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Influenza Vaccination and Risk of Ischemic Stroke: A Population-Based Case-Control Study

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ABSTRACT

Background and objectives: To assess the relationship between influenza vaccination in the general population and risk of a first ischemic stroke (IS) during pre-epidemic, epidemic and post-epidemic periods.

Methods: A nested case-control study was carried out in a Spanish primary care database over 2001-2015. Subjects aged 40-99 years with at-least 1-year registry and no history of stroke or cancer were selected to conform the source cohort, from which incident IS cases were identified and classified as cardioembolic or non-cardioembolic. Five controls per case were randomly selected, individually matched with cases for exact age, sex and date of stroke diagnosis (index date). A patient was considered vaccinated when he/she had a recorded influenza vaccination at least 14 days before the index date within the same season. Adjusted odds ratios (AOR) and their respective 95% confidence intervals (CI) were computed through a conditional logistic regression. Pneumococcal vaccination was used as a negative control.

Results: From a cohort of 3,757,621 patients, we selected 14,322 incident IS cases (9,542 non-cardioembolic and 4,780 cardioembolic) and 71,610 matched controls. Of them, 41.4% and 40.5%, respectively, were vaccinated yielding a crude OR of 1.05(95%CI:1.01-1.10). Vaccinated subjects presented a higher prevalence of vascular risk factors, diseases and comedication than non-vaccinated and, after full adjustment, the association of influenza vaccination with IS yielded an AOR of 0.88(95%CI:0.84-

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0.92) was found, appearing early ($AOR_{15\text{-}30\ days}$=0.79;95%CI:0.69-0.92) and slightly declining over time ($AOR_{>150\ days}$=0.92;95%CI:0.87-0.98). A reduced risk of similar magnitude was observed with both types of IS, in the three epidemic periods and in all subgroups analyzed (men, women, subjects below and over 65 years of age, and subjects with intermediate and high vascular risk). By contrast, pneumococcal vaccination was not associated with a reduced risk of IS (AOR=1.08;95%CI:1.04-1.13).

Discussion: Results are compatible with a moderate protective effect of influenza vaccine on IS appearing early after vaccination. The finding that a reduced risk was also observed in pre-epidemic periods suggests that either the "protection" is not totally linked to prevention of influenza infection, or it may be partly explained by unmeasured confounding factors.

INTRODUCTION

Though widely accepted that seasonal influenza increases the risk of ischemic stroke (IS)[1-4] the role of vaccine in its prevention is still under debate.[5-9] In a recent meta-analysis of observational studies, Lee et al[10] reported a pooled Odds Ratio of 0.82(95%CI:0.75–0.91) for stroke of any type (based on 10 studies) and 0.77(95%CI:0.60–0.98) for IS (based on 6 studies,[6,7,9-11-13] though 3[7,9,12] of them did not show any reduction), but the heterogeneity was high (I^2=75.6%) and most studies had less than 1000 stroke cases). Further, observational studies carried out in this area have been questioned for being presumably affected by several types of bias (i.e. healthy-user bias, adherent-user bias, or frailty bias) that could explain the favorable results found.[14-16] To circumvent this problem, or at least to reveal a hidden bias, Jackson et al[14] have proposed to analyze the data by time periods during the same season (before, during and after influenza waves), assuming that, in the postulated causal model, influenza vaccine

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would reduce the risk of IS via prevention of influenza infection and, thus, be mostly observed during the epidemic periods (as compared, for instance, to the pre-epidemic ones). As far as we know, none of the published studies examining the association of influenza vaccine with IS analyzed the data this way. Also, none of the studies has distinguished between the two main types of IS, cardioembolic and non-cardioembolic, which may be important to elucidate the pathogenic mechanism by which influenza vaccination would reduce the risk (e.g. stabilization of atherosclerotic plaque, prevention of a pro-coagulant state induced by the infection).[17] Assuming that influenza infection promotes the rupture of atherosclerotic plaques, we postulate that influenza vaccination would have a greater preventive effect on non-cardioembolic IS, mostly during epidemic periods.

The aim of the present study was to test the hypothesis that influenza vaccination reduces the risk of a first IS and to assess whether this effect is modified by different time periods (pre-epidemic, epidemic and post-epidemic), type of IS (cardioembolic *vs.* non-cardioembolic), and timing since vaccination. Finally, we used the pneumococcal vaccination as a negative control since it has been reported not to be associated with a decreased risk of vascular events,[8] while it may share the aforementioned biases.

PATIENTS AND METHODS

Study design and data source

We carried out a population-based case-control study using BIFAP (Base de datos para la Investigación Farmacoepidemiológica en el Ámbito Público), a Spanish healthcare database containing anonymized records of patients attended by primary care physicians (PCP) in the National Health System. The data recorded includes clinical events,

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prescriptions, laboratory tests and free-text annotations with their corresponding dates. BIFAP-2016 version contained around 7.6 million patients, with an average follow-up of 5.1 years, totaling 38.6 million person-years from nine different Spanish autonomous communities (out of 17).[18] BIFAP reflects the distribution of the Spanish population by sex and age, and has been validated through numerous studies,[19-20] showing comparable results to other well-known European databases.[21,22]

The study period spanned from January 1, 2002 to December 31, 2015. In a first step, we constructed the study cohort with all patients registered in the study period who were between 40 and 99 years old, had a record of at least one year with their PCP and had no history of cancer or IS. Patients entering the cohort were followed up until the occurrence of an incident diagnosis of IS, a diagnosis of cancer, 100 years of age, death, or the end of the study period, whichever occurred first.

Outcome definition and selection of cases

Potential IS cases were identified through the specific diagnosis codes from the International Classification of Primary Care (K90), and the International Classification of Diseases, 9th revision-clinical modification (434.x1 and 436), that PCPs included in the clinical records using the hospital discharge report. We also searched the free text associated with the diagnosis field, using strings related with IS. To ascertain that potential IS cases were true IS cases, we performed a validation procedure by classifying potential IS cases in categories according to the amount of information available and the specific part where this information appeared within the clinical record; then, we estimated the positive predictive value (PPV) of each case category through the manual review of clinical records from a random sample performed by two investigators (SRM and DBH), all the time blinded to drug/vaccine exposure, who used

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the criteria set by a validation group including a neurologist (LIE) and a cardiologist (AGLl). Discrepancies were solved by consensus of the whole team, reaching a final PPV of 87.1%. Then, such algorithm was applied to all potential cases yielding a total number of 14374 IS cases (eMethods in Supplement).

For some analyses we subclassified IS cases into cardioembolic and non-cardioembolic stroke, the latter including large artery atherosclerosis infarct, small vessel occlusion (lacunar stroke) and stroke of undetermined cause, by studying the PCP's annotations in the free text associated with the diagnosis before or within 3 months after the event. Texts of “cardioembolic”, “atrial fibrillation", as well as prescriptions for oral anticoagulants (OAC) before the event or within 3 months post-event, either alone or combined were used as the main criteria for supporting a cardioembolic stroke. Additionally, “mitral valve prosthesis”, or “mitral stenosis”, and use of class IC or III antiarrhythmics were used as complementary criteria when at least one of the main criteria were present (eMethods in the Supplement). Cases not fulfilling these criteria and those including texts such as “atherothrombotic”, “lacunar” or related terms were considered as non-cardioembolic. Strokes of unusual cause (eg, vasculitis, dissection, consumption of toxic substances) were excluded from all analyses.

For the present study, we decided to include TIA as a risk factor for IS and not as an outcome to avoid mixing TIA with IS and assure that all events considered were independent.

The index date of cases was the date of IS diagnosis recorded.

Selection of controls

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Five controls per case, individually matched to cases by exact age, sex and index date, were drawn from the study cohort following a risk-set sampling. This method assures that the odds ratios calculated are an unbiased estimate of the rate ratios of the underlying cohort, even in the presence of competing risks.[23,24] The index date for controls was the one of their matched cases.

Time periods definition

The study period included 15 influenza seasons (though the first and the last were not completed), the beginning of each season being set on September 1 and the end on August 31 of the following year.

Each influenza season was divided into three different periods: 1)pre-epidemic: from September 1 to the start of the influenza epidemic wave; 2)epidemic: the period of the epidemic wave defined for each season by the Influenza Surveillance System (https://vgripe.isciii.es/inicio.do); and 3)post-epidemic: from the end of the epidemic wave to the end of the season (August 31).[16] Dates and main characteristics of the different periods for each influenza season are shown in eTable 1 in the Supplement.

Exposure definition

A patient was considered exposed to influenza vaccination when received the vaccine beyond 14 days prior to the index date within the same influenza season (as 14 days is the time normally required to develop an immune response; see https://www.ecdc.europa.eu/en/seasonal-influenza/prevention-and-control/vaccines/timing). However, in a sensitivity analysis we extended this time to 30 days to additionally account for a potential mismatch between the true date of stroke

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onset and the recording date of stroke diagnosis by the PCP (which for some patients may correspond to the first office visit). Several time windows were established to assess the risk of IS from the time of vaccination (days 15-30, and every 30 days thereafter).

Likewise, patients were considered exposed to pneumococcal vaccine if they had a record beyond 14 days before the index date (any time before, but in an additional analysis, we only considered those vaccinated within the same season). Additionally, a sensitivity analysis was carried out using an exposure window of of beyond 30 days before the index date.

The influenza vaccination uptake in the population (overall and by age and background vascular risk) was estimated through the average prevalence of subjects vaccinated among controls during post-epidemic periods.

Potential confounding factors

We adjusted for the following potential confounding variables collected prior to the index date: 1)number of PCP´s visits in the year prior to the index date (as a general indicator of comorbidity); 2)lifestyle factors: body mass index (BMI), smoking status and alcohol abuse; 3)history of the following comorbidities (recorded any time before the index date): transient ischemic attack (TIA), ischemic heart disease (including acute myocardial infarction (AMI) or angina pectoris -including the use of nitrates as an indicator of angina-), thromboembolic disease, heart failure, atrial fibrillation, peripheral artery disease, hypertension, diabetes (recorded as such and/or use of glucose-lowering drugs), dyslipidemia (recorded as such and/or use of lipid-lowering drugs), hyperuricemia (either asymptomatic or in the context of gout), chronic obstructive pulmonary disease, rheumatoid arthritis and chronic renal failure; and

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4)current use (last prescription lasting to the 30 days prior to the index date) of the following drugs: antiplatelet drugs, oral anticoagulants, beta-blockers, alfa-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), calcium-channel blockers (CCBs), diuretics, paracetamol, metamizole, non-steroidal anti-inflammatory drugs (NSAIDs), opioids, corticosteroids, proton pump inhibitors (PPIs) and H2-receptor blockers.

Statistical analysis

The association between influenza vaccination and incident IS was assessed through conditional logistic regression models to estimate the crude Odds Ratios (ORs) and their corresponding 95% confidence intervals (CIs), including only the influenza vaccination status, and the adjusted OR (AOR) adding all the potential aforementioned confounders.

We also assessed the interaction with the following factors: age (under 65 years and 65 years or over), sex and background vascular risk. The latter was categorized as: 1)patients with an established vascular disease: TIA, ischemic heart disease, atrial fibrillation, peripheral artery disease or diabetes; 2)patients with vascular risk factors but no vascular diseases: current smoking, obesity (defined as BMI≥30kg/m2), hypertension, dyslipidemia or chronic renal failure; and 3)patients without vascular risk factors: those not classified in the above categories. Interaction assessment was performed by applying the fully adjusted regression models across different strata of each interacting variable and comparing the AORs associated with influenza vaccination with the interaction test described by Altman and Bland.[25] Results were considered statistically significant when the p-value was less than 0.05. For the stratified analysis by background vascular risk, we performed an unconditional logistic

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regression (including the matching variables in the model) because conditional logistic regression provided unstable estimates.

Missing values of smoking and BMI variables were identified in a specific category. To address this, we applied multiple imputation by chained equations models (MICE)[26,27] to estimate the association measures.

Sensitivity analysis

1)Excluding from the cardioembolic type those cases subclassified as such with just one of the main criteria; 2)considering the patients exposed to influenza vaccination when received the vaccine 30 days or beyond; 3)excluding hyperuricemia, gout, rheumatoid arthritis and current use of alfa-blockers, paracetamol, metamizole, opioids, PPIs and H2 receptor blockers from the model (in order to run a more parsimonious one); 4)using the missing-indicator method for missing data of smoking and BMI.

We conducted all analyses using STATA version 15/SE (StataCorp. College Station, Texas. 77845, USA).

Standard Protocol Approvals, Registrations, and Patient Consents

The project was approved by by the Scientific Committee of BIFAP on 26 February, 2020 and by the Research Ethics Committee of the University Hospital Príncipe de Asturias, (Ref FLU-ATERO-CACO; # 01/20) on Oct 2th, 2020, which granted a waiver to obtain the informed consent, as investigators had no access to personal data.

Data Availability

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Anonymized data not published within this article will be made available by reasonable request from any qualified investigator.

RESULTS

From a primary base of 3,8 million patients who met the inclusion criteria we identified 14,322 incident cases of IS and randomly extracted a total of 71,610 matched controls; 30.6% of cases fell in pre-epidemic periods (82.64 cases per 4-weeks), 20.4% in epidemic periods (74.82 cases per 4-weeks) and 49.0% in post-epidemic periods (66.20 cases per 4-weeks) (Figure 1). Most patients were vaccinated between week 38 and week 49 of the year (from early October to mid-December), with no differences in the calendar time of vaccination between cases and controls (additional data are shown in eFigure 1 in the Supplement).

At the time of the index date, the pattern of comorbidities and comedication between cases and controls was assessed and, as expected, the former had a higher comorbidity burden. Of note, the cases presented a higher number of visits to the PCP in the last year, and a higher prevalence of alcohol-abuse recording, history of TIA, AMI, atrial fibrillation, peripheral artery disease, and use of cardiovascular drugs such as antiplatelet agents (Table 1).

The comorbidity and comedication patterns of vaccinated *vs.* unvaccinated subjects were explored among controls. Compared with unvaccinated, vaccinated subjects were older (mean age [±SD]:78.8[±9.0] *vs.* 71.8[±13.5]); p<0.001), had more visits to their PCP in the last year before the index date (mean number of visits [±SD]:17.8[±14.1] *vs.*

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11.6[±12.8]; p<0.001) and presented a higher prevalence of vascular disease and risk factors, as well as a higher use of comedication (additional data are shown in eTable 2 and eFigure 2 in the Supplement). Such differences between vaccinated and unvaccinated subjects were even more pronounced in people aged <65 years old (eFigure 3 in the Supplement).

The proportion of influenza vaccination in the same season was slightly higher among cases (41.40%), than among controls (40.46%), yielding a crude OR of 1.05(95%CI:1.01-1.10), but decreased to 0.88(95%CI:0.84-0.92) when fully adjusted for vascular risk factors and comedication (Table 2). A significant association of influenza vaccination with a lower risk of IS was observed in the three epidemic periods, AOR of 0.79(95%CI:0.71-0.88) in pre-epidemic, AOR of 0.88(95%CI:0.81-0.97) in epidemic and AOR of 0.91(95%CI:0.86-0.97) in post-epidemic period. No significant interaction by period was observed (interaction test: p=0.131 for epidemic *vs*. pre-epidemic and p=0.545 for epidemic *vs*. post-epidemic) (Table 2). The association of influenza vaccination with a reduced risk of IS was observed for both non-cardioembolic (AOR=0.88;95%CI:0.83-0.93) and cardioembolic IS (AOR=0.90;95%CI: 0.83-0.98) (Table 2).

The reduced risk of IS associated with influenza vaccination remained statistically significant in both age categories examined (younger than 65 years and equal to or older than 65 years), in both men and women, and in patients with vascular risk factors or established vascular disease, but not in patients without vascular risk factors. Nevertheless, no statistically significant interaction was found in any subgroup analysis (additional data are shown in Figure 2 and eTable 3 in the Supplement).

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The reduced risk of IS associated with influenza vaccination appeared early and slightly declined over time though persisted significant over the season (Figure 3).

As far as vaccination against pneumococcus, no association was found with IS when considered vaccination any time before (AOR=1.08;95%CI:1.04-1.13), nor when vaccination was restricted to the same season (AOR=1.04;95%CI:0.93-1.16). The inclusion of influenza vaccination in the model led to an increased risk (AOR=1.14;95%CI:1.09-1.19, for vaccination any time before; and AOR=1.10;95%CI:0.98-1.23 for vaccination within the same season) (Table 3). No association between pneumococcal vaccination and IS was observed in any time window since vaccination (eTable 4 in the Supplement).

The sensitivity analyses did not show any relevant difference with the main analysis: 1)the exclusion of cardioembolic cases with only one of the main criteria yielded an AOR=0.90;95%CI:0.82-0.99 (eTable 5 in the Supplement); 2)the change of the exposure window to beyond 30 days yielded an AOR=0.89;95%CI:0.85-0.94, for influenza vaccination (eTable 6 in the Supplement) and an AOR=1.09;95%CI:1.04-1.13, for pneumococcal vaccination (eTable 7 in the Supplement); 3)the most parsimonious model yielded an AOR=0.87;95%CI:0.82-0.94, (eTable 8 in the Supplement); and 4)the use of missing indicator yielded an AOR=0.88;95%CI:0.84-0.92 (eTable 9 in the Supplement).

The prevalence of influenza vaccination among control patients was 16.4% in subjects younger than 65 years and 59.6% in older subjects, being modulated by the presence of

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vascular risk factors and diseases (eFigure 4 in the Supplement). Even in the oldest patients with vascular diseases the prevalence of influenza vaccination was below 70%.

DISCUSSION

The main findings of the present study are as follows: 1)influenza vaccination was associated with a reduced risk of IS of around 10-15%, which started earlier and persisted over time up to next season; 2)the reduction in risk was observed for both non-cardioembolic and cardioembolic IS alike; 3)the risk reduction associated with influenza vaccination appeared to be similar in pre-epidemic, epidemic and post-epidemic periods; 4)no effect modification was observed by sex or age; 5)the risk reduction was observed in patients with established vascular diseases) or those with vascular risk factors, while it was not found in subjects with no risk factor; and 6)we did not find a similar decreased risk of IS in subjects who received the pneumococcal vaccine.

Observational studies are affected by biases that in many cases are difficult to control for, like healthy-user bias, so the question of whether influenza vaccination has a preventive effect against stroke can only be answered definitively with a randomized clinical trial (RCT). However, RCTs thus far have evaluated composite events like cardiovascular death or MACE (major adverse cardiovascular events),[28,29] but were unable to evaluate IS as a separate outcome due to the small number of events.[30] In a meta-analysis of RCTs carried out in high-risk patients, Udell et al[31] estimated a pooled relative risk (RR) of major MACE associated with influenza vaccine of

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0.64(95%CI:0.48–0.86). Thus, for IS the only available evidence is provided from observational studies.

In 2007, Lee et al[10] published a meta-analysis of 6 observational studies with various designs and estimated a pooled OR of 0.77(95%CI:0.60–0.98) for IS, but the heterogeneity found was high (I^2=75.6%) and the whole sample size rather small (barely more than 3,000 IS cases when all studies were combined), five times lower than the number of cases included in our study. In a more recent meta-analysis, Tsivgoulis et al[32] included 12 studies (RCTs and cohort studies) and found a lower risk of IS in influenza vaccinated subjects (pooled RR=0.87;95%CI:0.79-0.96) with moderate evidence of heterogeneity (I^2=53%) and a total number of events of around 5000 cases (largely from cohort studies). In the same meta-analysis, no reduced risk was found with the pneumococcal vaccine (pooled RR=1.38;95%CI:0.60-3.16).

Other studies not included in the previously mentioned meta-analyses also showed a reduced risk. Asghar et al,[33] in a self-controlled case-series study, found an incidence rate ratio of 0.45(95%CI:0.36-0.57) in the first 3 days after influenza vaccination, which remained statistically significant up to 59 days; unfortunately, they did not distinguish between ischemic and hemorrhagic strokes. Interestingly, early-season vaccination was associated with a greater reduction compared to late-season vaccination.[33] Chiang et al,[34] in a case-control study of an elderly population (≥ 65 years) in Taiwan found an AOR of 0.80(95%CI:0.77-0.82) associated with the influenza vaccine received in the previous year. Finally, Kao et al [35] in a propensity-score matched cohort study conducted in Taiwan in patients with atrial fibrillation observed that influenza vaccinated subjects, as compared to unvaccinated, presented an adjusted hazard ratio of

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0.59(95%CI:0.50-0.71) during the influenza season. During non-influenza season, they also found an important reduced risk (AOR=0.50;95%CI:0.40-0.61).

The results observed in the present study mirror those obtained in a previous research conducted by our group to evaluate the effect of influenza vaccination on the risk of incident AMI[36]: a 10-15% long lasting risk reduction, observed in the three epidemic time periods, and in all subgroups examined by age, sex and background vascular risk. Similarly, no association was observed with the pneumococcal vaccine. The recently published IAMI trial has shown a 28% reduction in a composite variable of all-cause death, MI, or stent thrombosis at 12 months[37] in patients who have sustained an acute coronary event and who were vaccinated within the first 3 days of either the coronary angiography or hospital admission. These results are consistent with previous studies performed in high-risk patients after an acute coronary event.[38,39]

The mechanisms underlying the vascular benefit are thought to be multifactorial.[40] There is compelling evidence suggesting that different types of infections, including influenza, may increase the incidence of acute coronary events,[41] and stroke.[42] Thus, it would be expected that avoiding the infection through vaccination a protection may ensue. Further, it has been reported that influenza infection induces a systemic inflammatory response that can precipitate atheroma plaque rupture mediated by elevated concentrations of reactive proteins and cytokines.[43] However, these mechanisms may explain the reduced risk observed during epidemic and, perhaps, post-epidemic periods, but not the one found during pre-epidemic periods. This finding suggests that other mechanisms different from the prevention of influenza infection (e.g. a direct biological effect) could account for the risk reduction found. Alternatively, the

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negative association can be explained by underlying biases, as pointed out by several authors.[14-16] Both explanations deserve a comment. Influenza vaccine administration has been associated with a reduction in tumor necrosis factor alpha,[44] release of interleukin 10,[45] and an increase in exhaled nitric oxide,[46] findings that are compatible with a short-term anti-inflammatory effect. Interestingly, in the IAMI trial the time-to-event curves for vaccination versus placebo started to diverge early after hospitalization and then stabilized around 3 months, supporting immediate benefits from vaccination.[37,40] As commented before, Asghar et al[33] reported data compatible with an early effect of vaccination. Regardless the precise underlying mechanism (either a direct or indirect effect, or both), it would be expected that influenza vaccination had a greater impact on the atherothrombotic type of IS (either on large or small vessels); thus, it is intriguing that the risk of cardioembolic stroke is also reduced by influenza vaccination. As aforementioned, Kao et al[35] have reported a reduced risk of IS associated with influenza vaccination among patients with atrial fibrillation which is consistent with our results. To further clarify the underlying mechanisms, it would be interesting to explore the association of hemorrhagic stroke with influenza vaccination which is planned to be our next step in this research line.

Regarding the potential for bias, it is important to note that we can reasonably rule out the possibility that vaccinated subjects were healthier than non-vaccinated (the so-called healthy-user bias), since vaccinated patients presented a heavier burden of comorbidity (and comedication as indicator of comorbidity) than unvaccinated, clearly evident in all age groups, but particularly in those younger than 65 years old. A risk reduction in pre-epidemic could also be explained if those who would be cases (frail patients or those with intercurrent diseases) were vaccinated later than those who would be selected as

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controls. However, the calendar time of vaccination for cases and controls completely overlapped (eFigure 1 in the Supplement) excluding this possibility. Finally, it can be argued that vaccinated subjects may be more adherent to general preventive measures, such as a healthier diet and regular exercise, variables that are not usually measured in databases and, thus, difficult or impossible to adjust for in observational studies. The same may happen with frailty or functionality (variables rarely reported that may condition vaccination). While, admittedly, a residual confounding may exist due to these unmeasured factors, the negative result obtained with the pneumococcal vaccine suggests that they may not be the sole explanation of the negative association found between influenza vaccine and IS, as the same should be expected for the association between pneumococcal vaccine and IS.

The uptake of influenza vaccine was lower than it would be desirable, particularly among high-risk populations, which is a worrying result. Nevertheless, Spain is among the European countries with the highest rates of vaccination among the elderly.[47]

Our study has the following strengths: 1)while the access to the data by the investigators was retrospective, it is important to stress that the information included in the database was collected prospectively by the primary care clinicians; 2)the study sample size was large (even larger than the overall sample size of previous meta-analyses) and allowed us to estimate risks with reasonable precision; 3)the investigators who conducted the validation of IS cases were blinded to the vaccination status of patients, thus avoiding a differential misclassification of the event; 4)we made a validation effort to determine the pathophysiologic subtype of IS (cardioembolic or non-cardioembolic), whereas some studies published to date do not even differentiate between ischemic and

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hemorrhagic stroke; and 5)controls were randomly drawn from the person-time of the underlying cohort, thus avoiding a selection bias and ensuring that the odds ratios obtained are unbiased estimates of incidence rate ratios.[48]

The limitations of the study should also be pointed out: 1)as in any observational study, there may be unmeasured factors (frailty, lack of functionality and adherence to healthier life-style measures, among others) that can behave as hidden confounding factors; 2)only vaccinations carried out through the National Health System were recorded, so patients vaccinated in private medical care would be misclassified as unexposed; however, in Spain there is universal coverage by the National Health System and access to the vaccine is free of charge, so it is likely that private vaccination happened in a negligible proportion of subjects (particularly among the elderly); actually, the uptake of vaccination in our study in the elderly was practically identical to the one officially provided; [47] at any rate, a misclassification of the exposure, if non-differential with respect to the case status, would have distorted the measure of association towards the null and, thus, against our working hypothesis; 3)despite our efforts to validate the pathophysiological subtype of IS, we must admit that this is a challenging task, in particular when done through computer records, and thus some misclassification may exist; and 4)for some cases, it is possible that the index date may not correspond to the true stroke onset but the one on which the PCP recorded it; to accommodate for such potential mismatch between the two dates we performed a sensitivity analysis looking at vaccinations performed beyond 30 days before the index date and the main results did not materially change.

CONCLUSIONS

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The findings of this study are consistent with a moderate protective effect of influenza vaccine on IS in the general population, which is consistent with other published studies. The fact that a reduced risk was also observed during pre-epidemic periods is, however, intriguing and suggests that other mechanisms different from the prevention of influenza infection may play a role. Alternatively, results can be explained by a hidden bias, though the negative results obtained with the pneumococcal vaccine do not support this explanation. The uptake of influenza vaccine is still lower than optimal in high-risk populations and should be encouraged.

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Figure 1. Flowchart of patient selection.

Abbreviations: PCPs: Primary Care Physicians.

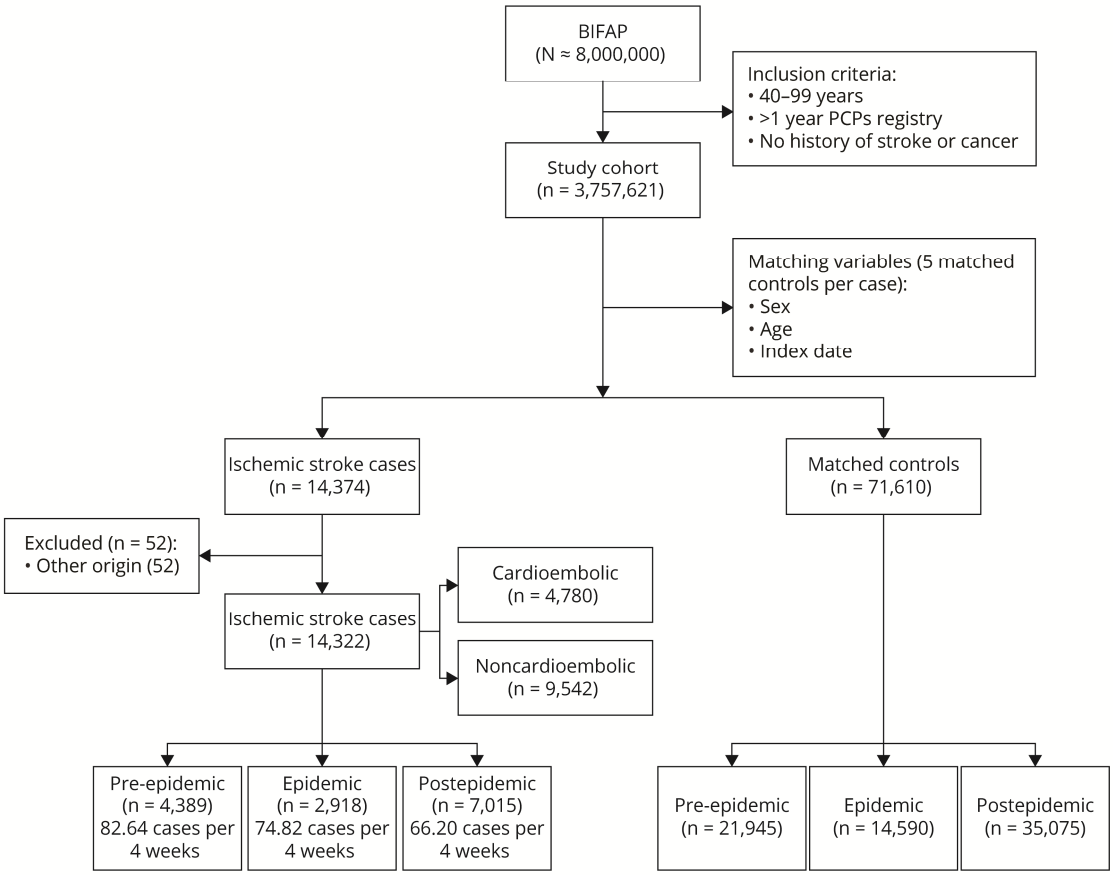

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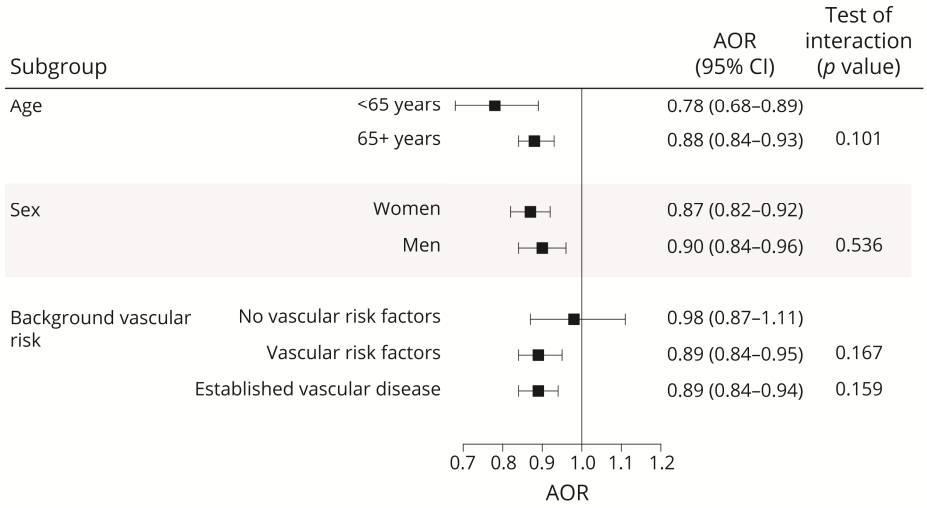
Figure 2: Influenza vaccine and risk of ischemic stroke by different subgroups (sex, age and background vascular risk).



Abbreviations: AOR: Adjusted Odds Ratio; CI: confidence interval.

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Figure 3: Risk of ischemic stroke and timing since influenza vaccination.

Abbreviations: AOR: Adjusted Odds Ratio; CI: confidence interval.

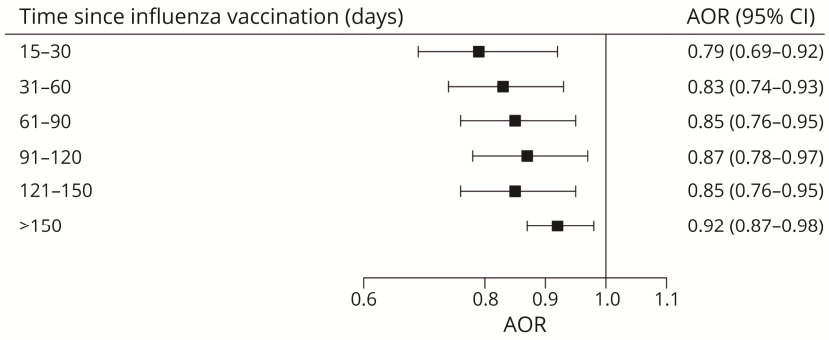

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Table 1. Baseline characteristics of cases and controls.

	Cases (%) **N=14,322**	**Controls (%)** **N=71,610**	**Non-adjusted OR*** **(95% CI)**
Age; mean (SD)	74.7 (± 12.3)	74.7 (± 12.3)	-
Men	7,166 (50.03)	35,830 (50.03)	-
PCP visits (last 12 months)			
Up to 5	2,485 (17.35)	19,857 (27.73)	1 (Ref.)
6-15	5,276 (36.84)	26,930 (37.61)	1.66 (1.58-1.75)
16-24	3,336 (23.29)	13,353 (18.65)	2.24 (2.11-2.37)
25+	3,225 (22.52)	11,470 (16.02)	2.65 (2.49-2.82)
BMI kg/m^2			
Up to 24.9	2,036 (14.22)	9,840 (13.74)	1 (Ref.)
25-29	4,227 (29.51)	20,904 (29.19)	0.98 (0.92-1.04)
30-34	2,565 (17.91)	11,882 (16.59)	1.05 (0.98-1.12)
35-39	749 (5.23)	3,104 (4.33)	1.17 (1.07-1.29)
40+	241 (1.68)	804 (1.12)	1.46 (1.26-1.70)
Unknown	4,504 (31.45)	25,076 (35.02)	0.86 (0.81-0.91)
Smoking			
Never smoking	4,760 (33.24)	23,693 (33.09)	1 (Ref.)
Current smoker	2,266 (15.82)	7,980 (11.14)	1.49 (1.41-1.59)
Past smoker	963 (6.72)	3,774 (5.27)	1.33 (1.23-1.44)
Unknown	6,333 (44.22)	36,163 (50.50)	0.88 (0.84-0.91)

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Alcohol abuse	416 (2.90)	1,095 (1.53)	1.95 (1.74-2.19)
Transient ischemic attack	790 (5.52)	1,643 (2.29)	2.52 (2.31-2.75)
Ischemic heart disease			
Myocardial infarction	871 (6.08)	2,490 (3.48)	1.89 (1.74-2.04)
Angor pectoris †	1,289 (9.00)	4,527 (6.32)	1.53 (1.43-1.64)
Thromboembolic disease	323 (2.26)	1,193 (1.67)	1.36 (1.20-1.54)
Heart failure	1,079 (7.53)	3,256 (4.55)	1.75 (1.63-1.88)
Atrial fibrillation	2,113 (14.75)	5,413 (7.56)	2.19 (2.07-2.31)
Peripheral artery disease	703 (4.91)	1,742 (2.43)	2.10 (1.92-2.30)
Hypertension	8,980 (62.70)	38,246 (53.41)	1.54 (1.48-1.60)
Diabetes §	4,086 (28.53)	13,563 (18.94)	1.73 (1.66-1.80)
Dyslipidemia ‖	6,344 (44.30)	28,288 (39.50)	1.23 (1.19-1.28)
Hyperuricemia			
Asymptomatic	1,132 (7.90)	5,110 (7.14)	1.14 (1.06-1.22)
Gout	709 (4.95)	2,757 (3.85)	1.32 (1.22-1.44)
COPD	1,230 (8.59)	5,208 (7.27)	1.21 (1.13-1.29)
Rheumatoid arthritis	124 (0.87)	638 (0.89)	0.97 (0.80-1.18)
Chronic kidney failure	825 (5.76)	2,682 (3.75)	1.59 (1.46-1.72)
Current use of			
Antiplatelet drugs	3,839 (26.80)	11,747 (16.40)	2.15 (2.05-2.25)
Oral anticoagulants	1,127 (7.87)	3,945 (5.51)	1.54 (1.43-1.65)
Beta-Blockers	2,115 (14.77)	6,366 (8.89)	1.88 (1.78-1.98)
Alfa-Blockers	362 (2.53)	1,553 (2.17)	1.19 (1.06-1.33)
ACE inhibitors	3,022 (21.10)	12,540 (17.51)	1.39 (1.33-1.45)
ARBs	2,552 (17.82)	11,143 (15.56)	1.25 (1.19-1.31)

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CCBs	2,230 (15.57)	8,909 (12.44)	1.41 (1.34-1.48)
Diuretics	2,903 (20.27)	11,488 (16.04)	1.50 (1.43-1.58)
Paracetamol	2,489 (17.38)	12,426 (17.35)	1.14 (1.08-1.21)
Metamizole	728 (5.08)	2,913 (4.07)	1.38 (1.27-1.50)
NSAIDs	1,350 (9.43)	7,226 (10.09)	0.99 (0.93-1.06)
Opioids	779 (5.44)	3,302 (4.61)	1.26 (1.16-1.37)
Corticosteroids	335 (2.34)	1,305 (1.82)	1.32 (1.17-1.49)
PPIs	4,759 (33.23)	19,815 (27.67)	1.42 (1.36-1.48)
H2 receptor blockers	336 (2.35)	1,229 (1.72)	1.39 (1.23-1.57)

*Adjusted only for matching factors (age, sex and calendar year).

[†]Recorded as such or when patients were using nitrates.

[§]Recorded as such or when patients were using glucose-lowering drugs.

[‖]Recorded as such or when patients were using lipid-lowering drugs.

Abbreviations: ACE: Angiotensin Converting Enzyme; ARBs: Angiotensin II-Receptor Blockers; BMI: Body Max Index, CCBs: Calcium-Channel Blockers; CI: Confidence Interval; COPD: Chronic Obstructive Pulmonary Disease; NSAIDs: Non-steroidal Anti-inflammatory Drugs; OR: Odds Ratio; PCP: Primary Care Physician; PPIs: Proton Pump Inhibitors; SD: Standard Deviation.

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Table 2. Risk of ischemic stroke and influenza vaccination overall and by different time periods and type of ischemic stroke (non-cardioembolic, cardioembolic).

Overall	**Cases (%) N=14322**	**Controls (%) N=71610**	**Non-adjusted OR[*] (95% CI)**	**Adjusted OR[†] (95% CI)**
Non-vaccinated Vaccinated	8392 (58.60) 5930 (41.40)	42635 (59.54) 28975 (40.46)	1 (Ref.) 1.05 (1.01-1.10)	1 (Ref.) 0.88 (0.84-0.92)
BY EPIDEMIC PERIOD				
Pre-epidemic	**Cases (%) N=4389**	**Controls (%) N=21945**	**Non-adjusted OR[*] (95% CI)**	**Adjusted OR[†] (95% CI)**
Non-vaccinated Vaccinated	3535 (80.54) 854 (19.46)	17507 (79.78) 4438 (20.22)	1 (Ref.) 0.93 (0.84-1.03)	1 (Ref.) 0.79 (0.71-0.88)
Epidemic	**Cases (%) N=2918**	**Controls (%) N=14590**	**Non-adjusted OR[*] (95% CI)**	**Adjusted OR[†] (95% CI)**
Non-vaccinated Vaccinated	1482 (50.79) 1436 (49.21)	7564 (51.84) 7026 (48.16)	1 (Ref.) 1.05 (0.96-1.15)	1 (Ref.) 0.88 (0.81-0.97)

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Post-epidemic	**Cases (%) N=7015**	**Controls (%) N=35075**	**Non-adjusted OR[*] (95% CI)**	**Adjusted OR[†] (95% CI)**
Non-vaccinated	3375 (48.11)	17564 (50.08)	1 (Ref.)	1 (Ref.)
Vaccinated	3640 (51.89)	17511 (49.92)	1.10 (1.04-1.16)	0.91 (0.86-0.97)
BY PATHOPHYSIOLOGICAL SUBTYPE				
Non-cardioembolic stroke	**Cases[§] (%) N=9542**	**Controls (%) N=47710**	**Non-adjusted OR* (95% CI)**	**Adjusted OR† (95% CI)**
Non-vaccinated	5819 (60.98)	29144 (61.09)	1 (Ref.)	1 (Ref.)
Vaccinated	3723 (39.02)	18566 (38.91)	1.01 (0.95-1.06)	0.88 (0.83-0.93)
Cardioembolic stroke	**Cases[§] (%) N=4780**	**Controls (%) N=23900**	**Non-adjusted OR* (95% CI)**	**Adjusted OR† (95% CI)**
Non-vaccinated	2573 (53.83)	13491 (56.45)	1 (Ref.)	1 (Ref.)
Vaccinated	2207 (46.17)	10409 (43.55)	1.15 (1.07-1.24)	0.90 (0.83-0.98)

Tests of interaction (ROR: Ratio of Adjusted ORs): epidemic *vs.* pre-epidemic: ROR=1.11 (95% CI 0.97 to 1.28), p=0.131; epidemic *vs*. post-epidemic: ROR=0.97

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(95% CI 0.87 to 1.08), p=0.545; pre-epidemic *vs*. post-epidemic: ROR=0.87 (95% CI 0.77 to 0.98), p=0.024.

Abbreviations: CE: cardioembolic; CI: confidence interval; non-CE: non-cardioembolic; OR: Odds Ratio; SD: standard deviation.

[*]Adjusted only for matching factors (age, sex and calendar year).

[†]Adjusted for matching factors (age, sex and calendar year) plus the covariates shown in table 1.

[§] Mean age CE cases: 76.8 (SD: ±11.2); mean age of non-CE cases: 73.6 (SD: ±12.7). This difference in age explains the higher prevalence of influenza vaccination in CE cases as compared to non-CE cases.

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Table 3. Risk of ischemic stroke and pneumococcal vaccination.

	Cases (%) N=14322	**Controls (%) N=71610**	**Non-adjusted OR[*] (95% CI)**	**Adjusted OR[†] (95% CI)**
Non-vaccinated	8979 (62.69)	48098 (67.17)	1 (Ref.)	1 (Ref.)
Vaccinated (any time before)	5343 (37.31)	23512 (32.83)	1.26 (1.21-1.31)	1.08 (1.04-1.13) 1.14 (1.09-1.19) [§]
Vaccinated (in the same season of the index date)	13874 (96.87) 448 (3.13)	69627 (97.23) 1983 (2.77)	1 (Ref.) 1.14 (1.03-1.27)	1 (Ref.) 1.04 (0.93-1.16) 1.10 (0.98-1.23) [§]

Abbreviations: CI: Confidence Interval; OR: Odds Ratio.

[*]Adjusted only for matching factors (age, sex and calendar year).

[†]Adjusted for matching factors (age, sex and calendar year) plus the covariates shown in table 1.

[§]Additionally adjusted for influenza vaccination.

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