between studies to include in the qualitative and quantitative (meta-analysis) evaluation, respectively. The inclusion criteria conformed to the PICOS model specified above for both evaluations. By contrast, the studies to include in the meta-analysis were selected based on the accountability of the COVID-19 diagnosis reported, according to the current WHO COVID-19 case definition [WHO 2020d]. In particular, only studies reporting cases confirmed in the following manner were included:

- Nucleic Acid Amplification Test (NAAT), either based on:
  - Reverse transcription polymerase chain reaction (RT-PCR)
  - Real-time loop-mediated isothermal amplification (RT-LAMP)
- Serologic tests detecting antibodies against SARS-CoV-2 in blood;
- Individuals with a positive SARS-CoV-2 antigen-detecting rapid diagnostic tests (Ag-RDTs) also meeting either the WHO probable or suspect case definition clinical criteria [WHO 2020d];

Studies reporting on SARS-CoV-2 infections attested by questionnaires/online surveys were excluded from the quantitative analysis. Moreover, only studies employing adjusted odds ratios (aORs) and adjusted hazard risks (aHRs) as effect size (ES) were considered eligible for meta-analysis.

The meta-analysis of ORs and HRs was carried out in order to obtain pooled estimates of various COVID-19-related outcomes. After a preliminary analysis, the random effects model was chosen as most reliable. However, in the event that the observed heterogeneity was absent or particularly low (i.e., with an $I2 < 40\%$), the fixed effects models were reapplied in order to see if the pooled effect changed significantly. The heterogeneity of the pooled estimates was quantified by means of I2. The outcomes were expressed as an appropriate measure of effect size (aOR with 95% CI).

A ‘‘leave-one-out” sensitivity analysis was carried out in order to ascertain that the pooled estimates were not driven by single studies. We planned a priori to conduct a subgroup analysis, in order to highlight those study characteristics that were significantly associated with heterogeneity among studies.

Statistical processing was carried out with the use of MetaXL 5.0 [Doi 2015].

Table 2.2.2 - Baseline characteristics of the studies included in the systematic review.

Study [Ref]	Study design[a]	Study location	Study period	Study population (age, years)	Sample size	Laboratory confirmation of COVID-19	COVID-19-related outcomes analyzed	% subjects with COVID19 diagnosis	% subjects vaccinated against influenza
Amato et al.	Ecological	Italy	10/03/2020–02/06/2020	Area level data for adults older >65 years	NA	NA	Infection, hospitalization, ICU, mortality	NA	NA
Arokiaraj	Ecological	India	2020	Area level data for adults older >65 years of different countries	NA	NA	Infection, mortality, severity	NA	NA
Azzi et al.	Retrospective cohort	US	03/03/2020–06/2020	Kidney transplant recipients with COVID-19 (median 59, range 49–68)	229	Yes	Mortality	100	89
Belingheri et al.	Retrospective EMR-based	Italy	05/2020	HCW (median 47, range 35–55)	3,520	Yes	Positive test (serology and RT-PCR)	8.60/3.60	23.20
Bersanelli et al.	Prospective multicenter observational INVIDIa-2 study	Italy	01/10/2020–31/01/2021	Advanced-cancer patients receiving immune-checkpoint inhibitors (ICIs) (median 69.5, range 61-76)	955	Yes	Infection	1.46	50.47
Caban-Martinez et al.	Cross-sectional study	US	16/04/2020–17/04/2020	Firefighters and paramedics (21-51+)	203	Yes	Infection	8.90	18.90

Candelli et al.	Retrospective cohort	Italy	01/03/2020–30/06/2020	Subjects with COVID-19 (VP mean 70.4, SD 16; UP mean 57.3, SD 15)	602	Yes	Endotracheal intubation (ETI); mortality (60 days)	100	24.91
Caratozzolo et al.	Retrospective EMR-based	Italy	21/02/2020–30/04/2020	Elderly with dementia (mean 79.7, SD 7.1)	848	Partial (hospitalized)	COVID-19 symptoms	11.2	54.60
Cocco et al.	Ecological	Italy	Until 31/03/2020	Area level data for adults older >65 years	NA	NA	Infection or mortality	NA	NA
Conlon et al.	Retrospective cohort	US	01/08/2019–15/07/2020	General population/EMR (mean 47.23, SD 22.07)	27,201	Yes	Positive test, hospitalization, length of stay, mechanical ventilation, ICU, mortality	4.50	47.80
de la Cruz Conty et al.	Prospective multicenter study	Spain	26/02/2020–05/11/2020	Pregnant women from with COVID-19 (median 33, range 28–37)	1150	Yes	Clinical presentation (asymptomatic, symptomatic, mild-moderate symptoms, pneumonia, ICU, mechanical ventilation, shock)	100	38.08
Fink et al.	Retrospective EMR-based	Brazil	01/01/2020–23/06/2020	Subjects with clinical or laboratory diagnosis of COVID-19/EMR (median 56, range 0–90+)	53,752	Partial	Invasive respiratory support; intensive care; mortality	100	31.20

Gobbato et al.	Retrospective EMR-based cohort	Italy	01/03/2020–15/05/2020	Subjects with COVID-19 (mean 60)	3,010	Yes	Hospitalization; mortality	100	37.17
Greco et al.	Retrospective and multicenter	Italy	15/03/2020–13/06/2020	Subjects with COVID-19 (VP mean 75, SD 17; UP mean 51, SD 19)	952	Yes	Hospitalization; hospitalization in older patients; mortality (30-day); mortality (30-day)	100	38.97
Green et al.	Retrospective EMR-based	Israel	01/02/2020–30/04/2020	General population/EMR (mean 39.2, SD 22.5)	22,563	Yes	Positive test	8.08	10.23
Ilic et al.	Retrospective cohort	Serbia	20/03/2020–22/04/2020	HCWs (mean 39.1, SD 11.4)	107	Yes	Any symptomatic case; hospitalization; pneumonia with ground glass opacifications and consolidations on CT	100	70.10
Jehi et al.	Prospective EMR-based cohort	US	02/04/2020–16/04/2020	General population/EMR (all ages)	11672 (D cohort); 2295 (V cohort)	Yes	Infection	7 (D cohort); 12.63 (V cohort)	54.18 (D cohort); 15.42 (V cohort)
Kindgen-Milles et al.	Multicenter	Germany		HCWs (physicians) (mean 31.1, SD 6.5)	516	Yes	Infection	3.20	50.80
Liu et al.	Case-control study	China	28/01/2020–12/03/2020	Pediatric patients with COVID-19 (median 6.82, range 2.08-10.20)	304	Yes	Clinical presentation (asymptomatic disease, fever or respiratory symptoms)	100	23.03
Marín-Hernández et al.	Ecological	Italy	Until 02/05/2020	Area level data for adults older >65 years	NA	NA	Mortality	NA	NA
Martínez-Baz et al.	Prospective and test-	Spain	01/03/2020–31/05/2020	HCW/EMR (18–55+)	10,555	Yes	Positive test	8.40	34.40

	negative case-control								
Noale et al.	Web survey based observational study	Italy	04/2020–06/2020	Participants who filled the online survery EPICOVID19 (mean 48 SD 14.7)	198,828	Partially (6,680 participants underwent SARS-CoV-2 NPS test)	Infection	25.10	4.80
Oliveira et al.	Prospective cohort	Brazil	30/06/2020–04/08/2020	General population/EMR (all ages)	435	Yes	Infection	14.02	68.96
Patwardhan et al.	Retrospecti-ve EMR-based	US	01/02/2020–30/08/2020	COVID-19 positive children/EMR (≤ 20)	905	Yes	Any symptomatic case; respiratory symptoms; severe disease	100	48.51
Pedote et al.	Retrospecti-ve EMR-based	Italy	02/2020–05/2020	COVID-19 positive subjects/EMR (median 55, IQR 39–71, range 0–100)	662	Yes	Hospitalization; mortality	100	28.70
Ragni et al.	Prospective and test-negative case-control	Italy	15/02/2020–22/05/2020	General population/EMR (all ages)	17,608	Yes	Positive test, hospitalization, mortality	27.50	30.80
Rivas et al.	Retrospecti-ve cohort	US	11/05/2020–28/06/2020	HCWs (mean 41.46, SD 12.01)	6,201	Yes	Positive test (Anti–SARS-CoV-2 IgG index >0.4)	4.80	96.90
Vila-Córcoles et al.	Retrospecti-ve cohort	Spain	1/03/2020–23/05/2020	Community-dwelling individuals and nursing-home residents aged ≥50	79,083	Yes	Positive test	0.49	28.58
Wehenkel	Ecological	Mexico	2020	Area level data of 39 countries for adults older >65 years	NA	NA	Deaths per million inhabitants, case fatality ratio (both in Europe and worldwide)	NA	NA

Wilcox et al.	Retrospecti-ve EMR-based	England	01/01/2020–31/07/2020	Subjects with COVID-19 (mean 52.4, SD 24.5)	6,921	Partial (11.2%)	Hospitalization and/or mortality	100	37.80
Yang et al.	Retrospecti-ve EMR-based cohort	US	03/2020–08/2020	Subjects with COVID-19 (mean 40.7, SD 16.3)	2,005	Yes	Hospitalization, ICU	100	10.67
Zanettini et al.	Ecological	US	22/01/2020–10/06/2020	Area level data of 2034 counties for adults older >65 years	NA	NA (counties with at least 10 COVID-19 cases were included)	Mortality	NA	NA
[a]Defined by authors or deducible; EMR= electronic medical record; VP= vaccinated population: UP= unvaccinated population; D cohort= development cohort, V cohort= validation cohort; a= Only 2019/20 season influenza vaccination was considered; HCWs= Healthcare workers;									

2.3. **Results**

A total of 33 studies were included in this review. Among them 16 studies described the association between influenza vaccination and SARS-CoV-2 infection [Ragni et al. 2020; Martínez-Baz et al 2020; Oliveira et al. 2020; Noale et al. 2020; Caratozzolo et al. 2020; Belingheri et al. 2020; Conlon et al. 2021; Green et al. 2020; Rivas et al. 2021; Vila-Córcoles et al. 2020; Bersanelli et al. 2020; Jehi et al. 2021; Caban-Martinez et al. 2021; Kindgen-Milles et al. 2021; Arokiaraj 2020; Amato et al. 2020] and 19 studies described the association between influenza vaccination and COVID-19 clinical outcomes. These were assessed considering different endpoints: hospitalization [Ragni et al. 2020; Conlon et al. 2021; Wilcox et al. 2021; Yang et al. 2021; Pedote et al. 2021; Gobbato et al. 2020; Greco et al. 2021.; Amato et al. 2020], admission to the intensive care unit [Conlon et al. 2021; Fink et al. 2020; Yang et al. 2021; Wilcox et al. 2021; Candelli et al. 2021; Amato et al. 2020], administration of mechanical ventilation [Conlon et al. 2021; Fink et al. 2020; Candelli et al. 2021] and mortality [Ragni et al. 2020; Fink et al. 2020; Wilcox et al. 2021; Gobbato et al. 2020; Pedote et al. 2021; Candelli et al. 2021; Conlon et al. 2021; Azzi et al. 2020; Greco et al. 2021; Arokiaraj et al. 2020; Amato et al. 2020; Cocco et al. 2020; Marín-Hernández et al. 2021; Wehenkel 2020; Zanettini et al. 2021].

Nine of the 16 studies regarding the association between influenza vaccination and SARS-CoV-2 infection and 10 of the 19 studies regarding the association between influenza vaccination and clinical outcomes of SARS-CoV-2 infection, contained adjusted estimates (aOR or aHR). Among these, those that also met the inclusion criteria set for the quantitative analysis were meta-analyzed.

The quantitative analysis of the association between influenza vaccination and SARS-CoV-2 infection comprised a total of 167,579 people (36,537 vaccinated and 131,042 unvaccinated. The quantitative analysis of the association between influenza vaccination and COVID-19 clinical sequelae, assessed by different outcomes (hospitalization, intensive care, mechanical ventilation and mortality), comprised a total of 112,713 COVID-19 patients.

The adjusted variables were not the same across all studies, however, the majority included age, sex and comorbidities. The baseline characteristics of the studies included in the review, organized by endpoint, are listed in **Table 2.3.1.** and **Table 2.3.2**, respectively.

2.3.1. Infection

Table 2.3.1 - Studies that assessed the association between influenza vaccination and SARS-CoV-2 infection.

Study	Study design[a]	Study location	Sample size	Effect Size (ES)	Crude Estimate (if applicable)	Adjusted Estimate (if applicable)	P value (if applicable)	Adjusted Factors
Ragni et al.	Prospective and test-negative case-control	Italy	17,608	OR	1.26 [95%CI 1.17-1.34]	0.89 [95%CI 0.80-0.99]	-	Age, sex, Charlson index, time of the swab test
Martínez-Baz et al.	Prospective and test-negative case-control	Spain	10,555	OR	-	1.07 [95%CI 0.92-1.24]	-	Age, sex, major chronic conditions, profession, any ILI diagnosis in the previous five years
Oliveira et al.	Prospective cohort	Brazil	435	OR	0.51 [95%CI 0.29-0.87]	-	-	-
Noale et al.	Web survery based observational study	Italy	198,828	OR	1.02 [95%CI 0.91-1.15]	0.89 [95%CI 0.78-1.01]	-	Age, sex, education, area of residence, comorbidities, smoking status
Caratozzolo et al.	Retrospective EMR-based	Italy	848	OR	-	0.47 [95%CI 0.29-0.74]	-	Age sex, comorbidities, Clinical dementia rating scale
Belingheri et al.	Retrospective EMR-based	Italy	3,520	OR	0.92 [95%CI 0.60-1.42]	0.41 [95%CI 0.07-2.39]	-	Age, sex, interaction between age and the vaccination intake in 2019/2020
Conlon et al.	Retrospective cohort	US	27,201	OR	0.82 [95%CI 0.73-0.92]	0.76 [95%CI 0.68-0.86]	-	Age, sex, ethnicity, race, BMI, Elixhauser score, comorbidities, smoking status

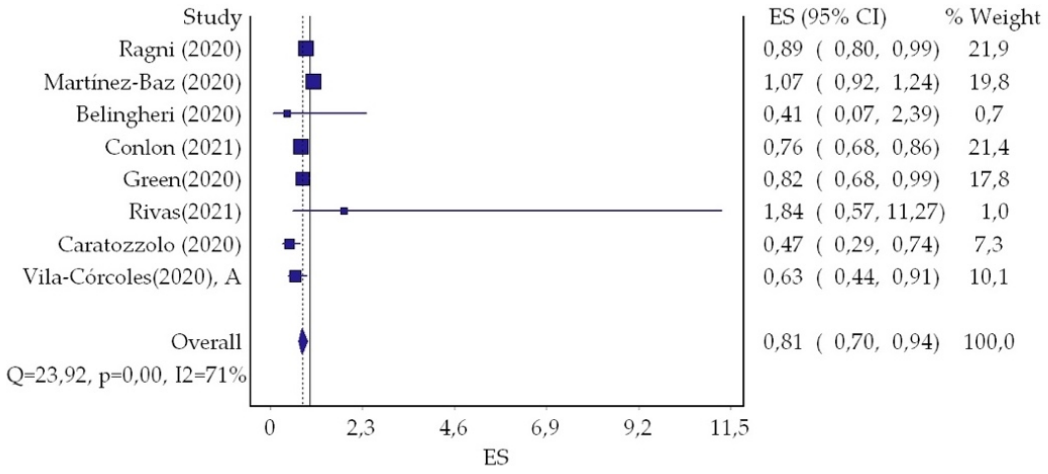
Green et al.	Retrospective EMR-based	Israel	22,563	OR	0.65 [95%CI 0.54-0.77]	0.79 [95%CI 0.67-0.98]	-	Age, ethnicity, socioeconomic status, comorbidities, smoking status
Rivas et al.	Retrospective cohort	US	6,201	OR	-	1.84 [95%CI 0.57-11.27]	-	Age, sex
Vila-Córcoles et al.	Retrospecti-ve cohort	Spain	79,083	HR	1.21 [95%CI 0.91-1.61]	0.63 [95%CI 0.44 -0.91]	-	Age, sex, comorbidities
Bersanelli et al.	Prospective multicenter observational INVIDIa-2 study	Italy	955	Calcu-lated OR*	0.73 [95%CI 0.25-2.13]	-	-	-
Jehi et al.	Prospective EMR-based cohort	US	11672 (D cohort);	Calcu-lated OR*	0.73 [95%CI 0.16-0.84]	-	-	-
			2295 (V cohort)	Calcu-lated OR*	0.59 [95%CI 0.01-0.88]	-	-	
Caban-Martinez et al.	Cross-sectional study	US	203	%	0.0% vs 21.0%		0.027	-
				Calcu-lated OR*	0.01 [95%CI 0.01-1.71]	-	-	
Kindgen-Milles et al.	Multicenter	Germany	516	%	3.1% vs 3.3%		-	-
				Calcu-lated OR*	0.97 [95%CI 0.36-2.61]	-	-	

Arokiaraj	Ecological	India	NA	r	-0.53	-	-
				R2	0.28	-	
Cocco et al.	Ecological	Italy	NA	r	0.546	0.006	-
Amato et al.	Ecological	Italy	NA	beta	-130 [-198 – -62]	0.001	-

[a]Defined by authors or deducible; EMR= electronic medical record; D cohort= development cohort, V cohort= validation cohort; Calculated OR*= The ES was calculated using the data available.

As mentioned before, meta-analyses were conducted where possible and reasonable, by applying the inclusion criteria described in paragraph 2.2. As a result, influenza vaccination was shown to be associated with a lower risk of SARS-CoV-2 infection (Figure 2.3.1.1 – model A): random effects model pooled adjusted OR (aOR) 0.81, 95%CI: 0.70–0.94.

Figure 2.3.1.1 - Forest plot for the association between influenza vaccination and SARS-CoV-2 infection: aOR by random effects model (A).



In order to ascertain that the pooled estimates were not driven by single studies a ‘‘leave-one-out” sensitivity analysis was carried out.

Firstly, the study by Vila-Corcoles *et al*. was excluded seen as it is the only study regarding the association between influenza vaccination and SARS-CoV-2 infection to present HR as measure of effect size (Figure 2.3.1.2 – model B). The results, however, did not significantly differ from the ones previously obtained: random effects model pooled aOR 0.84, 95%CI: 0.72–0.98.

Secondly, the study performed by Rivas *et al*. was excluded seen as it used the IgG index in place of RT-PCR to assess SARS-CoV-2 positivity (Figure 2.3.1.3 – model C). Similarly, the results did not significantly differ from the base case: random effects model pooled aOR 0.83, 95%CI: 0.71–0.97.

Figure 2.3.1.2 - Forest plot for the association between influenza vaccination and SARS-CoV-2 infection: aOR by random effects model (B).

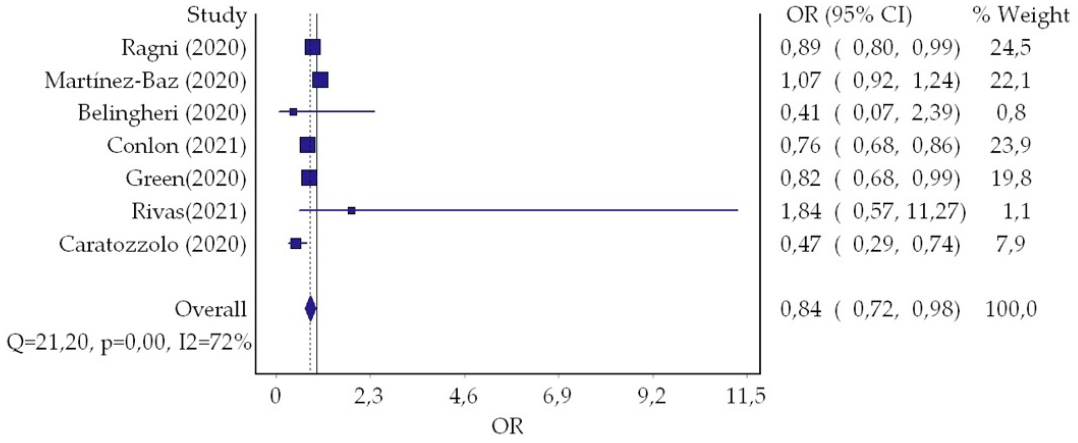
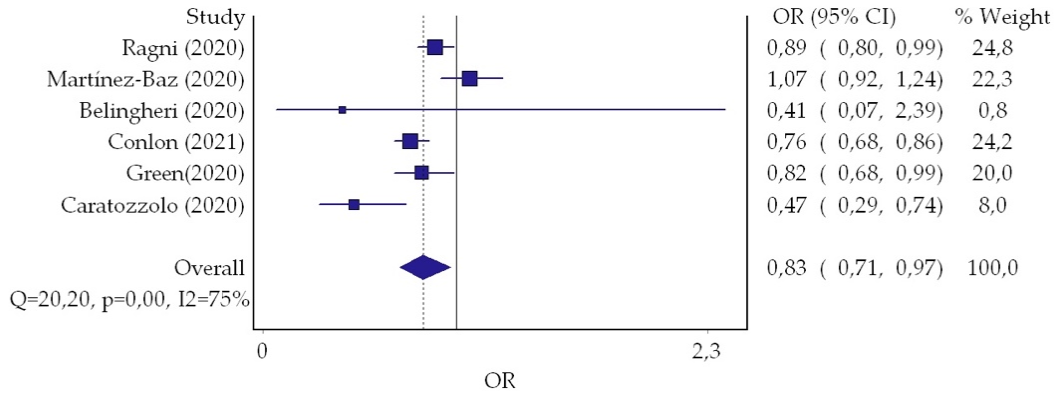


Figure 2.3.1.3 - Forest plot for the association between influenza vaccination and SARS-CoV-2 infection: aOR by random effects model (C).



When any one of the studies was omitted, the pooled estimates were consistent, demonstrating the robustness of the results and confirming the initial assumption according to which individuals vaccinated against influenza have a lower risk (81% of the odds of the unvaccinated group) of being infected with SARS-CoV-2.

Among studies excluded from quantitative analysis, some, nonetheless, endorsed our findings. In particular, Oliveira *et al.* [Oliveira et al. 2020] conducted a prospective cohort study in Brazil. Even if the authors did not provide adjusted measures of effect size, they found a 49% reduction for individuals vaccinated against influenza in the odds of being infected with SARS-CoV-2 (OR 0.51, 95%CI: 0.29-0.87).

Among the studies employing OR as ES, the findings of the study conducted by Noale *et al.* [Noale et al. 2020], excluded seen as it reports SARS-CoV-2 infections attested through an online questionnaire, did not reach statistical significance (OR 0.89 95%CI 0.78-1.01).

Other studies [Bersanelli et al. 2020; Jehi et al. 2020; Caban-Martinez et al. 2021; Kindgen-Milles et al. 2021], that did not provide relative measures of effect on the association between influenza vaccination and SARS-CoV-2 infection did, however, provide sufficient data to calculate the crude OR for this association. These were, respectively, 0.73 [95%CI 0.25-2.13], 0.59 [95%CI 0.01-0.88], 0.01 [95%CI 0.01-1.71], 0.97 [95%CI 0.36-2.61]. As attested by the confidence intervals, most of them did not reach statistical significance. An exception to this is represented by the study, prospective and multicentric, conducted by Bersanelli *et al*. in Italy on a cohort of 955 advanced-cancer patients in therapy with immune-checkpoint inhibitors (ICIs), for which the calculated reduction in odds of being infected with SARS-CoV-2 was 27%.

Additionally, three ecological studies conducted, respectively, in India by Arokiaraj and in Italy by Cocco *et al*. and Amato *et al*., found an association between influenza vaccination and reduced risk of SARS-CoV-2 infection (r -0.53, R2 -0.28 [Arokiaraj 2020]; r = 0.546 (p = 0.006) [Cocco et al. 2020]; Beta -130 (95%CI -198 – -62) (p=0.001) [Amato et al. 2020]). These results further underline that the coverage rate of the influenza vaccination is associated with a reduced spread of COVID-19.

2.3.2. COVID-19 clinical outcomes

To investigate the association between influenza vaccination and COVID-19 related clinical outcomes, meta-analyses investigating separately each one of the four clinical outcomes taken into consideration (hospitalization, mechanical ventilation, intensive care and mortality) were conducted.

Table 2.3.2 - Studies that assessed the association between influenza vaccination and Covid-19 clinical outcomes.

Study	Study design[a]	Study location	Sample size	Effect Size (ES)	Crude Estimate	Adjusted Estimate	P value	Adjusted Factors
Hospitalization								
Ragni et al.	Prospective and test-negative case-control	Italy	17,608	HR	-	1 [95%CI 0.84-1.92]	-	Age, sex, Charlson index, time of the swab test
Conlon et al.	Retrospective cohort	US	27,201	HR	-	0.58 [95%CI 0.46-0.63]	-	Age, sex, ethnicity, race, BMI, Elixhauser score, comorbidities, smoking status
Wilcox et al.	Retrospective EMR-based	England	6,921	OR	-	0.85 [95%CI 0.75-0.97]	-	Age, sex, BMI, socioeconomic status, frailty score, medications, comorbidities, smoking status
Yang et al.	Retrospective EMR-based cohort	US	2,005	OR	2.84 [95%CI 2.03-4.07][R]	0.41 [95%CI 0.28-0.59][R]	-	Age, sex, ethnicity, comorbidities
Pedote et al.	Retrospective EMR-based	Italy	662	OR	1.2 [95%CI 0.70-1.90]	1.03 [95%CI 0.56-1.92]	0.510	Age, sex, chronic disease
Gobbato et al.	Retrospective EMR-based cohort	Italy	3,010	OR	-	0.62 [95%CI 0.44-0.85]	-	Age, sex, comorbidities, medications, health district
Greco et al.	Retrospective and multicenter	Italy	952	OR	-	1.44 [95%CI 1.01-2.05]	0.04	Age, sex
				%	68.7% vs. 33.2%		<0.001	
Amato et al	Ecological	Italy	NA	beta	-4.61 [-6.27 – -2.05]		0.001	-

Intensive Care								
Conlon et al.	Retrospective cohort	US	27,201	OR	-	0.64 [95%CI 0.41-1]	-	Age, sex, ethnicity, race, BMI, Elixhauser score, comorbidities, smoking status
Fink et al.	Retrospective EMR-based	Brazil	53,752	OR	-	0.93 [95%CI 0.87-0.98]	-	Age, sex, race, educational level, treatment facility, comorbidities
Yang et al.	Retrospective EMR-based cohort	US	2,005	OR	5.64 [95%CI 2.11-23.01][R]	3.29 [95%CI 1.18-13.77][R]	-	Age, sex, ethnicity, comorbidities
Wilcox et al.	Retrospective EMR-based	England	6,921	OR	-	0.85 [95%CI 0.75-0.97]	-	Age, sex, BMI, socioeconomic status, frailty score, medications, comorbidities, smoking status
Amato et al.	Ecological	Italy	NA	beta	-0.58 [-1.05 – -0.12]		0.017	-
Mechanical Ventilation								
Conlon et al.	Retrospective cohort	US	27,201	OR	-	0.45 [95%CI 0.27-0.78]	-	Age, sex, ethnicity, race, BMI, Elixhauser score, comorbidities, smoking status
Fink et al.	Retrospective EMR-based	Brazil	53,752	OR	-	0.83 [95%CI 0.77-0.88]	-	Age, sex, race, educational level, treatment facility, comorbidities
Candelli et al.	Retrospective cohort	Italy	602	OR	-	0.73 [95%CI 0.36-1.56]	-	Age, sex, comorbidities

Mortality								
Ragni et al.	Prospective and test-negative case-control	Italy	17,608	HR	-	1.14 [95%CI 0.95-1.37]	-	Age, sex, Charlson index, time of the swab test
Fink et al.	Retrospective EMR-based	Brazil	53,752	OR	-	0.84 [95%CI 0.78-0.90]	-	Age, sex, race, educational level, treatment facility, comorbidities
Wilcox et al.	Retrospective EMR-based	England	6,921	OR	-	0.85 [95%CI 0.75-0.97]	-	Age, sex, BMI, socioeconomic status, frailty score, medications, comorbidities, smoking status
Gobbato et al.	Retrospective EMR-based cohort	Italy	3,010	OR	-	0.62 [95%CI 0.44-0.85]	-	Age, sex, comorbidities, medications, health district
Pedote et al.	Retrospective EMR-based	Italy	662	OR	1.6 [95%CI 0.80-3.20]	1.70 [95%CI 0.80-3.60]	0,165	Age, sex, chronic disease
Candelli et al.	Retrospective cohort	Italy	602	OR	-	0.20 [95%CI 0.08-0.51]	-	Age, sex, comorbidities
Conlon et al.	Retrospective cohort	US	27,201	HR	0.84 [95%CI 0.51-1.36]	0.76 [95%CI 0.68-0.86]	-	Age, sex, ethnicity, race, BMI, Elixhauser score, comorbidities, smoking status
Azzi et al.	Retrospective cohort	US	132	OR	-	1.13 [95%CI 1.04-1.43]	-	Age, type of kidney transplant
				%	76% vs 93%		1⁄4 0.0015	

Greco et al.	Retrospective and multicenter	Italy	952	OR	-	1.06 [95%CI 0.60-1.88]	00.85	Age, sex
				%	14,3% vs. 4,3%		<0.001	
Arokiaraj	Ecological	India	NA	r	−0,367		-	-
				R2	0.13		-	
Amato et al.	Ecological	Italy	NA	beta	-3.29 [-5.66– -0.93]		0,010	-
Cocco et al.	Ecological	Italy	NA	r	0.546		-	-
Marín-Hernández et al.	Ecological	Italy	NA	r	−0,5874 (n = 21)		0.0051	-
				R2	0.345		0.01	
Wehenkel	Ecological	Mexico	NA	DPMI	+ 0.487		0.0017	-
				CFR	+0.629		0.00075	
Zanettini et al.	Ecological	US	NA	MMR	-	0.95 [95% CI: 0.92–0.98]	-	A set of 40 potential confounders
Other								
Ilic et al.	Retrospective cohort	Serbia	107	OR (bilateral pneumo-nia on CT)	0.297 [95%CI 0.082-1.074]	0.207 [95%CI 0.05-0.847]		BMI, comorbidities

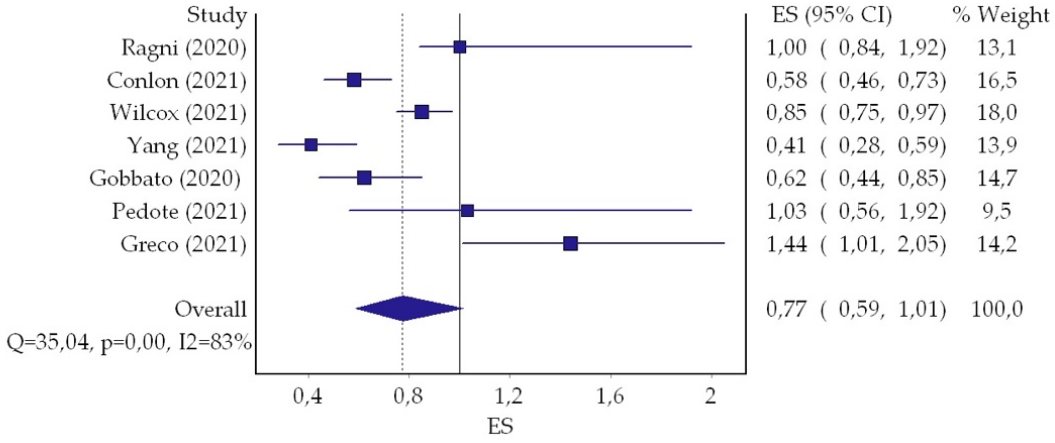
de la Cruz Conty et al.	Prospective multicenter study	Spain	1,150	% (asymp-tomatic disease)	26.vs. 73.9%		0.051	-
Liu et al.	Case-control study	China	304	% (asymp-tomatic disease)	24.29% vs. 34.25%		0.267	-
				% (fever or respirato ry simp-tomps)	71.43% vs. 63.01%		0.267	
Patwardhan et al.	Retrospective EMR-based	US	905	OR (simp-tomatic disease)	-	0.714 [95%CI 0.529-0.964]	-	Age, sex, race, age (month) at diagnosis, allergies/asthma, comorbidities, BMI, exposure to smoke
				OR (respira-tory simp-tomps)	-	0.678 [95%CI 0.492-0.934]	-	
				OR (severe disease)	-	0.672 [95%CI 0.50-0.903]	-	

[a]Defined by authors or deducible; EMR= electronic medical record; D cohort= development cohort, V cohort= validation cohort; Calculated OR*= The ES was not explicitly reported and it was calculated using the data available; [R]=Reversed OR; MMR = mortality rate ratio; DPMI= deaths per million inhabitants; CFR = case Fatality Ratio

2.3.3. Hospitalization

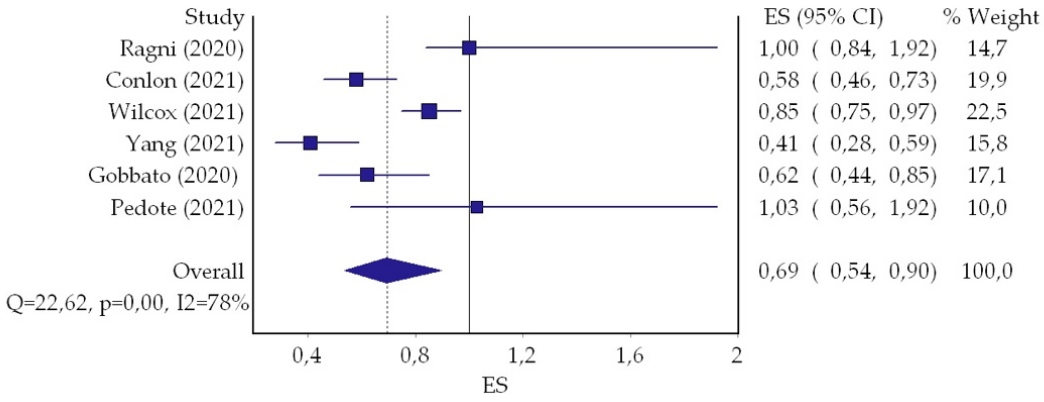
The quantitative analysis of the studies investigating the association between influenza vaccination and COVID-19 related hospitalization did not reach, at first, statistical significance (Figure 2.3.3.1 – model A): random effects model pooled aOR 0.77 95%CI 0.50-1.01.

Figure 2.3.3.1 - Forest plot for the association between influenza vaccination and COVID-19 related hospitalization: aOR by random effects model (A).



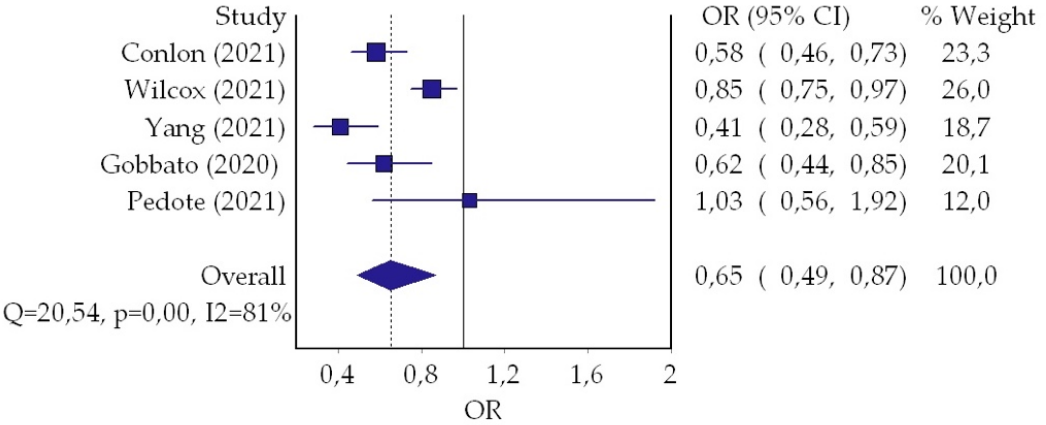
However, among the studies reporting adjusted measures of effect, one outlier stands out. In particular, the study performed by Greco et al. [Greco et al. 2021], pointing out vaccination against influenza to be an independent risk factor for undergoing hospitalization, presented a high imbalance, in terms of age, between the cohorts of vaccinated and unvaccinated individuals. Since the risk of hospitalization was strongly influenced by patients' age, the information on vaccinal status alone, as stated by the authors, may not a be a reliable predictor for hospitalization. As a consequence, a ''leave-one-out'' sensitivity analysis was carried out (Figure 2.3.3.2 – model B). This model showed, for the vaccinated cohort, a 31% reduction in odds of being hospitalized for COVID-19 (aOR 0.69, 95%CI: 0.54-0.90).

Figure 2.3.3.2 - Forest plot for the association between influenza vaccination and COVID-19 related hospitalization: a OR by random effects model (B).



Additionally, as it was previously done for the risk of infection, the single study on the association between influenza vaccination and COVID-19 related hospitalization employing HR instead of OR as a measure of effect size [Ragni et al. 2020] was excluded (Figure 2.3.3.3 – model C). The pooled estimates were consistent with model B, demonstrating the robustness of the results: random effects model pooled aOR 0.65, 95%CI: 0.49–0.87.

Figure 2.3.3.3 - Forest plot for the association between influenza vaccination and COVID-19 related hospitalization: aOR by random effects model (C).



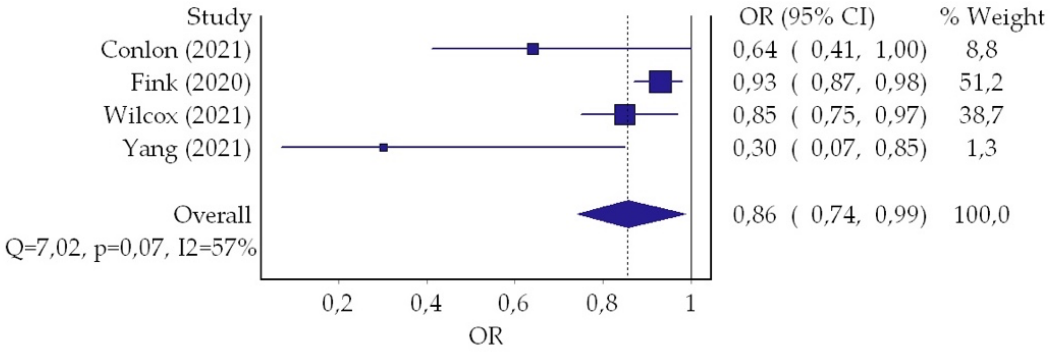
In addition, an association between influenza vaccination and reduced risk of COVID-19 related hospitalization was, likewise, demonstrated in one study conducted in Italy

by Amato et al. [Amato et al. 2020]. This study, excluded from the meta-analysis due to its design, found a correlation between vaccination and risk of hospitalization equal to Beta -4.61 (95%CI -6.27 – -2.05) (p = 0.001).

2.3.4. Intensive Care

A meta-analysis of the association between influenza vaccination and risk for COVID-19 patients of being treated in the intensive care unit was performed. As a result, even if only four studies [Conlon et al. 2021, Fink et al. 2020, Yang et al. 2021, Wilcox et al. 2021] employing adjusted measures of effect size investigated this association, influenza vaccination was shown to be associated with a decreased risk of being treated in the intensive care unit (Figure 2.3.2.4): random effects model pooled aOR 0.86, 95%CI: 0.74–0.99).

Figure 2.3.4 - Forest plot for the association between influenza vaccination and need for intensive care in COVID-19 patients: aOR by random effects model.

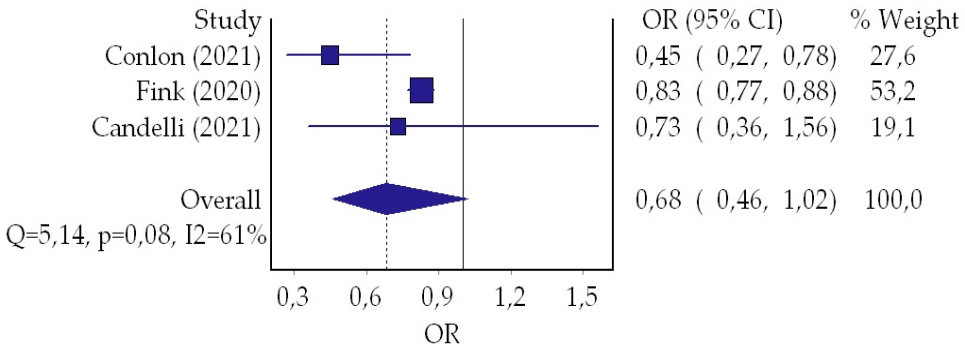


As for the risk of hospitalization, an association between influenza vaccination and reduced risk of intensive care treatments was demonstrated in the ecological study conducted by Amato et al. [Amato et al. 2020], reporting a correlation equal to Beta -0.58 (95%CI -1.05 – -0.12) (p = 0.017).

2.3.5. Mechanical Ventilation

An investigation of the association between influenza vaccination and risk for COVID-19 patients of undergoing mechanical ventilation was carried out only by three [Conlon et al. 2021, Fink et al. 2020, Candelli et al. 2021] of the included studies. Nevertheless, a meta-analysis was performed and, as expected because of the substantial heterogeneity between the small number of studies, the pooled adjusted OR did not reach statistical significance (Figure 2.3.5.1): random effects model pooled aOR 0.68, 95%CI: 0.46–1.02).

Figure 2.3.5 - Forest plot for the association between influenza vaccination and need for mechanical ventilation in COVID-19 patients: aOR by random effects model



2.3.6. Mortality

A meta-analysis of the studies reporting adjusted measures of effect of the association between influenza vaccination and COVID-19 related mortality was performed. The quantitative analysis did not reach, at first, statistical significance (Figure 2.3.6.1 – model A): aOR 0.86 95%CI 0.73-1.02. However, as previously seen for studies reporting on the association between vaccination and hospitalization, one outlier stands out.

In particular, the study performed by Pedote et al. [Pedote et al. 2021], that did not demonstrate any significant association between influenza vaccination and risk of

hospitalization or death, but found a statistically significant association between health outcomes in COVID-19 patients, age >65 and chronic disease, did not take into account the impact of comorbidities on disease severity / mortality predictions (by employing, for example, the Charlson Comorbidity Index (CCI) Score). As a consequence, a ''leave-one-out" sensitivity analysis was carried out (Figure 2.3.6.2 – model B). As a result, vaccination was shown to be associated with a 16% reduction in odds of death by COVID-19 (aOR 0.84, 95%CI: 0.71-0.99).

Figure 2.3.6.1 - Forest plot for the association between influenza vaccination COVID-19 related mortality: aOR by random effects model (A).

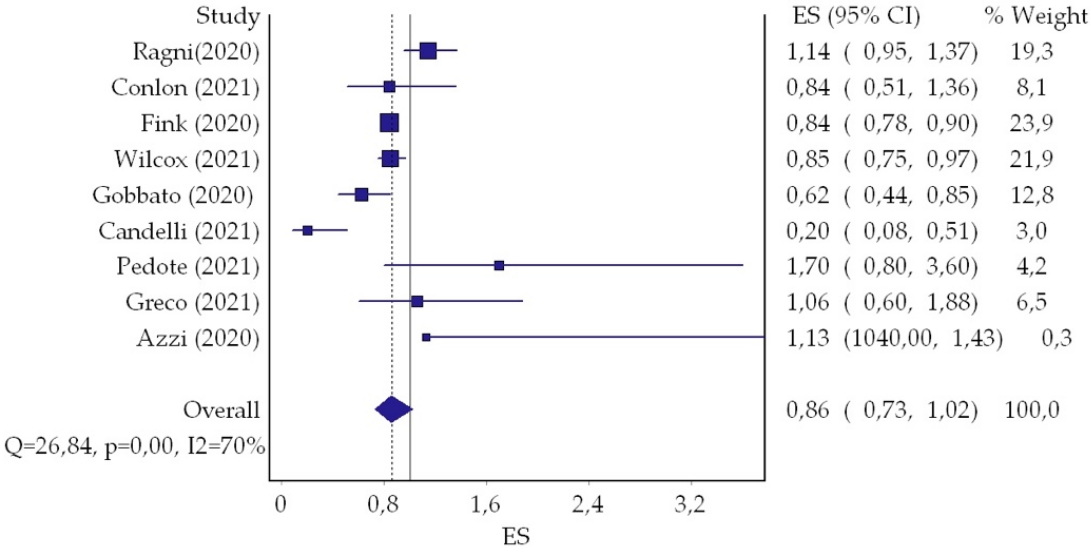
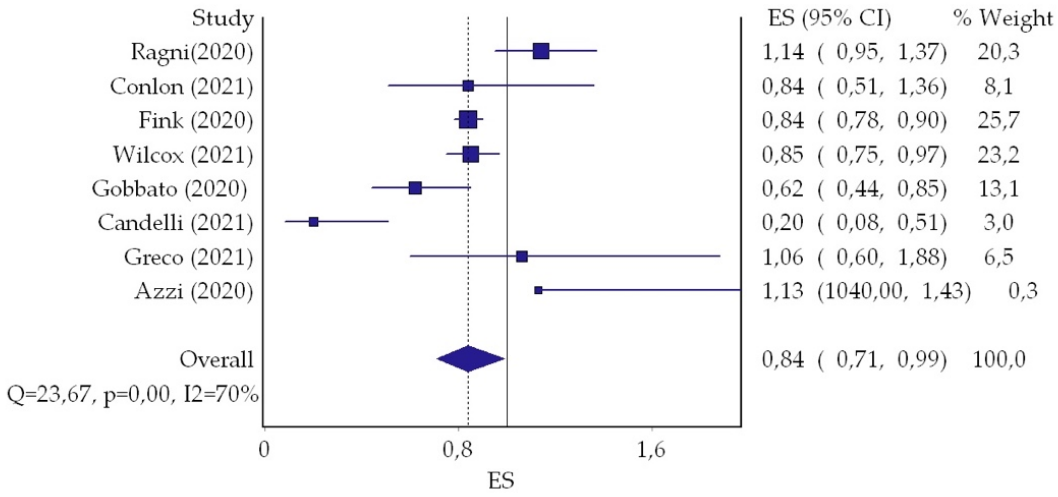
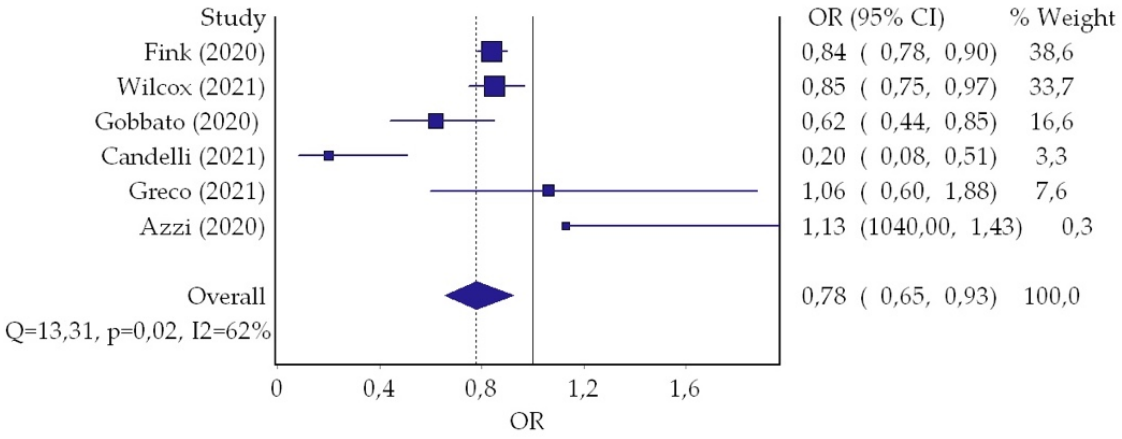


Figure 2.3.6.2 Forest plot for the association between influenza vaccination COVID-19 related mortality: aOR by random effects model (B).



Additionally, the two studies on the association between influenza vaccination and death in COVID-19 patients employing HRs instead of ORs as a measure of effect size [Ragni et al. 2020; Conlon et al. 2021] were excluded (Figure 2.3.6.3 – model C). The pooled estimates were consistent with model B, demonstrating the robustness of the results: random effects model pooled aOR 0.78, 95%CI: 0.65–0.93.

Figure 2.3.6.3 - Forest plot for the association between influenza vaccination COVID-19 related mortality: aOR by random effects model (C).



2.3.7. Association between influenza vaccination and COVID-19 clinical outcomes in the elderly

COVID-19 disproportionately affects the elderly due to the high proportion of individuals presenting frailties and underlying chronic conditions whithin this age group [Fisman et al. 2020]. Because older adults are at increased risk of morbidity and mortality, it is important to evaluate the effect that additional preventive measures, such as vaccination against influenza, may have in this age group.
In **Table 2.3.7**, studies specifically describing any association between influenza vaccination and Covid-19 related endpoints in adults older than 65 years old, are reported.

Table 2.3.7 - Covid-19 related endpoints in the elderly vaccinated against influenza

Study	Endpoints	Sample size	Effect Size (ES)	Crude Estimate	Adjusted Estimate	P value
Caratozzolo et al. 2020	Infection	848	OR	-	0.47 [95%CI 0.29-0.74]	-
Noale et al. 2020	Infection	198,828	OR	0.83 [95%CI 0.60-1.14]	0.87 [95%CI 0.59-1.28]	-
Ragni et al. 2020	Hospitalization	17,608	HR	-	0.66 [95%CI 0.44-0.98]	-
Ragni et al. 2020	Mortality		HR	-	0.70 [95%CI 0.50-1]	-
Zanettini et al. 2021	Mortality	NA	MMR	-	0.95 [95% CI: 0.92–0.98]	-
Arokiaraj 2020	Mortality	NA	r	−0,367		-
			R2	0.13		
Cocco et al. 2020	Mortality	NA	r	0.546		-
Marín-Hernández et al. 2021	Mortality	NA	r	−0,5874 (n = 21)		0.0051
			R2	0.345		0.01
Wehenkel 2020	Mortality	NA	DPMI	+ 0.487		0.0017
			CFR	+0.629		0.00075
MMR = mortality rate ratio; DPMI= deaths per million inhabitants; CFR = case fatality ratio.						

Because of the small number of studies reporting adjusted measures of effect on the association between influenza vaccination and any COVID-19 outcomes in adults older than 65 years old, and because of considerable heterogeneity between study designs and participants involved in each outcome, the initially planned subgroup analysis was not carried out. However, even single studies on the topic seem to underline the importance of this preventive measure in the elderly population.

For instance, the study performed by Ragni et al. [Ragni et al. 2020] that, after adjusting for confounding factors, found no association between influenza vaccination and hospitalization or death in the general population, obtained, for patients aged ≥65, HRs 0.66 (95% CI: 0.44–0.98) and 0.70 (95% CI 0.50–1.00) for hospitalization and death, respectively.

Moreover, since older adults are often eligible for free-of-charge influenza vaccination, a number of databases reporting vaccination coverages in this population are publicly available. This is probably this the reason for which various ecological studies [Zanettini et al. 2021; Arokiaraj 2020; Cocco et al. 2020; Marín- Hernández et al. 2021; Wehenkel 2020] specifically focused on the population aged ≥65 and found an association between influenza vaccination and reduced risk of death by COVID-19.

2.3.8. Association between influenza vaccination and COVID-19 clinical outcomes in children

The pediatric population (particularly children aged <12) tends to be affected by COVID-19 less frequently than adults [Dong et al. 2020]. However, although the clinical manifestations are usually mild, severe cases, including hypotension and multisystem involvement, have been reported [Deville et al. 2021]. For this reason, investigating whether preventive measures, such as influenza vaccination, may have an impact on COVID-19 outcomes in children is of clinical relevance. Among the included studies, two focused on the association between influenza vaccination and COVID-19 clinical outcomes in children and are reported in **Table 2.3.8**.

While Liu et al. [Liu et al. 2021] found that initial symptoms are not related with immunization against influenza (P=0.267), the results obtained by Patwardhan et al. [Patwardhan et al. 2021] have shown that children who were vaccinated for influenza had lower odds of having symptomatic diseases than those not vaccinated (p=0.028, aOR 0.714, 95% CI [0.529, 0.964]).

Table 2.3.8 - Covid-19 related endpoints in children vaccinated against influenza

Study	Endpoints	Sample size	Effect Size (ES)	Crude Estimate	Adjusted Estimate	P value
Patwardhan et al. 2021	Symptomatic disease	905	OR	-	0.714 [95%CI 0.53-0.96]	-
	Respiratory symptoms		OR	-	0.678 [95%CI 0.49-0.93]	-

	Severe disease		OR	-	0.672 [95%CI 0.50-0.90]	-
Liu et al. 2021	Asymptomatic disease	304	%	24.29% vs. 34.25%		0.267
	Respiratory symptoms / Fever		%	71.43% vs. 63.01%		

Although more evidences on the topic are needed, immunizing children, who spread flu easily, can help reduce the risk of co-infection and severe COVID-19 clinical presentation. For this reason, carrying out and enforcing vaccination campaigns worldwide remains of fundamental importance among people of every age.

2.3.9. Association between influenza vaccination and COVID-19 clinical outcomes during pregnancy

Among the studies included in the review, one [de la Cruz Conty et al. 2021] investigated 1150 SARS-CoV-2 positive pregnant women from 78 Spanish hospitals. Although no association was observed between the influenza vaccination status of patients and the clinical presentation / severity of symptoms of SARS-CoV-2, maternal vaccination programs, especially in the actual pandemic, are imperative. This is particularly true in light of the current available data suggesting that, while pregnancy does not increase susceptibility to SARS-CoV-2 infection, it tends to worsen the clinical course of the disease compared with nonpregnant women of the same age [Zambrano et al. 2020].

2.3.10. Association between influenza vaccination and SARS-CoV-2 infection in healthcare workers

According to data on healthcare workers' infection in the context of COVID-19 disease, 14% of COVID-19 cases reported worldwide, prior to the availability of SARS-Cov-2 vaccines, were among HCWs [WHO 2020 e]. Since HCWs, by working in close proximity to patients and coworkers, are at higher risk for contracting COVID-19, it is imperative to provide them all with preventive measures available, able to reduce the

burden of respiratory infections. In order to do this, as mentioned in Chapters 1 and 3, influenza vaccination campaigns were enforced worldwide and various countries introduced mandatory flu vaccination for HCWs.

In the context of these events, a possible association between influenza vaccination and SARS-CoV-2 infection was investigated by various authors. These evidences are collected in **Table 2.3.10**.

Table 2.3.10 - SARS-CoV-2 infection in HCWs vaccinated against influenza

Study	**Study location**	**Population**	**Sample size**	**Effect Size (ES)**	**Crude Estimate**	**Adjusted Estimate**	**P value**
Belingheri et al.	Italy	HCWs	3,520	OR	0.92 [95%CI 0.60-1.42]	0.41 [95%CI 0.07-2.39]	-
Ilic et al.	Serbia	HCWs	107	OR (bilateral pneumonia on CT)	0.297 [95%CI 0.082-1.074]	0.207 [95%CI 0.05-0.847]	
Martínez-Baz et al.	Spain	HCWs	10,555	OR	-	1.07 [95%CI 0.92-1.24]	-
Rivas et al.	US	HCWs	6,201	OR	-	1.84 [95%CI 0.57-11.27]	-
Kindgen-Milles et al.	Germa-ny	Physicians	516	%	3.1% vs 3.3%		-
				Calcula-ted OR*	0.97 [95%CI 0.36-2.61]	-	-
Caban-Martinez et al.	US	Firefighters and paramedics	203	%	0.0% vs 21.0%	-	0.027
				Calcula-ted OR*	0.01 [95%CI 0.01-1.71]	-	
HCWs = Healthcare workers; Calculated OR*= The ES was not explicitly reported and it was calculated using the available data.							

Because, among the studies included, only a small number reported adjusted measures of effect, and because of the substantial heterogeneity between study designs and participants, it was not possible to carry out a subgroup analysis.

Moreover, studies conducted in different settings have produced contrasting results. In particular, four studies [Martínez-Baz et al. 2020, Belingheri et al. 2020, Rivas et al. 2021, Kindgen-Milles et. al 2021] have not found any association between the 2019/20 influenza vaccination and SARS-CoV-2 positivity rate. On the other hand, Ilic et al. reported a negative association between influenza vaccination and the finding, in a cohort of HCWs, of bilateral pneumonia on CT (aOR 0.207, 95%CI: 0.05-0.847). Furthermore, in the US, Caban-Martinez et al. found a statistically significant difference (p=0.027) in terms of SARS-CoV-2 infection rates, between vaccinated and unvaccinated paramedics and firefighters. Both of these studies, however, are limited by the small sample size.

2.4. **Discussion**

The studies included in this systematic review and meta-analysis differ in many aspects, including their design, study population and sample size. Because of the testing policy implemented in the area in which the study was conducted, also the testing criteria to assess SARS-CoV-2 positivity noticeably differed. Furthermore, six studies [Marín- Hernández et al., Cocco et al., Zanettini et al., Arokiaraj, Amato et al., Wehenkel] only employed area-level data with a potential for ecological fallacy and unmeasured confounding. Because of the diversity of study populations and the prospective nature of the systematic review the quality of the studies was not assessed, although we attempted to minimize this bias by including only peer reviewed studies. Furthermore, because not all studies reported measures of association adjusted by the relevant confounders, more stringent inclusion criteria for the quantitative analysis (meta-analysis) were adopted to include only those which did.

It must be noted that all reviewed studies are observational, and thus more likely to be subject to bias and confounding. However, in the context of a global pandemic in which the concern for respiratory comorbidities in SARS-CoV-2 patients is extremely

high, randomized controlled trials (RCTs) denying patients in the control arm an effective preventive measure would be unethical.

Finally, most of the included studies were conducted in the 2019-20 flu season alone. Since influenza varies substantially across years, it is not clear whether the results of this study can be applied for different influenza seasons.

Recently, another systematic review and meta-analysis on this topic was published [Wang et al. 2021]. While the authors have systematically analyzed the included studies, assessing the risk of bias and performing subgroup analysis by sample size, regions and study design, it should be noted that the number of included studies, in comparison to this systematic review and meta-analysis, is significantly lower, reflecting a less comprehensive search strategy. This is probably the reason for which, as stated by the authors, and in contrast with the results we obtained, the association between influenza vaccination and reduced risk of COVID-19 clinical outcomes was found to be not statistically significant by random effects model and resulted only somehow significant by employing fixed effects model.

Another relevant dissimilarity between our reviews regards the inclusion criteria applied. In order to ascertain the accountability of the COVID-19 diagnosis according to the current WHO COVID-19 case definition [WHO 2020d], a set of more stringent inclusion criteria were applied to our study. For this reason, the study conducted by Noale *et al*. was - by contrast with the quantitative analysis performed by Wang *et al.* - excluded, seen as it reports SARS-CoV-2 infections attested by an online questionnaire.

For these above-mentioned reasons, and despite the discussed limitations, this systematic review and meta-analysis is, to our knowledge, the first study that provides such in-depth insight on the association between seasonal influenza vaccination and COVID-19 related outcomes. Although not all included studies demonstrated it, our findings clearly indicate that influenza vaccination has an overall protective effect against COVID-19 related outcomes, including risk of infection, risk of hospitalization, need for intensive care and death.

One of the primary reasons why some of the included studies have reported different results, among those listed hereafter, there are the considerable differences found in study design and populations.

In particular, the study performed by Greco et al. [Greco et al. 2021] found influenza vaccination to be an independent risk factor for undergoing hospitalization, however, as stated by the authors, since vaccinated patients were significantly older than un-vaccinated patients, the risk of hospitalization was strongly influenced by patients age.

One study [Pedote et al. 2021] that did not demonstrate any significant association between influenza vaccination and risk of hospitalization or death, but found a statistically significant association between health outcomes in COVID-19 patients, age >65 and chronic disease, did not however employ the Charlson Comorbidity Index (CCI) Score to assess the impact of comorbidities on disease severity / mortality predictions. Since COVID-19 disease severity is affected by comorbidity, we believe that taking into account comorbidities using this simple index may reduce the risk of bias.

Finally, the contrast between studies investigating the risk of infection in HCWs, among which some [Martínez-Baz et al. 2020, Belingheri et al. 2020, Rivas et al. 2021, Kindgen-Milles et. al 2021] did not find any significant association between influenza vaccination and the risk of SARS-CoV-2 infection, underlines the need for further research to explore this association. It should be taken into account that most of these studies did not adjust for risk exposure, which we believe to be a critical aspect to take into account when evaluating such high-risk study populations. It is precisely for this reason that, with the aim of implementing this comprehensive analysis, we decided to further investigate the association between influenza vaccination and SARS-CoV-2 positivity by carrying out on retrospective study on a cohort of HCWs working at the San Martino Polyclinic Hospital, Genoa, Italy (Chapter 3).

In conclusion, our review confirms the plausibility of influenza vaccine-induced immunity against SARS-CoV-2 and proves the vaccines overall protective effect against SARS-CoV-2 infection (pooled aOR 0.81, 95%CI: 0.70–0.94) and COVID-19 clinical sequelae. In particular, the cohort of vaccinated individuals was found to have a 31%

risk reduction [aOR 0.69 (95%CI: 0.54-0.90) - model B] for hospitalization, 14% risk reduction [aOR 0.86 (95%CI: 0.74–0.99) for intensive care, and 16% risk reduction [aOR 0.84 (95%CI: 0.71-0.99) - model B] in terms of mortality.

CHAPTER 3

SHORT-TIME EFFECT OF THE 2020/21 QUADRIVALENT INFLUENZA VACCINE ON THE SARS-COV-2 POSITIVITY IN A COHORT OF ITALIAN HEALTHCARE WORKERS.

3.1 **Background and Rationale**

The last 2020/21 season northern hemisphere influenza vaccination campaign was carried out during an unprecedented period characterized by the possible co-circulation of both influenza viruses and SARS-CoV-2 [WHO 2021d]. Due to the objective difficulties to perform a clinical differential diagnosis and the fear of higher rates of respiratory comorbidities in high-risk populations, attitudes towards influenza vaccination shifted worldwide.

Healthcare workers (HCWs), especially, have been reported to be more likely to get vaccinated against influenza during 2020 because of COVID-19 [Robbins et al. 2021] and new vaccination requirements for HCWs were introduced as well. For instance, in Italy the free-of-charge influenza vaccine offer for older adults was shifted from ≥65 to ≥60 years [Italian Ministry of Health 2020] and some Regions [Region of Latium, 2020; Region of Sicily, 2020; Region of Calabria, 2020] introduced a mandatory 2020/21 influenza vaccination for HCWs and/or institutionalized subjects.

Following these changes in vaccination policies, the first Italian estimates [Di Pumpo et al. 2021] have documented a significant increase in the vaccine uptake among HCWs.

This, together with the findings of the systematic review and meta-analysis we previously performed (Chapter 2), pointing out a negative association between seasonal influenza vaccination and COVID-19 related outcomes, led us to the attempt of substantiating this hypothesis carrying out a retrospective cohort study at San Martino Polyclinic Hospital (Genoa, Italy), investigating the association between 2020/21 season influenza vaccination and SARS-CoV-2 positivity rate in a cohort of healthcare workers (HCWs).

3.2. **Methods and Data Analysis**

3.2.1. Study design, Setting and Participants

This study adopted a retrospective cohort design and was conducted at San Martino Polyclinic Hospital (Genoa, Italy). This referral tertiary acute-care university hospital employs approximately five-thousand people (referred to as HCWs) which represented the study eligible population.

The study's intervention of interest was the seasonal 2020/21 influenza vaccination. The vaccination campaign started on 12th October 2020 and ended in mid-January 2021 even though most (90%) doses were administered in October and November 2020. The type of vaccines adopted were standard-dose egg-based quadrivalent (QIVe; Vaxigrip Tetra, Sanofi Pasteur) and standard-dose cell culture-derived quadrivalent (QIVc; Flucelvax, Seqirus). The vaccines, administered at San Martino Polyclinic Hospitals' Hygiene Unit, were actively recommended to all employees and were free-of-charge.

Vaccinal status was ascertained by linking vaccination cards to the signed informed consent. HCWs without available vaccination record and informed consent were considered non-vaccinated.

The study index date was determined *a priori* considering the lag of 14 days that is necessary to achieve protective immunity [Gross 1996] and the beginning of influenza vaccination campaign on 12th October. For this reason, the study started on 26th October and ended on 27th December 2020 when, the day after, a massive internal anti-SARS-CoV-2 vaccination campaign would have begun.

Having undergone at least two real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) tests during the study period was set as the basis for inclusion. Subjects not meeting this criterium were excluded from the base-case analysis because they may be systematically different from the rest of cohort (eg. being in smart working). A sensitivity analysis, including these HCWs in the best performing best-case model, was performed to assess this uncertainty.

The first positive test date was considered the event date for positive subjects, while the last negative test date was the event date for negative subjects [Conlon et al., 2021]. Subjects positive for SARS-CoV-2 before 26th October or before influenza vaccine administration were excluded. Analogously, vaccinated HCWs having had their last RT-qPCR test within 14 days post-vaccination were excluded.

All RT-qPCR tests were performed at the regional reference laboratory for COVID-19 diagnostic located in Polyclinic San Martino, Hygiene Unit (Genoa, Italy) within 8 hours upon the arrival of specimens. RT-qPCR was performed by using the extraction-free method on Nimbus IVD, (Seegene Inc., Republic of Korea) using the Allplex™ SARS-COV-2 Assay kit (Seegene Inc., South Korea), according to the manufacturer's instructions. RT-qPCR results are then transmitted to the Regional Healthcare Department on a daily basis.

3.2.2. Study outcome and variables

The study outcome was the incidence of SARS-CoV-2 as determined by RT-qPCR. The variable of interest was 2020/21 influenza vaccination status.

Age, sex, nationality (Italian vs foreigner), frequency of SARS-CoV-2 RT-qPCR testing and week of the last RT-qPCR test performed were considered as potential confounders. Since vaccinated subjects may be more exposed to both influenza virus and SARS-CoV-2, the frequency of RT-qPCR testing was thought to mitigate the effect of indication bias. Therefore, for each HCW, we carried out a count of performed RT-qPCR tests from the beginning of systematic testing (the first available test was performed on 7th March 2020) to the last available test performed on 27th December 2020. Since following a positive result, HCWs perform follow-up testing (usually on a weekly basis) until two consecutive negative results are obtained, these tests do not reflect the risk of exposure. For this reason, in the case of positive subjects only the number of tests undergone before their first positive test were counted. Finally, we adjusted for the week of last RT-qPCR test in order to account for the changing SARS-CoV-2 epidemiology.

3.2.3. Data analysis

The sub-cohorts of HCWs positive and negative for SARS-CoV-2 and vaccinated and non-vaccinated HCWs were described by expressing categorical data as proportions with 95% confidence intervals (CIs) and compared by the chi-square test with Yates's correction. The continuous variables were expressed as means and standard deviations (SDs) and compared using the t test. Crude risk ratio (RR) was the effect size used to express association between the SARS-CoV-2 RT-qPCR test readout and influenza vaccination status. Uni- and multivariable Cox proportional hazard modelling were employed to calculate crude (HR) and adjusted (aHR) hazard ratios respectively. Interaction terms between influenza vaccination and potential confounders were also tested in order to avoid bias and misinterpretation of the results [Vatcheva et al., 2015]. Model performance was compared by quantifying the concordance coefficient and Akaike information criterion (AIC).

All analyses were performed in R stats packages, version 4.0.3 [RStudio Team 2020].

3.3. **Results**

3.3.1. Characteristics of the cohort

At least one RT-qPCR test between 26/10/2020 and 27/12/2020 was performed by a total of 3,231 HCWs. 30 (0.9%) HCWs having had a previously documented positive RT-qPCR test, and 640 (19.8%) vaccinated HCWs having had the last available RT-qPCR test within the first two weeks following vaccination were excluded. Therefore, the final cohort included 2,561 HCWs that contributed a total of 94,438 person-day observations. 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%) had immigrant background. Influenza vaccine was administered to 35.6% (95% CI: 33.7–37.5%) of HCWs, of these 62.3% (95% CI: 59.1–65.5%) received QIVc.

290 SARS-CoV-2 infections were detected during the study period. Table 3.3.1.1 stratifies positive and negative HCWs according to sex, age, nationality, and SARS-CoV-2 testing frequency. Positive and negative subjects were uniformly distributed however, negative HCWs had about twice influenza vaccine coverage [RR = 0.43 (95% CI: 0.33–0.57)].

Table 3.3.1.1 - Comparison between positive and negative SARS-CoV-2 healthcare workers

Variable	**Positive (N = 290)**	**Negative (N = 2,271)**	**P**
Sex, % (95% CI) female	71.0 (65.4–76.2)	69.4 (67.5–71.3)	0.61
Age, mean (SD)	46.5 (11.3)	46.8 (11.5)	0.72
Immigrant background, % (95% CI)	4.8 (2.7–8.0)	3.8 (3.1–4.7)	0.51
Influenza vaccination, % (95% CI)	19.3 (14.9–24.3)	37.5 (35.5–39.5)	<0.0001
SARS-CoV-2 testing frequency, mean (SD)	5.3 (3.0)	5.2 (3.0)	0.45

Vaccinated and non-vaccinated cohorts differed from the point of view of nationality, with immigrant background having significantly lower vaccination coverage than natives [RR 0.60 (95% CI: 0.38–0.93)], and from the point of view of SARS-CoV-2 testing frequency, with vaccinated individuals performing on average more SARS-CoV-2 RT-qPCR tests ($P < 0.001$) than non-vaccinated HCWs (Table 3.3.1.2).

Table 3.3.1.2. - Comparison between vaccinated and non-vaccinated healthcare workers

Variable	**Vaccinated (N = 911)**	**Non vaccinated (N = 1,650)**	**P**
Sex, % (95% CI) female	67.7 (64.6–70.8)	70.6 (68.3–72.8)	0.14
Age, mean (SD)	46.3 (11.5)	47.1 (11.5)	0.09
Immigrant background, % (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

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

The incidence of SARS-CoV-2 was 1.62 (95% CI: 1.22–2.10) and 3.91 (95% CI: 3.43–4.45) per 1,000 person-days in vaccinated and non-vaccinated HCWs, respectively, with a HR of 0.41 (95% CI: 0.30–0.55). As shown in Table 3.3.2.1, the adjusted model 1 did not significantly differ from the un-adjusted model. A further adjustment for the week of the last RT-qPCR testing (model 2) revealed a greater effect size for the above association. Moreover, each additional molecular test was associated with a 15% increase in SARS-CoV-2 positivity. Model 2 was associated with both a substantial reduction in AIC (-22%) and an increase in concordance coefficient (from 0.62 to 0.92) with respects to model 1. In model 3, two significant interaction terms were established by testing different interactions between influenza vaccination and potential confounders. Firstly, among the vaccinated individuals the risk of SARS-CoV-2 positivity decreased by 3% with each 1-unit increase in age. Secondly, vaccinated HCWs that resulted positive to SARS-CoV-2 underwent significantly more RT-qPCR tests. Of note, the main effect of influenza vaccination turned out to be non-significant [two-tailed α<0.05 (P = 0.11)] (Table 3.3.2.1).

Table 3.3.2.1 - Multivariable Cox proportional hazard models on the association between 2020/21 influenza vaccination and SARS-CoV-2 first positive test.

Variable	Level	Model 1		Model 2*		Model 3*	
		aHR (95% CI)	P value	aHR (95% CI)	P value	aHR (95% CI)	P value
Influenza vaccine	No	Ref	–	Ref	–	Ref	–
	Yes	0.42 (0.31–0.56)	<0.001	0.19 (0.13–0.27)	<0.001	0.32 (0.08–1.30)	0.11
Sex	Female	Ref	–	Ref	–	Ref	–
	Male	1.00 (0.78–1.30)	0.99	1.00 (0.78–1.30)	0.99	0.97 (0.75–1.26)	0.84
Age	1-year increase	1.00 (0.99–1.01)	0.82	0.99 (0.98–1.00)	0.089	1.00 (0.99–1.01)	0.58
Nationality	Italian	Ref	–	Ref	–	Ref	–
	Immigrant	1.25 (0.73–2.14)	0.42	1.22 (0.71–2.10)	0.47	1.21 (0.70–2.08)	0.49
SARS-CoV-2 testing	1-unit increase	0.97 (0.93–1.00)	0.079	1.15 (1.10–1.20)	<0.001	1.11 (1.06–1.17)	<0.001
Influenza vaccine*Age		–	–	–	–	0.97 (0.94–0.99)	0.023
Influenza vaccine* SARS-CoV-2 testing		–	–	–	–	1.16 (1.06–1.28)	0.001
Concordance (standard error)		0.62 (0.02)		0.92 (0.01)		0.92 (0.01)	
AIC		4195		3269		3257	
*Models adjusted for the week of the last SARS-Cov-2 RT-qPCR test.							

Table 3.3.2.2 - Sensitivity analysis on the association between 2020/21 influenza vaccination and SARS-CoV-2 first positive test, by excluding subjects with a single SARS-CoV-2 test.

Variable	**Level**	**Model 1**		**Model 2***		**Model 3***	
		aHR (95% CI)	**P**	**aHR (95% CI)**	**P**	**aHR (95% CI)**	**P**
Influenza vaccine	No	Ref	–	Ref	–	Ref	–
	Yes	0.40 (0.29–0.54)	<0.001	0.18 (0.12–0.26)	<0.001	0.65 (0.49–0.86)	0.002
Sex	Female	Ref	–	Ref	–	Ref	–
	Male	0.99 (0.75–1.30)	0.92	0.98 (0.74–1.29)	0.88	0.96 (0.74–1.26)	0.77
Age	1-year increase	1.00 (0.99–1.01)	0.97	0.99 (0.98–1.00)	0.13	1.00 (0.99–1.01)	0.62
Nationality	Italian	Ref	–	Ref	–	Ref	–
	Immigrant	1.29 (0.75–2.22)	0.35	1.28 (0.74–2.21)	0.37	1.15 (0.66–2.00)	0.63
SARS-CoV-2 testing	1-unit increase	0.97 (0.93–1.02)	0.21	1.17 (1.12–1.23)	<0.001	1.06 (1.01–1.11)	0.009
Influenza vaccine*Age		–	–	–	–	0.99 (0.99–0.99)	0.023
Influenza vaccine* SARS-CoV-2 testing		–	–	–	–	1.05 (1.01–1.10)	0.013
Concordance (SE)		0.62 (0.02)		0.92 (0.01)		0.90 (0.01)	
AIC		3727		2855		3194	
*Models adjusted for the week of the last SARS-Cov-2 RT qPCR test.							

In the sensitivity analysis, by excluding HCWs that underwent only one molecular test (N = 236), no major changes occurred even though a slight decrease in AIC was observed and the main effect of influenza vaccination was found to be statistically significant (Table 3.3.2.2).

3.4. **Discussion**

Consistently with the systematic review and meta-analysis we previously performed (Chapter 2), the results of our study indicate that influenza vaccine is associated with decreased risk of SARS-CoV-2 infection.

We applied robust analytic techniques to reduce the risk of bias and confounding commonly found in observational studies. A strength of the study is that, by contrast with other studies of the same kind [Martínez-Baz et al. 2020, Belingheri et al. 2020, Rivas et al. 2021, Kindgen-Milles et. al 2021], we adjusted our results for the risk of exposure. In particular, because each additional molecular test was associated with a 15% increase in testing positive and vaccinated HCWs performed on average more SARS-CoV-2 RT-qPCR tests ($P < 0.001$), it is safe to affirm that vaccinated HCWs that resulted positive to SARS-CoV-2 also underwent significantly more RT-qPCR tests.

Furthermore, although literature clearly indicates that higher rates of COVID-19 are reported in older patients [CDC 2021b], we have found the risk of SARS-CoV-2 positivity to decrease by 3% with each 1-unit increase among the vaccinated individuals, reinforcing the hypothesis of an influenza vaccine overall protective effect against the risk of SARS-CoV-2 infection. These findings might have relevant implications from a public health perspective and highlight, now more than ever, the importance of pursuing worldwide effective influenza vaccination campaigns.

However, some limitations to our study must be considered. Firstly, Influenza and SARS-CoV-2 variability must be taken into account. In particular, our study was conducted during 2019-20 season and, since influenza vaccines vary substantially across

years, it is not clear whether the results of this study could be applied for different influenza seasons. Moreover, at the time of our study, three main SARS-CoV-2 clade (20E(EU1), 20B and 20A) were circulating in Italy and the variant of concern B.1.1.7 (also known as "British Variant") had just begun to spread [Capozzi et al. 2021]. It is possible that, with the evolving of the pandemic and the spreading and emerging of new variants of concern, the finding of our study may not apply to a new setting.

Finally, it should also be noted that vaccination cards and the informed consent forms employed to ascertain vaccination status were originally kept for administrative and non-epidemiological purposes.

Nonetheless, the results of this retrospective cohort study pointed out a significant reduction in the odds of testing positive for COVID-19 in patients who received an influenza vaccine compared to those who did not. These evidences reinforce the importance of promoting influenza vaccination campaigns, particularly while SARS-CoV-2 vaccines are still sub-optimally available worldwide, in order to reduce the burden of both influenza and COVID-19.

3.5 **Declarations**

The preliminary findings described in this chapter are a slightly modified version of the manuscript entitled "Short-time effect of the 2020/21 quadrivalent influenza vaccine on the SARS-CoV-2 positivity in a cohort of Italian healthcare workers" by Alexander Domnich, Allegra Ferrari, Matilde Ogliastro, Giancarlo Icardi, that will be later submitted to a relevant journal in the field of vaccination and infectious disease.

CHAPTER 4

CONCLUDING REMARKS

The aim of this thesis was to investigate the possible reduction in the incidence of SARS-CoV-2 positivity rate and other disease related outcomes among the cohort of people immunized against seasonal influenza. This hypothesis is supported by previous observations according to which the influenza vaccine could induce immunity against SARS-CoV-2, mainly due to the stimulation of trained innate immune memory able to trigger, when another respiratory pathogen is present, a local lung immune system rapid response [Netea et al. 2020; Salem 2020;] (Chapter 1 – *"A possible cross protective immunity*").

The objective was achieved in two consecutive steps: (i) a systematic review and meta-analysis of the previously published reports on this topic, and (ii) a retrospective cohort study conducted at San Martino Polyclinic Hospital (Genoa, Italy), investigating the association between 2020/21 season influenza vaccination and SARS-CoV-2 positivity rate in a cohort of healthcare workers (HCWs).

Three databases (Ovid MEDLINE®, Biological Abstracts, CAB Abstracts including Global Health) were searched from inception to 09 March 2021. Random effects models were used to pool adjusted estimates with 95% confidence intervals (CIs). 33 studies were included in the systematic review. Of them, 8 and 10 studies regarding the association between influenza vaccination and, respectively, SARS-CoV-2 infection and COVID-19 clinical outcomes, contained adjusted estimates (aOR or aHR) and were included in the quantitative analysis.

Influenza vaccination was found to be associated with a lower risk of SARS-CoV-2 infection (pooled aOR 0.81, 95%CI: 0.70–0.94) and COVID-19 clinical sequelae. In particular, the cohort of vaccinated individuals was found to have a reduced risk of hospitalization (pooled aOR 0.69, 95%CI: 0.54-0.90 - model B), need for intensive care (pooled aOR 0.86, 95%CI: 0.74–0.99) and death (pooled aOR 0.84, 95%CI: 0.71-0.99 - model B).

The available local data was then employed to carry out a retrospective cohort study (between 26th October and 27th December 2020), with the aim of evaluating the incidence of SARS-CoV-2 on a cohort of Italian HCWs working at the San Martino

Polyclinic Hospital. The final sample size included 2,561 HCWs among which 35.6% (95% CI: 33.7–37.5%) were immunized against seasonal influenza. The incidence of SARS-CoV-2 was 1.62 (95% CI: 1.22–2.10) and 3.91 (95% CI: 3.43–4.45) per 1,000 person-days in vaccinated and non-vaccinated HCWs, respectively, with an adjusted HR of 0.42 (95% CI: 0.31–0.56) (model 1).

Since vaccinated subjects may be more exposed to both influenza virus and SARS-CoV-2, the frequency (n.) of RT-qPCR testing was used to mitigate the effect of indication bias. Moreover, the results were adjusted for the week of last RT-qPCR test in order to account for the changing SARS-CoV-2 epidemiology. As a result, model 2 indicated a greater effect size for the above association, with HR of 0.19 (95% CI: 0.13–0.27).

Finally, model 3 showed that among the vaccinated individuals the risk of SARS-CoV-2 positivity decreases by 3% with each 1-unit increase in age, further backing up the hypothesis, mentioned in Chapter 2 (*"2.3.7 Association between influenza vaccination and COVID-19 clinical outcomes in the elderly"*), of an even greater protective effect of influenza vaccination in the frailer age group.

In order to compile an accurate and up-to-date report on the link between Influenza vaccination and SARS-CoV-2 infection, the previously performed meta-analysis (Chapter 2 – *"2.3.1. Infection"*) was implemented through the addition of the findings of the study conducted at San Martino Polyclinic Hospital. Although this study has not been yet peer reviewed, the addition of these preliminary results revealed an even greater reduction in the odds of being infected with SARS-CoV-2 [aOR 0.66 (95%CI: 0.51–0.85) – model D], further reinforcing results obtained thus far regarding influenza vaccine's protective effect against this virus.

Despite the robust analytic techniques applied and the consistency between the findings of the study conducted at San Martino Polyclinic Hospital and the pooled adjusted estimates of the systematic review and meta-analysis we performed, it should be considered that the employed data mostly refer to 2019-20 influenza season alone. Since influenza vaccines vary substantially across years, also considering the possible

emerging of new coronavirus variants, it is not clear whether our findings could be applied for different seasons.

Nonetheless, these results are particularly important in the context of the ongoing global pandemic and, in particular, in light of the phenomenon, which has become increasingly common in the past decades, of vaccine hesitancy. In fact, despite the availability of safe vaccines and the high burden of seasonal influenza, which alone leads to an estimated 3-5 million cases of severe disease and 250-500,000 deaths each year [WHO 2012], influenza vaccine coverage rates within specific risk groups are still well below the recommended rate of 75% [Council of the European Union 2009; ECDC 2015]. Since patients with influenza have an increased risk of severe disease and mortality during a co-infection with SARS-CoV-2 [Sarkar et al. 2020], this issue was exacerbated when last 2019/20 northern hemisphere and 2020 southern hemisphere seasonal influenza epidemics overlapped with the COVID-19 pandemic [WHO 2016b; Domnich et al. 2020].

In the context of the impending influenza-COVID-19 "twindemic", carry out influenza vaccination campaigns is fundamental for reducing the pressure on healthcare systems. For this reason, we hope that the findings reported in this thesis, underlining the overall protective effect that influenza vaccines may have against SARS-CoV-2 disease and related sequelae, may help boost robust educational campaigns and integrated social and health policy initiatives, particularly towards “hard to reach”, at high-risk and marginalized populations.

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