Master of Public Health

Master de Santé Publique

Use of the test-negative design case control study for estimating influenza vaccine effectiveness (VE) and relative vaccine effectiveness (rVE) in Europe: a review of the literature and quantitative bias assessment

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List of Acronyms

ARI	Acute respiratory infection
Covid-19	Coronavirus disease 2019
GP visits	General practitioner visits
HD	High-dosed
ILI	Influenza like illness
IPTW	Inverse probability treatment weighting
LAIV	Live attenuated inactivated vaccine
MH Odds ratio	Mantel-Haenszel Odds ratio
OR	Odds ratio
ORV control	Other respiratory viruses control
PAN control	All panel negative control
PSM	Propensity score matching
RT-PCR	Real-time polymerase chain reaction
rVE	Relative vaccine effectiveness
SARI	Severe acute respiratory infection
SD-egg	Standard-dosed egg-based vaccine
TND	Test-negative design case-control study
TND control	Test-negative control
VAHNSI	Valencia Hospital Surveillance Network for the Study of Influenza and other Respiratory Viruses
VE	Vaccine effectiveness
VIF	Variance Inflation Factor
WHO	World Health Organization

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Abstract

Introduction: Seasonal influenza is a vaccine-preventable illness taking more than 300,000 lives annually around the world. The test-negative design case-control study (TND) is a method to assess the effectiveness in real-world setting. Objectives are 1) to explore the use of TND in vaccine effectiveness (VE) studies and identify the biases, 2) to describe the potential biases and its impact on the TND relative vaccine effectiveness (rVE) studies, and 3) to analyze the impact of the biases and bias correction methods.

Methods: Literature review on TND VE studies and systematic literature review on TND rVE studies were conducted. Patients 60 year or older who had respiratory symptoms during 2010 influenza season were analyzed. The cases were those positive for influenza viruses. Logistic regression with different control groups were conducted: 1) TND control – negative for influenza viruses, 2) ORV– positive for other respiratory viruses, and 3) PAN– negative for all respiratory viruses. Sensitivity analyses were conducted using the propensity scores for matching, stratifying, and weighting, and for the participants with wider criteria than the main analysis.

Results: VE estimates with the ORV controls showed higher estimates than other controls. In rVE analyses, the ORV controls showed the lowest estimate. Vaccination history was different among cases and controls in VE analysis. Sensitivity analyses showed similar results in both VE and rVE analyses. Age acts an effect modifier in VE analyses. Covariates measured in rVE analyses did not make more than 10% changes to the crude estimates.

Conclusion: Health-seeking behaviour difference should still be expected in TND studies for influenza VE. In rVE, the cases and controls are more comparable. The study design is fairly resistant to information bias. However, caution is still needed for clinical criteria and seasonal definition of the study inclusion as very few cases were observed in these participants.

Utilisation de l'étude cas-témoins de type test négatif pour l'estimation de l'efficacité du vaccin contre la grippe (VE) et de l'efficacité relative du vaccin (rVE) en Europe: revue de la littérature et évaluation quantitative des biais

Résumé

Introduction: La grippe saisonnière est une maladie évitable par la vaccination qui fait plus de 300 000 victimes chaque année dans le monde. L'étude cas-témoins de type test-négatif (TND) est une méthode d'évaluation de l'efficacité dans un contexte réel. Les objectifs sont 1) d'explorer l'utilisation du TND dans les études sur l'efficacité des vaccins (VE) et d'identifier les biais, 2) de décrire les biais potentiels et leur impact sur les études sur l'efficacité relative des vaccins (rVE) du TND, et 3) d'analyser l'impact des biais et des méthodes de correction des biais.

Méthodes: Une revue de la littérature sur les études de l'efficacité relative du vaccin contre le tétanos et une revue systématique de la littérature sur les études de l'efficacité relative du vaccin contre le tétanos ont été réalisées. Les patients âgés de 60 ans ou plus qui ont présenté des symptômes respiratoires pendant la saison grippale 2010 ont été analysés. Les cas étaient ceux qui étaient positifs pour les virus de la grippe. Une régression logistique avec différents groupes de contrôle a été effectuée : 1) contrôle TND - négatif pour les virus de la grippe, 2) ORV- positif pour d'autres virus respiratoires, et 3) PAN- négatif pour tous les virus respiratoires. Des analyses de sensibilité ont été réalisées en utilisant les scores de propension pour l'appariement, la stratification et la pondération, et pour les participants ayant des critères plus larges que ceux de l'analyse principale.

Résultats: Les estimations de la VE avec les contrôles ORV ont montré des estimations plus élevées que les autres contrôles. Dans les analyses rVE, les contrôles ORV ont montré l'estimation la plus basse. L'historique de vaccination était différent entre les cas et les contrôles dans l'analyse VE. Les analyses de sensibilité ont montré des résultats similaires dans les analyses VE et rVE. L'âge agit comme un modificateur d'effet dans les analyses VE. Les covariables mesurées dans les analyses rVE n'ont pas modifié de plus de 10% les estimations brutes.

Conclusion: Une différence dans le comportement de recherche de la santé devrait toujours être attendue dans les études TND pour la VE de la grippe. Dans les rVE, les cas et les témoins sont plus comparables. La conception de l'étude est assez résistante au biais d'information. Cependant, il faut rester prudent quant aux critères cliniques et à la définition saisonnière de l'inclusion dans l'étude, car très peu de cas ont été observés chez ces participants.

Introduction

Influenza epidemiology

Seasonal influenza is an acute respiratory illness caused by viruses in the family *Orthomyxoviridae*. There are 3 to 5 million severe cases annually worldwide according to the World Health Organization (WHO), and it is estimated that 290, 000 to 650 ,000 respiratory deaths were related to influenza (Iuliano et al., 2018; Paget et al., 2019). This estimate does not account for deaths from other diseases such as cardiovascular disease, which can be influenza-related. In Europe, a burden of 4 -50 million symptomatic cases and 15,000 to 70,000 deaths has been estimated annually (European Centre for Disease Prevention and Control, 2017).

Influenza epidemics are seasonal in nature and can also occur as pandemics when novel strains emerge against which humans have no pre-existing immunity. Seasonal epidemics occur between November and April in the Northern hemisphere, and between June and October in Southern hemisphere with year-round transmission in tropical areas. There are four types of influenza virus (A, B, C, & D). Currently, H1N1 and H3N2 subtypes of A, and Victoria and Yamagata lineages of B circulate causing seasonal epidemics following antigenic drift whereby the viruses escape vaccine- or infection-induced immunity (Paules & Subbarao, 2017).

Influenza spreads predominantly by droplet transmission and, also by contact transmission and aerosol transmission. The symptoms range from asymptomatic/mild to severe and can lead to death in rare cases. Most frequently reported symptoms are weakness, myalgia, cough, and nasal congestion (Monto et al., 2000) and classical complications are predominantly pulmonary expressing as pneumonia (by influenza virus itself or secondary bacterial infection), exacerbations of underlying pulmonary and cardiac disease, and, less frequently, neuromuscular and cardiac complications (Rothberg et al., 2008).

Specific populations are at increased risk of exposure to influenza, (health care workers), and others are considered at increased risk of severe and fatal outcomes (pregnant women, children under 5 years, elderly, people with chronic diseases and immunocompromised people) and are considered priority groups for influenza vaccination (World Health Organization, 2021).

Influenza vaccine

Influenza vaccination is considered the most effective way to prevent infection and subsequent disease (Paules & Subbarao, 2017) while some non-pharmacological measures (such as personal hygiene and social distancing) are also effective. Influenza vaccines have been available since the 1940s (CDC, 2021) and there are different vaccine types based on

how they are produced. Most vaccines are produced in embryonated hens' eggs (egg-based) but more modern methods include growing the influenza virus in cell culture (cell-based), using or recombinant technology to synthesize the protein antigens. Additional differences include how many subtypes of viruses are included (trivalent or quadrivalent), and in what forms (inactivated – high dose and standard dose, or live-attenuated). Adjuvanted, egg-based vaccines are also available and provide better immunogenicity in children or otherwise naïve individuals. Details can be seen in *Figure 10* of Annexes.

Vaccine effectiveness and relative vaccine effectiveness

Every year, influenza vaccines are produced to match the antigenic structure of circulating viruses. Considerable effort has gone into understanding the protective benefits of these vaccines in preventing disease. Randomized control trials (RCT) have been conducted to measure influenza vaccine "efficacy" but it has been much more common to measure vaccine "effectiveness" in real-world conditions using observational study designs because of the ethical problem.

Influenza vaccine effectiveness (VE) varies in each season depending on the matching of the vaccine with circulating viruses and other factors and is estimated to be within 40% to 60% if the antigen is well matched (Centers for disease control and prevention, 2020). Other factors affecting VE and seasonal difference are the person's characteristics (age as confounder or effect modifier, sex, health status), swabbing time since the symptom onset, use of antiviral, and timeliness of vaccination (Darvishian et al., 2017; Sullivan et al., 2014; Young et al., 2018).

VE studies compare the disease incidence between those vaccinated and unvaccinated to generate a measure of vaccine performance. Relative vaccine effectiveness (rVE) measures the relative performance of different vaccines available in the market. rVE studies are also important as there might be different health status among vaccinated and unvaccinated in VE studies which is more comparable in rVE studies' participants resulting in reduced potential for confounding (Mannino et al., 2012). Because multiple influenza vaccines have seldom been used in the same country at the same time, relatively few rVE studies have been conducted and published in the literature.

VE and rVE studies

Observational studies used for vaccine effectiveness monitoring normally follow cohort, case-control, and pseudo-ecologic designs such as the screening method (or their variations such as nested case-control studies or the test-negative design, discussed below). Different outcomes can be incorporated depending on the primary study objectives including either classical specific outcomes (applying laboratory confirmation) or broader, non-specific,

symptomatic disease endpoints. Non-specific outcomes are influenza-like illness (ILI), severe acute respiratory infection (SARI) for hospitalization, pneumonia, admission to intensive care units (ICU), death, and adverse birth outcomes. Again depending on the study objectives, they may be performed in outpatient or inpatient settings (*Evaluation of Influenza Vaccine Effectiveness A Guide to the Design and Interpretation of Observational Studies Immunization, Vaccines and Biologicals*, 2017).

To increase comparability of the treated and untreated in the observational studies, the propensity scores as the probability to get vaccinated were calculated based on measured covariates (Rosenbaum & Rubin, 1983) and used in matching, stratifying, or weighting (inverse probability for treatment weighting – IPTW) (Månsson et al., 2007). These methods were increasingly used in cohort studies, and also in some case-control studies (Balasubramani et al., 2020; Mannino et al., 2012; Månsson et al., 2007).

Test Negative Design case-control studies

Test Negative Design case-control studies (TND) is a modified case-control using test results (e.g; PCR) to select as the cases or the controls. It can be used in both outpatient and inpatient settings, and patients are enrolled based on the predefined clinical criteria. Depending on the test results, those who detected as flu positive became cases and others became controls. There are other alternative controls - the positives for other respiratory viruses, or negative to all respiratory viruses including influenza which have advantages in reducing biases associated with laboratory testing performance and the viral interference making vaccinated persons susceptible to other respiratory viruses (Feng et al., 2018).

TND has been used increasingly overtime after the first use in the pneumococcal vaccines due to its advantages: and reduce biases – health-seeking behaviour difference, misclassification of disease status due to its advantages: relatively cheaper and faster to conduct than cohort and traditional case-control studies using the existing surveillance system to collect the data, reduced biases like community-level variation, health-seeking behaviours, and misclassification of disease(Sullivan et al., 2016). It is also economically feasible compared to the cohort studies.

Although TND is robust in theory, the variations in the VE estimates were observed while using TND not only because of external factors like different circulating viruses and locations but also because of the study design and the statistical model (Sullivan et al., 2014). There are attempts to look at the biases in test-negative design influenza vaccine effectiveness studies (Ainslie et al., 2017, 2019; Foppa et al., 2013; M. L. Jackson & Nelson, 2013; Sullivan et al., 2014).

No previous studies have explored biases and confounders which may be present in TND rVE studies. As the number of differentiated influenza vaccines being used increases, more and more rVE studies will be conducted and so this becomes a topic of research interest. Similar questions may apply to other vaccines (e.g: Covid-19 vaccines) where TND will be the preferred design due to feasibility and design advantages.

Objectives of study

- To explore previous use of influenza test-negative design VE/rVE studies and identify and categorize the biases described in the literature
- To describe how potential biases and confounders in TND rVE studies have impacted on rVE estimates
- To quantitatively analyze these biases and confounders by conducting an influenza rVE study using a Spanish respiratory virus surveillance database and assess the impacts of using different statistical methods to adjust for biases/confounders.

Materials and methods

Study design

Literature review and systematic literature review

An initial scoping literature review on TND VE studies was conducted to understand biases and confounders described in previously published systematic reviews of TND VE papers catalogued within the PubMed database on 15 February 2021. It was intended to be a background for the next step systematic review.

This initial review provided the information to design a systematic literature review strategy to identify papers on the use TND influenza rVE studies within PubMed and Embase databases, on 9 March 2021. After several exploratory searches, the following search terms were used to retrieve the papers on PubMed and Embase.

Search strategy

1. “Influenza” OR “flu” (including Mesh term in PubMed)
2. Vaccine
3. **1** And **2** OR “influenza vaccine”
4. “Relative effectiveness” OR “Comparative effectiveness” OR “rVE” OR “comparison” OR “comparing”
5. “test-negative” OR “test negative” OR “TND” OR “case control” OR “case-control”
6. **3** AND **4** AND **5**

The reference lists of the retrieved articles were reviewed to identify missing articles.

Study selection

Titles and abstracts of identified papers were reviewed to identify the potential papers to be included in the article review. In title reviews, broader inclusion criteria were set to be sensitive and these included the papers with two or more influenza vaccines OR TND vs case-control studies OR different control groups or different settings of TND studies. The abstract review was aimed to be specific and the papers with two or more vaccines in TND designs were included. No exclusion criteria were set due to the relative paucity of the papers available.

After the initial literature review, several potentially biasing/confounding variables in TND rVE studies had been identified and were specifically extracted to enable the comparison of studies. These parameters were: Publication years, location, season, target population and eligible criteria, types of vaccines, outpatient or inpatient setting, outcome (e.g: test positive or hospitalization for flu), type of vaccination record, adjusted variables, analysis models, and biases and confounders observed.

Data Analysis

Design overview and Data sources

Test-negative design case-control studies were conducted to compare the odds of vaccination among the cases and controls as the VE analysis. The rVE analyses between two groups were conducted: 1) who received adjuvanted vaccines and 2) who received other vaccines, virosomal or split. The participants were the 60 years or older patients who were admitted to five hospitals in Valencia, Spain during the 2010-11 influenza season. These five hospitals provided healthcare services to 975,174 inhabitants aged 18 years or older (J. Puig-Barberà et al., 2012).

Valencia Hospital Network for the Study of Influenza (VAHNSI) has been conducting active surveillance hospital-based data collection during the influenza season and conducting studies for influenza vaccine effectiveness since 2009. Data are collected by full-time field researchers identifying the patients who meet inclusion criteria: ILI episodes (definition of WHO at that time) with sudden onset, no reported egg allergy, resident of the hospital catchment area, not institutionalized, not hospitalized within 30 days, and no previous lab-confirmed influenza infection (J. Puig-Barberà et al., 2012). The dataset for 2010 season contains 1,045 patients hospitalized starting from week 45 of 2010 to week 11 of 2011.

Setting and Participants

The study limited the participants' age to 60 years or older at the date of hospitalization. The influenza season was defined as a week with at least 5% positivity rate of influenza among all participants tested in the dataset and started from week 50 of 2010 until week 11 of 2011

to reduce nondifferential misclassification of diagnosis (Ozasa, 2008), details in *Figure 11* (Annexes). The clinical criteria set for the study were fever or cough within 7 days of onset of the symptoms. Patients who had taken antiviral treatment before hospitalization were excluded.

Exposure and outcome

Vaccination status and time were obtained by asking the patients and ascertained with the records in Valencia's population-based Vaccine Information System (J. Puig-Barberà et al., 2012). Individuals were considered vaccinated if they had received the vaccine at least 14 days prior to the ILI symptom onset.

A nasopharyngeal swab and a pharyngeal swab were obtained from each participant and multiplex real-time Reverse-transcription polymerase chain reaction (RT-PCR) were performed accordingly. The viruses tested were influenza viruses, coronavirus, metapneumovirus, bocavirus, respiratory syncytial virus (RSV), adenovirus, parainfluenza virus, and rhinovirus (J. Puig-Barberà et al., 2012).

The outcome was determined by the RT-PCR results and influenza A or B or mixed influenza infections were regarded as cases, while influenza negatives as TND controls. In alternative control groups, the different measures were used for the control selection: positive for other respiratory viruses as the ORV control group, and negative for all viruses in the panels as the PAN control group.

Baseline characteristics

The following baseline characteristics of each patient were collected and recorded during admission: age, sex, indications for inclusion, hospitalization date, the time elapsed from symptoms onset to swabbing, presence of major underlying medical conditions, long-term treatments, contact with children, smoking habits, occupation, number of physician encounters in the last three months, number of hospitalizations in the last year, and prescription of antivirals. Intensive care unit admission, death in hospital, and length of stay were also recorded in the dataset.

Statistical analysis

Baseline characteristics of cases and controls, and also the vaccinated and unvaccinated were compared and tested with Fisher’s exact test for categorical variables and Wilcoxon rank-sum (Mann-Whitney) test for non-parametric continuous variables.

A priori adjustment variables were set depending on the literature reviews observed in the first part of the study. Among them, effect modifier and highly correlated variables were excluded in the model building leaving sex, study site, and presence of comorbidity as *a*

priori variables for VE analyses. Other potential covariates in the dataset were assessed with univariate regression, and p<0.25 cut-point were used to include as the confounders in the multivariate regression model.

Tabular analyses were assessed with these variables, and the potential effect modifiers were identified if the odds ratios in the strata were different from the crude odds ratio. Collinearity among the covariates was checked with Variance Inflation Factor (VIF) value and tolerance, and correlated ones (high VIF value and low tolerance) are excluded in the model building.

For VE analyses, the following logistic regression models were performed: 1) crude models, 2) models with the *a priori* covariates excluding the correlated ones, 3) full models with *a priori* covariates and other covariates with p-value less than 0.25 in univariate regressions. The backward stepwise selection was performed to get the adjusted model by excluding the variables with p-values >0.05 one by one starting from the highest p-value until all variables in the model were significant at the 0.05 level. During these steps, likelihood ratio tests and the standard error of vaccination's odds ratio were tested and observed to evaluate the fit of model compared to the previous one. Hosmer-Lemeshow test was also used to assess the goodness of fit of the models.

For rVE study, *a priori* variables were the variables identified during the literature review: age, sex, presence of comorbidity, vaccination within 120 days before symptom onset, and prior season vaccination. The study site was not included as the different vaccines were used in the different study sites. The same method as in VE analysis was used to get the potential cofounders in the dataset with the univariate regressions.

For model building, the rVE estimates were compared with the crude estimates by adding each variable at one time and observed the changes from the crude rVE estimate. The models with *a priori* variables were used to compare among different control groups and different methods.

VE and rVE were calculated with the formula of (1 – Odds ratio) / 100. The Odds ratio in VE is vaccinated against unvaccinated, and in rVE is vaccinated with the adjuvanted vaccine against other vaccines.

Sensitivity analyses

Additional measures were applied to assess their impact on adjusted VE and rVE for test-negative design case-control studies. First, the propensity score to be vaccinated was calculated with the *a priori* variables which are related to both outcomes and exposure status (Garrido et al., 2014) (sex, presence of comorbidity, and study site for VE analysis, and age, sex, presence of comorbidity, prior season vaccination, and time since vaccination).

The balance of propensity score between vaccinated and unvaccinated was checked with the graph by looking at the number of people by vaccination status in each group of propensity scores.

The propensity scores were used for different methods to assess the VE and rVE estimates: matching, stratifying, and weighting. The propensity scores were used to create four groups, and conditional logistic regression was conducted by matching in these groups. Mantel-Haenszel combined odds ratio and stratified odds ratios were assessed using these propensity score groups.

For inverse probability treatment weighting (IPTW), the propensity scores were used to create the sampling weight: 1/propensity score for vaccinated ones, and 1/(1-propensity score) for unvaccinated ones. Then this sampling weight was used to observe the VE and rVE estimates in the logistic regression.

In the main analyses of the study, the participants who did not meet the criteria were left due to different reasons: other respiratory symptoms rather than fever or cough, out of epidemic period, and usage of antiviral before hospital admission. VE and rVE estimates were assessed for all patients aged 60 years or older including these excluded ones to observe the impact of potential misclassification of disease status.

Stata version 15.0 was used for these analyses and the values of VE and rVE were plotted in each graph respectively to observe the differences based on the setting, method, and covariates included.

Results

Literature review on test-negative influenza VE studies

During the literature review on test-negative design VE studies, 9 systematic reviews were found in PubMed. Together with these reviews, the WHO guide on influenza vaccine effectiveness and six papers found in the reference of these systematic reviews were studied to learn about the biases and confounders observed in the test-negative design influenza vaccine effectiveness studies.

Details of those biases and confounders will be discussed together with the results of the systematic literature review on test-negative design rVE studies in the following section.

Systematic literature review on test-negative influenza rVE studies

Search results on PubMed and Embase are described in figure 1. 81 articles in PubMed and 76 articles were found. Including 65 duplicates, leaving 92 articles included for title review. After excluding 64 papers during the title review step, 15 during the abstract review step, and

finally 2 during the article review step for not matching the predefined criteria a total of 11 papers were included in the systematic literature review. 5 papers studied vs standard-dose, egg-based vaccine (SD-egg) was compared with live attenuated vaccines (LAIV) in 5 papers, with high-dose (HD) in 2 papers, with cell-based in 2 papers with adjuvanted in one paper, and with two vaccines, virosomal and intradermal, in one paper.

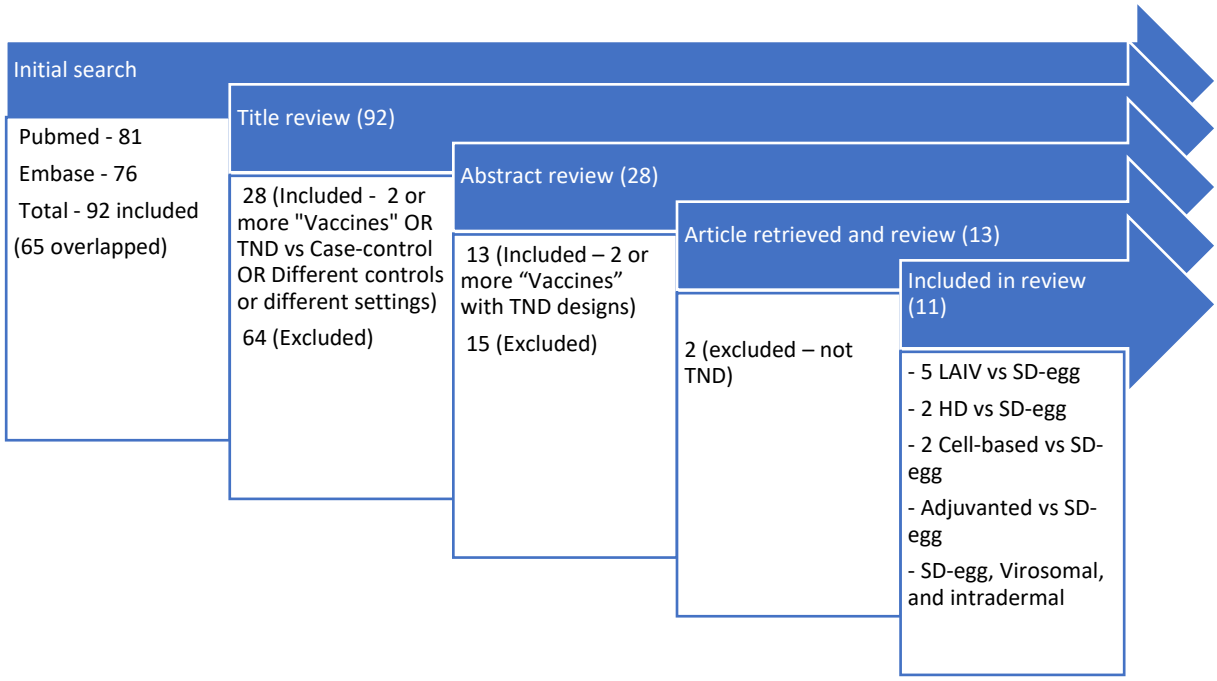

Figure 1 Identification of the articles

Identified biases and confounding factors

Selection bias

The difference in health-seeking behaviour is an important bias for influenza VE studies which can result in an imbalance in healthcare consultation rates between the cases and controls. TND case-control studies can reduce it assuming healthcare-seeking behaviour is similar as being compared among those who sought medical care and stayed in the same communities, although it cannot be eliminated (Sullivan et al., 2014).

Healthy vaccinee bias, where a person with better health conditions is more likely to get vaccinated, and confounding by indication, where a person with chronic diseases is more likely to get vaccinated, impacted on the VE estimates with different directions can be observed in the observational studies (Remschmidt et al., 2015). These biases can also be found in TND VE studies ((Bruxvoort et al., 2019; Joan Puig-Barberà et al., 2014; Treanor et al., 2012; Van Buynder et al., 2013), but (Eick-Cost et al., 2012) suggested that TND is more

appropriate than using healthy controls having more similar health-seeking behaviour with the cases.

For rVE studies, the differences due to these biases are less likely to occur as it is comparing between two vaccinated persons. Because all study participants chose to receive the vaccine, we can assume they are more similar than those individuals who choose not to receive the vaccine at all. However, it is still observed in some cases like comparing the efficacious and expensive vaccine (HD vaccine) against the usual vaccine where older and more vulnerable people got the HD vaccine in the United States (Balasubramani et al., 2020).

Test-negative design case-control studies are based on the assumption that the influenza vaccination has no effect on non-influenza ILI (*Evaluation of Influenza Vaccine Effectiveness A Guide to the Design and Interpretation of Observational Studies Immunization, Vaccines and Biologicals*, 2017), but this may not be strictly true; children may have a higher risk of infection by other respiratory viruses in the time immediately following influenza vaccination (Cowling et al., 2012). This bias can impact VE estimates substantially only if the incidence of influenza is more than 10 times of non-influenza ARI (acute respiratory infection) and the duration of non-specific immunity lasted longer than 3 months (Foppa et al., 2013). Although there is no evidence yet on the adults with this mechanism, using of different control groups can be used to observe whether there is any impact on VE estimates.

Vaccination reduces the severity of symptoms and therefore vaccinated persons are less likely to seek healthcare services following an influenza infection. A simulation study explored the implications of this on VE from TND studies concluding that if healthseeking is lower in vaccinees, this could result in significantly elevated VE. (Ainslie et al., 2017).

The other selection bias noted in the test-negative design rVE study concerns individuals excluded from study due to a lack of information on the type of vaccines received. While this would not affect VE if similar participants were excluded from case/control groups, a study in US, found that excluded individuals had different characteristics which could be associated with vaccine type (Doyle et al., 2021).

Information bias

Misclassification of disease status

Test sensitivity and specificity to determine whether as cases or controls is important to reduce the information bias due to the nondifferential misclassification of disease status. The sensitivity of the test can vary with the study site and the swabbing time since symptoms onset, but the bias is minimal if the test specificity is high (Sullivan et al., 2014). RT-PCR test

is the most sensitive and specific test to detect influenza infection and is now in routine use in most settings, so this risk is limited (Weinberg et al., 2004).

Misclassification of exposure status

This can occur if vaccination status is ascertained through patients' recalls without verification with vaccination records. This misclassification most commonly results in over or under estimated VE depending on differential or nondifferential in nature (DeMarcus et al., 2019; Sullivan et al., 2014).

Confounding factors

Age is an important confounding factor as older people are more likely to get infected or hospitalized with influenza, and more or less likely to get vaccinated (Sullivan et al., 2016; Van Buynder et al., 2013). It can also act like an effect modifier (Joan Puig-Barberà et al., 2014), and the stratification of VE estimates by age is, therefore, important (*Evaluation of Influenza Vaccine Effectiveness A Guide to the Design and Interpretation of Observational Studies Immunization, Vaccines and Biologicals*, 2017). Low protection of vaccination among the elderly for hospitalization with influenza was observed in a systematic review (Rondy et al., 2017) and immunosenescence is an acknowledged challenge to the protective benefit of influenza vaccines in older populations.

Similarly, individuals at high-risk status, having more comorbidities and/or frailty, can be confounded by indications for influenza vaccination (Ainslie et al., 2017; Joan Puig-Barberà et al., 2014; Sullivan et al., 2016), and adjustment in the analysis will be needed. Women are more likely to get vaccinated for influenza and higher risk of having contact with the kids, however, sex is frequently omitted in the studies (Sullivan et al., 2016).

Time since vaccination is also important as the immunity may wane over time (Belongia et al., 2015), and the VE estimate dropped in those within 91 to 180 days comparing to those within 15 – 90 days (Young et al., 2018). Strong clustering effects of the study site and epidemiological week were observed in a study in Spain for the 2011 season (Joan Puig-Barberà et al., 2014).

Prior season vaccination is related to the outcome and also with the current season vaccination (Joan Puig-Barberà et al., 2014) and it acted as the effect modifier in a study in children for the live attenuated vaccine (McLean et al., 2017). VEs also vary with different strains of influenza and vaccination is generally less effective against H3 (Rondy et al., 2017; Young et al., 2018).

TND case-control studies with different settings were reviewed, and the out-patient vs in-patient setting and using different control groups (TND for influenza negatives, ORV for other

respiratory viruses positives, PAN for negative for all respiratory viruses tested) showed no substantial difference in VE estimates (Feng et al., 2016, 2018).

One study in the US for the 2010 season found that there was little evidence of confounding effects of included covariates as evidenced by small differences between the estimates of the crude and the adjustment models observed for SD-egg and LAIV (Treanor et al., 2012).

Table 1 Biases and confounders in TND VE and rVE studies

Bias	VE studies	rVE studies	Correction
Selection bias			
Vaccination	Vaccination lowers the probability of seeking medical care in influenza ARI patients	Similar effects between vaccines	
	Definition of outcome for cases and non-cases (flu more severe than other ARIs)	Same	Not suitable for the outcome as death
	Vaccination affects the probability of non-influenza ARI	Similar effects between vaccines	Compare with different control groups (TND, ORV, PAN)
Exclusion		Excluded have different characteristics (lack of the type of vaccines information)	Sensitivity analysis
Healthy vaccinee effect	Unvaccinated - have higher healthcare utilization	NA	Adjustment, Propensity scores
Confounding by indication	Vaccine recipients are more vulnerable, and more treated/tested	HD vaccines were prioritized for more vulnerable and older because of the price	Adjustment, Propensity scores
Information bias			
Misclassification of vaccination status	Recall bias if rely on patients' recall	Vaccination records were used to ascertain	Using vaccination record
Misclassification of disease status	Test sensitivity and specificity (high specificity - minimal bias)	Same as VE	Use PCR and strict case definitions
	No shedding of the virus after 4 days of symptoms	Same as VE	Restrict or adjust
Confounders			
Age	Older - more vaccinated, more likely to get infections, higher comorbidities	Age in deciles - an effect modifier	Restriction for study, Adjustment, Propensity scores

Calendar time	The campaign before or at the start of a season, and protection wane as the season progresses	Same as VE	Restriction for study, Adjustment, Propensity scores
Sex	Women may have higher vaccination and higher exposure through children (but frequently omitted)	NA	Adjustment
Hospital/site		Strong clustering effect	Restriction for study, Adjustment, Propensity scores
Prior season vaccination	Modifier in LAIV	Associated with flu positive and vaccination	Adjustment, Propensity scores
Influenza types	Less effective against H3	Same as VE	OR for all influenza, and for different strains
Epidemic season	Effective during epidemic season irrespective of vaccine match status	Same as VE	Adjustment for multi-seasons study

Data analysis

FISABIO dataset contains 1045 patients who attended five hospitals in Valencia, Spain. After applying inclusion and exclusion criteria, 576 patients remained, 58 of whom tested influenza-positive. The process of exclusion and inclusion can be seen in *Figure 2**Figure 2*.

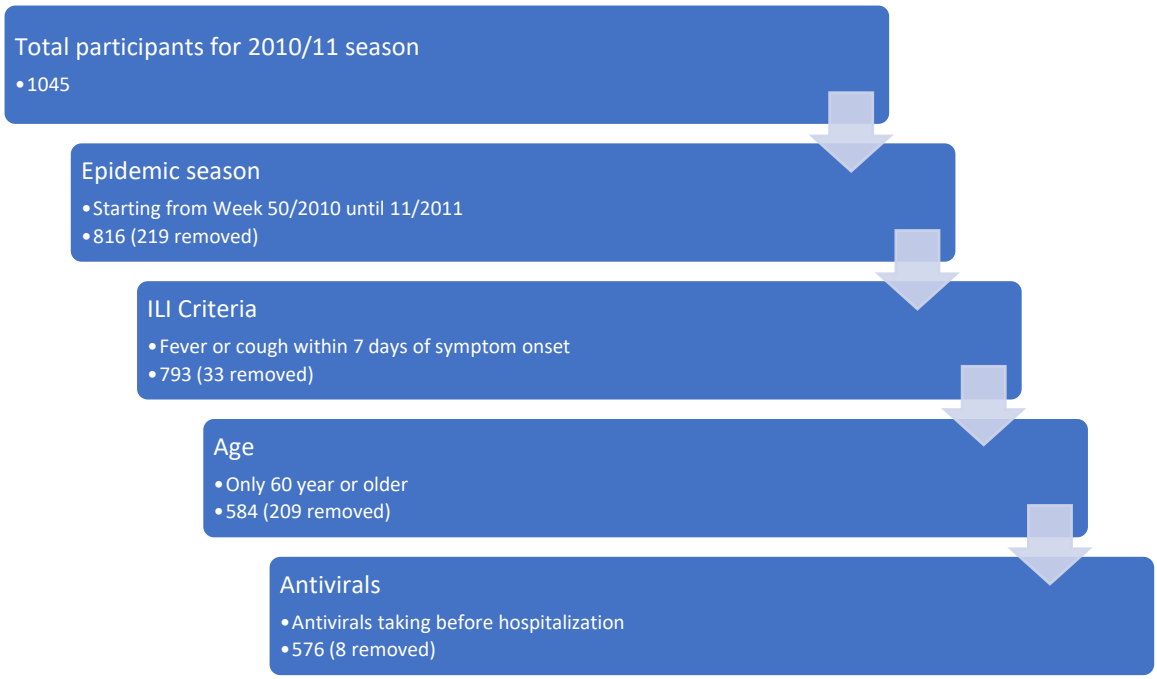

Figure 2 Flow chart of the study process

Table 2 Characteristics of participants by the outcome, and by the exposure status

	Control 518 (%)	*Case 58 (%)*	*P Value*	*Un-vaccinated 221 (%)*	*Vaccinated 355 (%)*	*P Value*
Hospital			0.039			0.234
La Plana	162(31)	15(26)		70(32)	107(30)	
Arnau De Vilanova	82(16)	14(24)		44(20)	52(15)	
La Ribera	73(14)	3(5)		22(10)	54(15)	
San Juan	38(7)	9(16)		16(7)	31(9)	
General Elda	163(31)	17(29)		69(31)	111(31)	
Age (mean)	77	73	0.000	74	78	0.000
Age group			0.064			0.000
60 - 74	185(36)	28(48)		105(48)	108(30)	
>= 75	333(64)	30(52)		116(52)	247(70)	
Female	225(43)	23(40)	0.675	107(48)	141(40)	0.047
Obesity (BMI >=30)	160(31)	17(29)	0.881	69(31)	108(30)	0.853
Presence of comorbidity	466(90)	51(88)	0.647	191(86)	326(92)	0.047
Smoking status			0.054			0.004
Never	262(51)	23(40)		109(49)	176(50)	
Ex-Smoker	202(39)	23(40)		75(34)	150(42)	
Current Smoker	54(10)	12(21)		37(17)	29(8)	
GP Consultation Last 3 Months?			0.110			0.607
None	80(15)	12(21)		39(18)	53(15)	
One	133(26)	20(34)		60(27)	93(26)	
Two or More	305(59)	26(45)		122(55)	209(59)	
Occupation			0.110			0.443
Skilled Non-Manual	75(14)	13(22)		39(18)	49(14)	
Skilled Manual	66(13)	10(17)		27(12)	49(14)	
Unskilled	377(73)	35(60)		155(70)	257(72)	
Contact with Children	183(35)	25(43)	0.251	79(36)	129(36)	0.929
Hospitalized Last 12 Months?	224(43)	22(38)	0.486	95(43)	151(43)	0.931
Swab taking within 4 Days of Symptoms	292(56)	33(57)	1.000	116(52)	209(59)	0.142
Flu Vaccine 2009-10	350(68)	30(52)	0.019	61(28)	319(90)	0.000
Flu Vaccine 2010-11 Type			0.115			0.000
No vaccine	191(37)	30(52)		221(100)	0(0)	
Mf59	166(32)	11(19)		0(0)	177(50)	
Virosomal	112(22)	12(21)		0(0)	124(35)	
Split	49(9)	5(9)		0(0)	54(15)	
Pneumococcal Vaccine	113(22)	11(19)	0.737	22(10)	102(29)	0.000
Pandemic Flu Vaccine	191(37)	17(29)	0.313	27(12)	181(51)	0.000
ICU Admission	17(3)	1(2)	1.000	5(2)	13(4)	0.463
Death	23(4)	2(3)	1.000	11(5)	14(4)	0.538

The different characteristics between cases and controls, and between exposed and unexposed can be seen in

Table 2. Among the cases and the controls, statistical differences with a p-value ≤ 0.05 were observed in hospital (study site), age group, fever, and prior season vaccination variables. The test-negative (TND) controls were older and more likely to get a flu vaccination in the previous season compared to the cases.

For the vaccination status, vaccinated study participants are older, have more comorbidities, and are more likely to get flu vaccination in the prior season (90% comparing to 28%), pandemic flu vaccination (51% to 12%), and pneumococcal vaccination (29% to 10%). It was also found that more men were in the vaccinated group. The participants in the vaccinated group are more likely to quit smoking comparing to the unvaccinated group although the ratios of non-smokers are similar.

In the other respiratory viruses (ORV) control group, 98 participants who were tested positive for other respiratory viruses were included as the controls in the analysis. 420 participants who were tested negative for both influenza and other respiratory viruses were included as the controls in negative for all viruses (PAN) control group.

Table 3 Some characteristics of different control groups (VE)

	TND Control 518 (%)	*ORV Control 98 (%)*	*PAN Control 420 (%)*
Age group			
60 - 74	185(36)	37(38)	148(35)
>=75	333(64)	61(62)	272(65)
Female	225(43)	48(49)	177(42)
Comorbidity	466(90)	92(94)	374(89)
Smoke			
Never	262(51)	55(56)	207(49)
Ex-Smoker	202(39)	34(35)	168(40)
Current Smoker	54(10)	9(9)	45(11)
GP visits in last 3 months			
0	80(15)	18(18)	62(15)
1	133(26)	21(21)	112(27)
2	305(59)	59(60)	246(59)
Has Been Hospitalized Last 12 Months?	224(43)	40(41)	184(44)
Prior season Flu Vaccine	350(68)	69(70)	281(67)
Flu Vaccine 2010-11 Type			
None	191(37)	28(29)	163(39)

Mf59	166(32)	31(32)	135(32)
Virosomal	112(22)	26(27)	86(20)
Split	49(9)	13(13)	36(9)
Pneumococcal Vaccine	113(22)	29(30)	84(20)
Pandemic Flu Vaccine	191(37)	29(30)	162(39)

With the predefined p-value of <0.25, the following variables were considered to include in the model building: Age group, Smoking status, GP visits within 3 months, Occupation status, Contact with kids, and Pandemic flu vaccination were included in TND control groups. Univariate regression results of the different control groups can be seen in Annexes, Table 8

Some *a priori* variables identified from the literature review- sex and presence of comorbidity, were also included in the full model although their p-value is larger than 0.25.

Age in two groups (60 – 74, and >=75) was acting as the effect modifier having different odds ratios in the different strata during tabular analysis and showed a significant positive association with vaccination. Including prior season vaccination showed a huge increase in the standard error of vaccination effect during logistic regression, and relatively higher VIF and lower tolerance indicating possible collinearity and was therefore excluded from the model.

Influenza vaccine effectiveness

The final adjusted model of the TND control contained smoking status covariate.

The VE estimates in the TND control and the PAN control groups showed similar estimates while in the ORV control showed higher estimates, details in AnnexesTable 7, Table 9, andTable 11. The models of 60 to 74 years age group showed similar significant VE estimates around 70% except for the full model of the ORV controls (53%) and the full model of the PAN controls (79%). VE estimates in participants aged 75 years or older showed no significant results with wide confidence intervals.

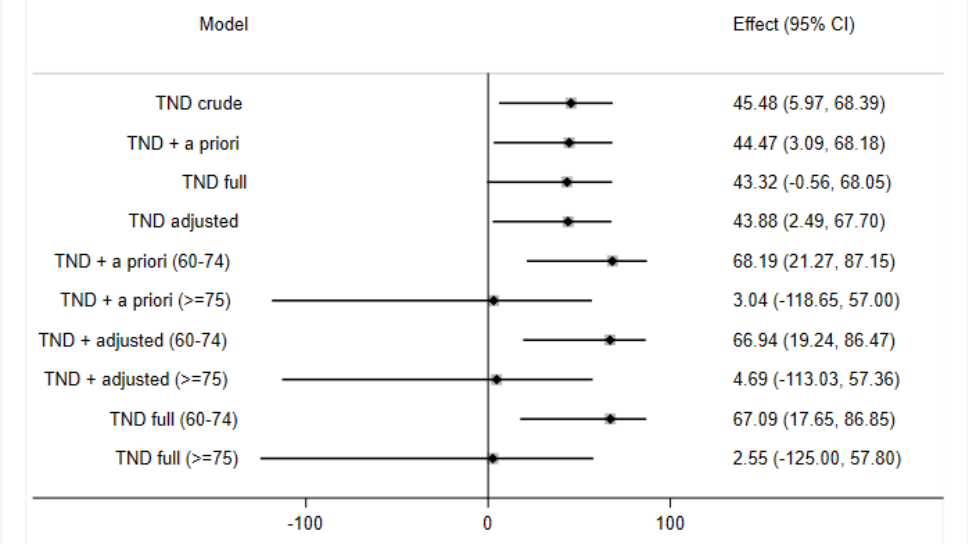

Figure 3 VE estimates of different models with TND control

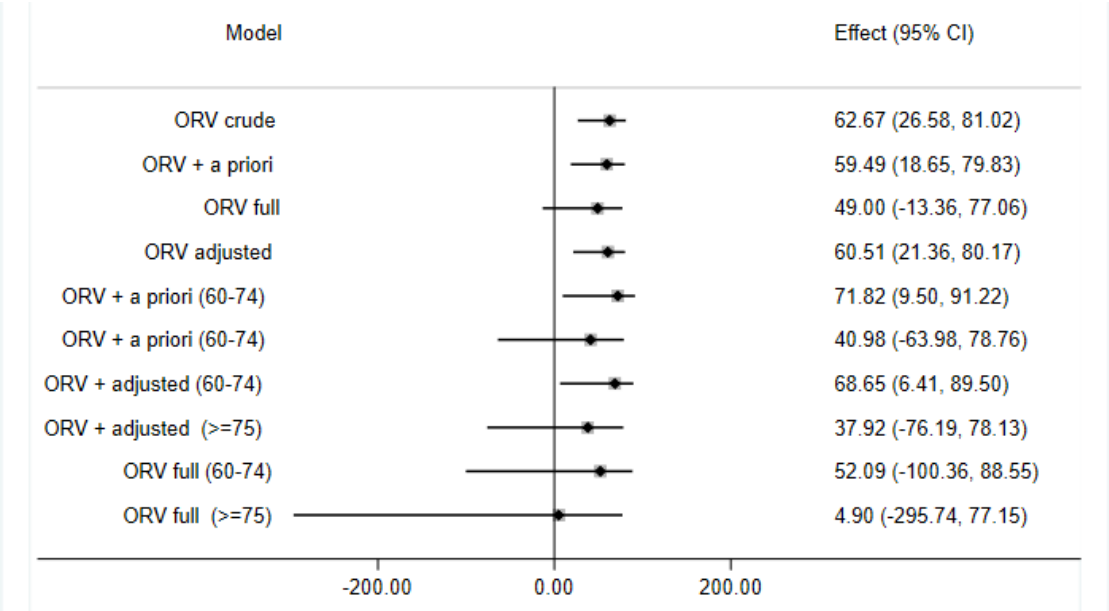

Figure 4 VE estimates of different models with ORV control

The adjusted model with ORV control group included the occupation covariate.

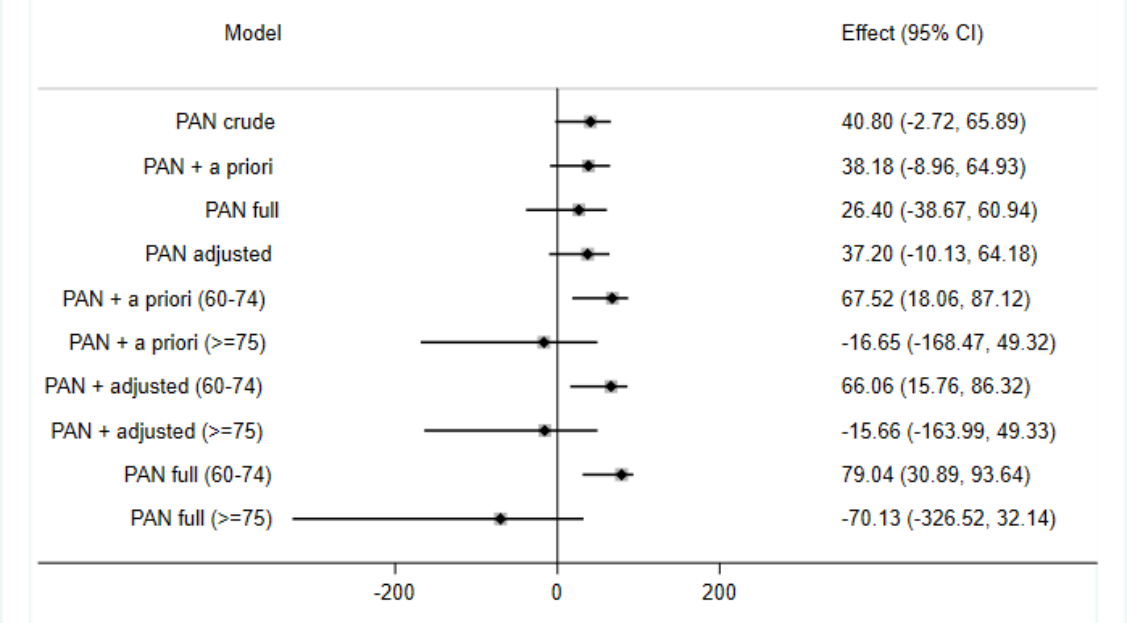

Figure 5 VE estimates of different models with PAN control

The adjusted model with PAN control group included the study site covariate.

Propensity scores and different methods

Propensity score were calculated using the *a priori* variables and the balance of propensity scores between vaccinated and unvaccinated groups had been observed. Overlaps of these groups were seen in the propensity score groups as in Figure 6 showing a good choice of the variables.

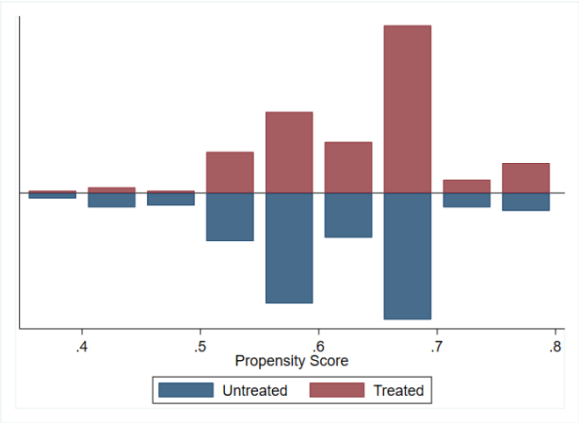

Figure 6 Balance of propensity score

The propensity score matching model (PSM) with four groups of propensity scores gave the same result as the multivariate logistic regression model. The combined Mantel-Haenszel

(MH) odds ratio with these four groups and weighted logistic regression (Inverse probability of treatment weighting – IPTW) showed similar results too.

The VE estimates with excluded participants aged 60 year or older showed minimal changes to the main analyses. Among a total of 753 participants in the model of all participants, 59 were found to be positive for influenza.

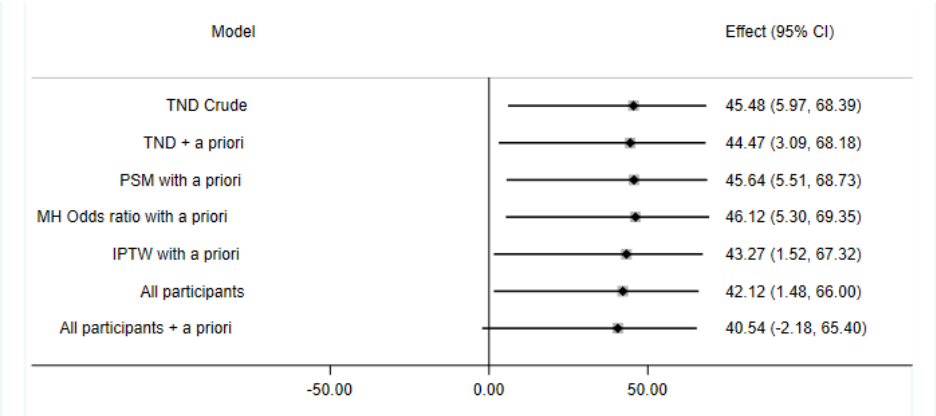

Figure 7 VE estimates by different methods and different participants

Results of rVE Analysis

Characteristics of the participants

A total of 355 participants was included in the analysis who met the criteria mentioned previously. Among them, 28 participants (7.89%) were found to be influenza positive. Exposure status on the vaccine types was equally distributed, 178 participants received virosomal or split vaccines (124 and 54 respectively) while 177 participants received adjuvanted vaccines. Adjuvanted vaccines were administered in 3 hospitals only while virosomal was administered in 2 other remaining hospitals with 3 people in La Ribera hospital. Split vaccines were administered in all hospitals.

No significant differences between cases and controls were observed for all variables in the dataset. However, there are differences between those who received the adjuvanted vaccines and other vaccines. Although current smoking status was not much different between these two groups, those who received the adjuvanted vaccine are more likely to be the ex-smokers. They are mostly unskilled labour in occupation status and consulted with general practitioners 2 times or more within 3 months although the percentage of the population having one or more comorbidities were not different.

Table 4 Characteristics participants by the outcome, and the exposure statuses (rVE)

	Control 327 (%)	*Case 28 (%)*	*P-value*	*Other vaccines 178 (%)*	*Adjuvanted 177 (%)*	*P-value*
Hospital			0.174			0.000
La Plana	101(31)	6(21)		8(4)	99(56)	
Arnau De Vilanova	47(14)	5(18)		9(5)	43(24)	
La Ribera	51(16)	3(11)		19(11)	35(20)	
San Juan	25(8)	6(21)		31(17)	0(0)	
General Elda	103(31)	8(29)		111(62)	0(0)	
Age (mean)	78	76	0.189	77	78	0.142
Age group			1.000			0.205
60 - 74	100(31)	8(29)		60(34)	48(27)	
>=75	227(69)	20(71)		118(66)	129(73)	
Female	128(39)	13(46)	0.547	73(41)	68(38)	0.665
1 or more Comorbidities	300(92)	26(93)	1.000	164(92)	162(92)	0.849
Smoke			0.408			0.005
Never	163(50)	13(46)		103(58)	73(41)	
Ex-Smoker	139(43)	11(39)		61(34)	89(50)	
Current Smoker	25(8)	4(14)		14(8)	15(8)	
More Than Three	98(30)	6(21)		24(13)	80(45)	
Gp Consultation Last 3 Months			0.322			0.000
None	47(14)	6(21)		32(18)	21(12)	
One	84(26)	9(32)		62(35)	31(18)	
Two or more	196(60)	13(46)		84(47)	125(71)	
Occupation			0.348			0.000
Skilled Non-Manual	47(14)	2(7)		25(14)	24(14)	
Skilled Manual	43(13)	6(21)		38(21)	11(6)	
Unskilled	237(72)	20(71)		115(65)	142(80)	
Contact With Children	119(36)	10(36)	1.000	73(41)	56(32)	0.078
Hospitalized Last 12 Months?	140(43)	11(39)	0.843	77(43)	74(42)	0.830
Vaccination within 120 Days before symptoms	233(71)	22(79)	0.514	135(76)	120(68)	0.100
Swab taking within 4 Days Of Symptoms	193(59)	16(57)	0.844	117(66)	92(52)	0.010
Prior season vaccination	294(90)	25(89)	1.000	164(92)	155(88)	0.164
Flu Vaccine 2010-11 Type			0.457			0.000
Mf59	166(51)	11(39)		0(0)	177(100)	
Virosomal	112(34)	12(43)		124(70)	0(0)	
Split	49(15)	5(18)		54(30)	0(0)	
Pneumococcal Vaccine	91(28)	11(39)	0.199	60(34)	42(24)	0.046
Pandemic Flu Vacc	168(51)	13(46)	0.695	83(47)	98(55)	0.112
ICU admission	12(4)	1(4)	1.000	10(6)	3(2)	0.086
Death	14(4)	0(0)	0.614	6(3)	8(5)	0.599

In the ORV control group, a total of 98 participants was included in the analysis with 70 ORV controls. In the PAN control group, 257 participants were tested negative for all viruses and set as control resulting in a total of 285 participants.

Table 5 Different characteristics of different control groups in rVE analyses

	TND Control 327 (%)	*ORV Control 70 (%)*	*PAN Control 257 (%)*
Age group			
60 - 74	100(31)	23(33)	85(33)
>=75	227(69)	47(67)	172(67)
Female	128(39)	34(49)	94(37)
Comorbidity	300(92)	66(94)	234(91)
Smoking status			
Never	163(50)	41(59)	122(47)
Ex-Smoker	139(43)	24(34)	115(45)
Current Smoker	25(8)	5(7)	20(8)
GP visits in last 3 months			
0	47(14)	12(17)	35(14)
1	84(26)	16(23)	68(26)
2 or more	196(60)	42(60)	154(60)
Has Been Hospitalized Last 12 Months?	140(43)	28(40)	112(44)
Prior season Flu Vaccine	294(90)	61(87)	233(91)
Flu Vaccine 2010-11 Type			
Mf59	166(51)	31(44)	135(53)
Virosomal	112(34)	26(37)	86(33)
Split	49(15)	13(19)	36(14)
Pneumococcal Vaccine	91(28)	27(39)	64(25)
Pandemic Flu Vaccine	168(51)	26(37)	142(55)

Changes to crude rVE estimate were less than 10% for all models with each variable for the TND control group. The change was more than 10% in the full model only.

With the ORV control group, no variable was associated with influenza test status at the p ≤ 0.25 level in univariate analysis. Details can be found in Annexes *Table 14*.

With PAN control group, time since vaccination and pneumococcal vaccination had p-value ≤ 0.25 in univariate analyses. Details can be found in Annexes *Table 16*.

10% change to the crude odds ratio was not observed in the models with each variable.

rVE estimates by TND and PAN control groups showed similar results and ORV control group showed lower estimates. More than 10% change to the crude rVE was not observed in the models with each variable with the different control groups. The details of the models can be seen in Table 13, *Table 15*, and *Table 17*of Annexes.

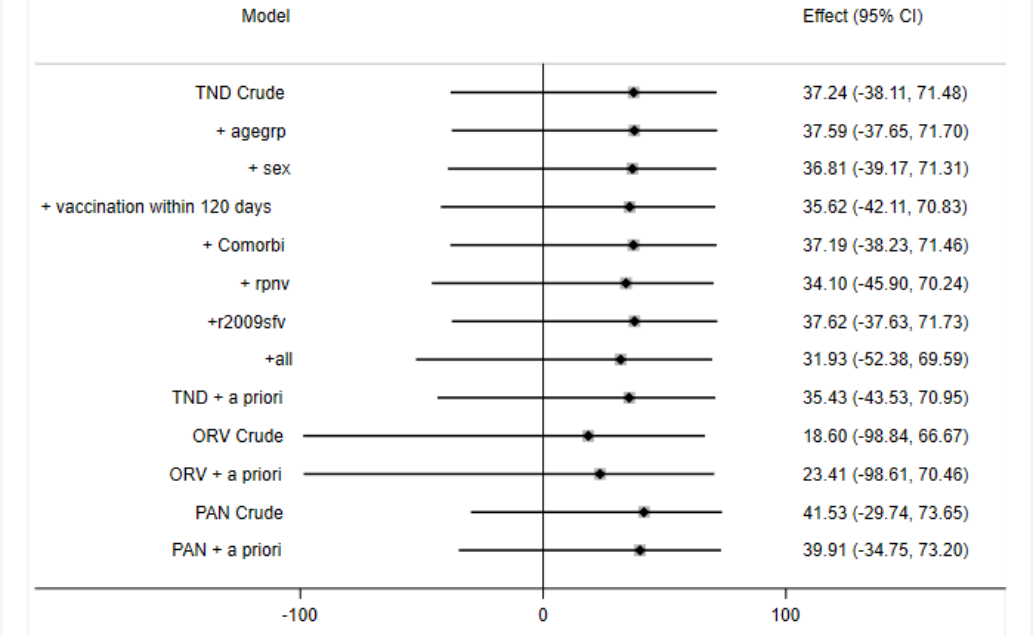

Figure 8 Different rVE estimates

Propensity scores and different methods

The results of the different models using propensity scores, matching, stratified, and weighting, showed similar results with the logistic regression models. The models including all participants aged 60 years or older from the dataset showed similar results too. Among a total of 479 participants in the model of all participants, 28 were found to be positive for influenza.

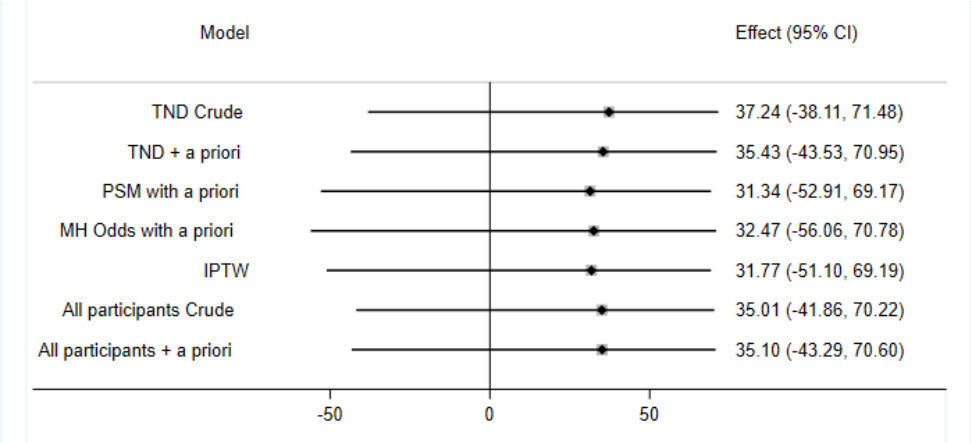

Figure 9 rVE estimates with different methods and different participants

Discussion

TND studies have been in frequent use to monitor vaccine effectiveness and other exposures and offer advantages over other study designs in their ability to reduce community level variation and health-seeking behaviour differences between cases and controls. However, these are observational study designs and assessments for bias and confounders are still

needed to assess the risk of distorted results. This study attempted to look at these biases and confounders observed in VE and rVE TND studies by searching the literature and analyzing a real-world dataset.

Selection bias

VE studies

Selection bias can occur if patients hospitalized due to chronic conditions are more likely to get vaccinated for influenza (Feng et al., 2018). In this VE study, the difference in vaccination status among those who have at least one comorbidity and who have none was statistically significant (almost equally among those without comorbidity, and 63.1% vaccinated among those with comorbidity). However, the presence of the comorbidity did not make a statistically significant effect in the multivariate models. The change to crude odds ratio was also minimal (0.545 to 0.549) which is in line with the previous finding (Remschmidt et al., 2015). Our dataset was comprised entirely of hospitalized patients and this population may have a high prevalence of comorbid conditions, irrespective of the reason for their hospitalization, which may explain this finding.

Older people were more likely to get vaccinated and the age groups acted as an effect modifier which was also observed in the same dataset during the 2011 season (Joan Puig-Barberà et al., 2014). Stratified OR showed higher significant VE in 60 to 74 years aged group compared to the overall VE, confirming the challenges of protecting older adults.

Lower VE estimates with the PAN control group and higher estimates with the ORV control group were observed compared to the flu negative control group. It can be due to the virus interference where the flu vaccination made susceptible to other viruses, although it is less likely to observe in older adults due to poor immunological responses (Feng et al., 2018). It might also be because of the confounding by indication where vaccinated people were more prone to infection as the proportion of patients who have at least one comorbidity in ORV control is higher than in other control groups in this dataset. The fact that results were sensitive to choice of controls underlines the importance of careful study design and multiple sensitivity analysis when conducting observational VE studies.

rVE studies

The findings in rVE analyses were contrary to the results of VE: higher estimates with PAN control group, and lower estimates with ORV control group in crude models while the differences in adjusted models were much smaller. However, the confidence interval in ORV control group was wider than other control groups showing the limited number of participants. The difference might not be much if the dataset is big enough as the upper limit of confidence intervals showed similar results, though this would be difficult to do in practice because

hospitalized influenza is a rare outcome and few healthcare settings routinely use >1 influenza vaccine in the same population groups.

Information bias

Misclassification of vaccination status could not be checked in the analysis as there was no information concerning the discrepancies between the patients' recalls and ascertainment by the records.

Although the misclassification of disease, false negative, is more likely to occur if the swabbing time is more than four days after the onset of the symptoms due to poor sensitivity (Sullivan et al., 2014), the swabbing time difference did not make significant changes to estimates in these VE and rVE analyses. It confirmed the importance of the specificity of the test rather than the sensitivity (Endo et al., 2020).

Including participants which likely to be more negative (out of the epidemic season, prior antiviral treatment, and broader definition of ILI) did not have much effect on estimates of both VE and rVE analyses. However, these excluded participants had only one case in a total of 223 patients in VE analysis, and no case at all in rVE analysis. Again, the sensitivity may not be a problem for the TND studies, however, it will be better to use the sensitive case definition criteria including the restriction of the epidemiological weeks.

Confounders

VE studies

The significant differences in prior season vaccination, pandemic flu vaccination, and pneumococcal vaccination status among vaccinated and unvaccinated for 2009 season influenza vaccination showed the difference in health-seeking behaviour which has been previously cited as an important limitation of observational VE studies (L. A. Jackson et al., 2006). Quitting smoking occurred significantly more in the vaccinated group, which may confirm this "healthy vaccinee bias". This indicated that TND in VE studies are still impacted by the health-seeking behaviour difference between cases and controls (Sullivan et al., 2016)

As discussed earlier, age is an important confounder as widely accepted (Sullivan et al., 2014) and effect modifier even in the analysis of people aged 60 years or more. Sex might be an important confounder as being different on exposure status, but it did not show the significance in the logistic regression models supporting the decision by many authors to omit it (Sullivan et al., 2016).

Study site difference can still be an important confounder in TND VE studies as stated in the previous study (Joan Puig-Barberà et al., 2014), however, its statistical difference was not observed in these analyses. It may be due to similarity in vaccination coverage in all study

sites due to the free vaccination policy in Spain (J. Puig-Barberà et al., 2012) as the statistical difference was observed for the disease status. Additionally, these data were collected from a relatively small area (Valencia, Spain) where we might expect the population to be relatively homogeneous as compared to over the entire country.

rVE studies

Vaccination for pandemic flu and pneumococcal virus, hospitalization within one year, and GP visits within three months were not significantly different between cases and controls showing more similar health-seeking behaviour comparing to the VE analysis. And the small differences to crude estimates by each potential confounder also showed a quite similar characteristics between cases and controls, and between exposed and unexposed. This is expected because individuals choosing to be vaccinated are likely similar to each other; and vaccine allocation is decided based on public health policy, not individual choice. For this reason this season represented an interesting 'natural experiment' to measure the performance of different vaccines.

Implication of study

TND studies have been increasingly used for influenza vaccine effectiveness studies due to their advantages of feasibility and reduction of bias. It might also be considered to use in other vaccine effectiveness studies as it can be conducted with the existing surveillance data, such as in Covid-19 vaccination these days.

Availability of different vaccines with new technologies for influenza will make policymakers think of using more effective vaccines, and the relative vaccine effectiveness between different vaccines will need to be assessed. Test-negative design case-control may be used mostly in these studies due to above-mentioned advantages and the findings in this study will be helpful for the design and analysis plan.

There might also be an opportunity to compare the effectiveness of different vaccines in today's context for Covid-19. rVE studies on Covid-19 will become important for the policymakers as multiple vaccines with different technologies are available widely in the same geographic region. In such situation, the findings from this study can be helpful to design and analyze the TND VE and rVE studies.

Limitations of study

The generalizability of the VE and rVE estimates is limited as being conducted on the hospitalized elderly people in this study. Any extrapolation to other populations should be treated with caution. The limited number of cases in this study especially for rVE analyses

(where the effect size is likely to be small) made the insignificant estimates. This is a general limitation of rVE studies from databases, particularly for rare, hospitalized outcomes. Vaccines were allocated on a regional bases which may have obscured differences in baseline characteristics between sites.

Further studies concerning the biases and confounders in the TND rVE studies should be carried out in different settings such as out-patient settings, for multiple seasons, and with other age groups. A large sample size with an adequate number of events to explain the impact of the covariates can still be done in rVE studies to understand the real difference among the different vaccines for influenza, and also for other vaccine-preventable diseases.

Conclusion

The test-negative case-control studies can be used to assess the vaccine effectiveness and relative effectiveness in the hospitalized patients using pre-existing real-world data. The estimates from TND are still valid concerning the information bias if highly specific tests are used to decide the disease status. Although the reduction of confounding by health-seeking behaviour is expected in the TND VE studies, it is not completely eliminated. In TND rVE studies, cases and controls are more comparable and health-seeking behaviours are not different. Age can still be an important confounder or the effect modifier even in the study of people aged 60 years or older. Other potential confounders like prior season vaccination for influenza, sex, presence of comorbidity, and the study site can make significant differences on VE and rVE estimates depending on the circumstances. Considering these biases and limitations, individual observational rVE study results should be treated with caution.

List of Annexes - figures

List of Annexes - tables

Annexes

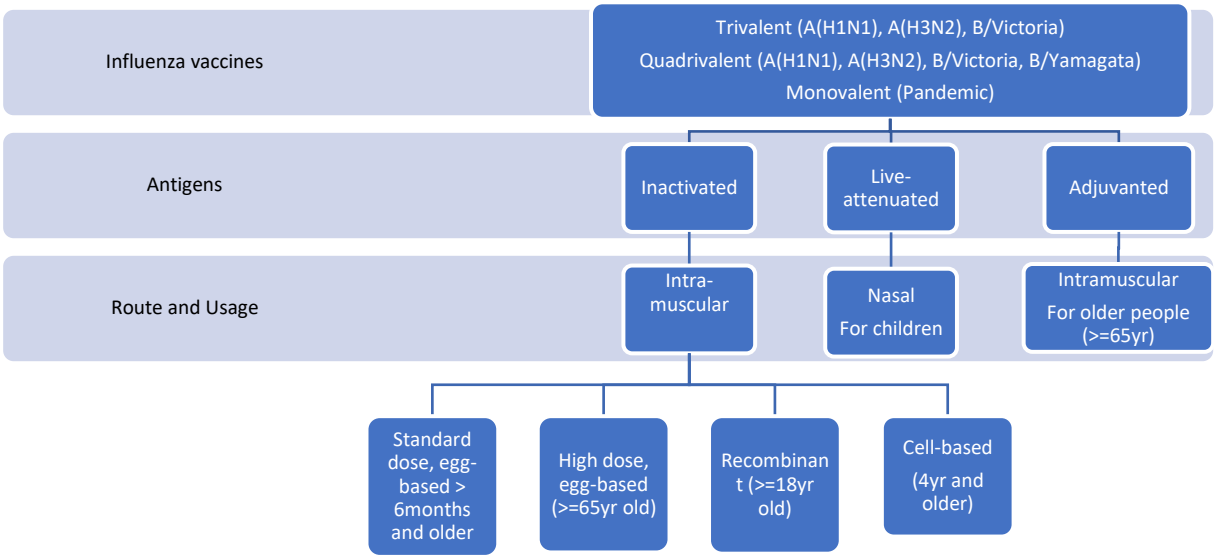

Figure 10 Types of influenza vaccines

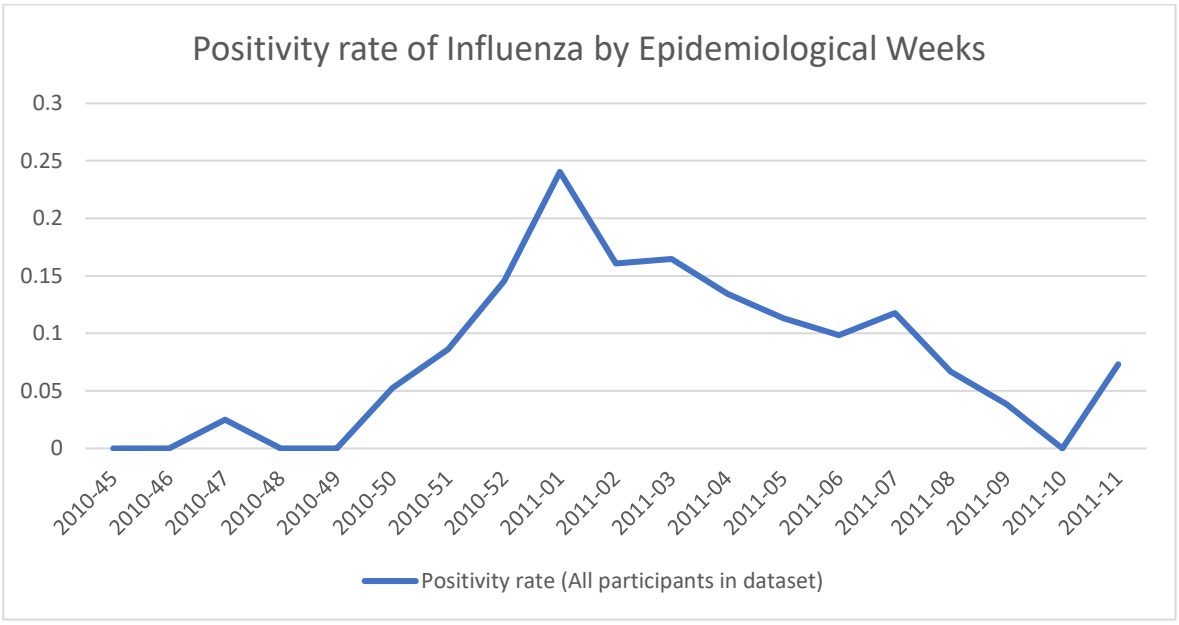

Figure 11 Positivity rate among all participants in the dataset

Table 6 Univariate regression with outcome TND (flu positive or negative)

Variable	*Odds ratio*	*95% CI*		*LR Chi2*	*P*
Crude (vaccination)	0.55	0.32	0.94		
Age group (60 – 74)	1				(Reference)
>=75	0.60	0.34	1.03	3.43	0.0639
Sex	0.86	0.49	1.49	0.31	0.5801
Hospital (La Plana)	1				(Reference)
Arnau de Vilanova	1.84	0.85	4.00		
La Ribera	0.44	0.12	1.58		
San Juan	2.56	1.04	6.28		
General Elda	1.13	0.54	2.33	10.00	0.0404
Obesity	0.93	0.51	1.68	0.06	0.8042
Comorbidity	0.81	0.35	1.88	0.22	0.6361
Smoking status (Non-smoker)	1				(Reference)
Ex-smoker	1.30	0.71	2.38		
Current smoker	2.53	1.19	5.40	5.32	0.07
GP visits (None)	1				(Reference)
1 visit	1.00	0.47	2.16		
2 or more visits	0.57	0.27	1.18	4.16	0.1249
Occupation (Skilled Non-Manual)	1				(Reference)
Skilled manual	0.87	0.36	2.12		
Unskilled	0.54	0.27	1.06	3.83	0.1474
Contact with kids	1.39	0.80	2.40	1.34	0.2474
Hospitalization within 12 months	0.80	0.46	1.40	0.61	0.4357
Vaccination within 120days before symptoms	1.60	1.06	2.41	5.42	0.0199
Swab taking within 4days of symptom onset	1.02	0.59	1.77	0.01	0.9389
Prior season flu vaccination	0.51	0.30	0.89	5.58	0.0182
Pneumococcal vaccination	0.84	0.42	1.67	0.26	0.6118
Pandemic flu vaccination	0.71	0.39	1.28	1.33	0.2488

Table 7 Different models with TND control

	(Crude)	(2)	(3)	(4)	(5)	(With *a priori*)	(Full)	(Adjusted)	(*a priori* 60-74)	(*a priori* >=75)	(Full 60-74)	(Full >=65)	(Adjusted 60-74)	(Adjusted >=65)
vac	0.545** (0.316 - 0.940)	0.535** (0.309 - 0.925)	0.549** (0.318 - 0.949)	0.561** (0.323 - 0.975)	0.546** (0.311 - 0.956)	0.555** (0.318 - 0.969)	0.567* (0.320 - 1.006)	0.561** (0.323 - 0.975)	0.318** (0.128 - 0.787)	0.970 (0.430 - 2.187)	0.329** (0.132 - 0.823)	0.974 (0.422 - 2.250)	0.331** (0.135 - 0.808)	0.953 (0.426 - 2.130)
6.hospital				1.786 (0.819 - 3.892)		1.797 (0.824 - 3.921)	1.821 (0.830 - 3.992)	1.786 (0.819 - 3.892)	1.363 (0.411 - 4.520)	2.198 (0.777 - 6.221)	1.367 (0.412 - 4.540)	2.209 (0.767 - 6.366)	1.343 (0.407 - 4.432)	2.135 (0.758 - 6.010)
11.hospital				0.472 (0.132 - 1.687)		0.479 (0.134 - 1.715)	0.485 (0.135 - 1.737)	0.472 (0.132 - 1.687)	0.317 (0.0364 - 2.767)	0.618 (0.126 - 3.040)	0.319 (0.0366 - 2.789)	0.620 (0.125 - 3.081)	0.325 (0.0374 - 2.818)	0.595 (0.121 - 2.914)
17.hospital				2.669** (1.080 - 6.596)		2.728** (1.101 - 6.760)	2.795** (1.111 - 7.033)	2.669** (1.080 - 6.596)	2.249 (0.561 - 9.016)	3.486** (1.018 - 11.94)	2.318 (0.574 - 9.366)	3.518* (0.974 - 12.70)	2.196 (0.550 - 8.761)	3.246* (0.967 - 10.90)
18.hospital				1.136 (0.547 - 2.357)		1.141 (0.549 - 2.371)	1.151 (0.553 - 2.398)	1.136 (0.547 - 2.357)	1.515 (0.525 - 4.368)	0.932 (0.324 - 2.677)	1.510 (0.523 - 4.360)	0.935 (0.322 - 2.718)	1.521 (0.528 - 4.379)	0.898 (0.314 - 2.565)
sex		0.809 (0.463 - 1.415)				0.794 (0.452 - 1.396)	0.797 (0.453 - 1.401)		0.824 (0.340 - 2.000)	0.852 (0.394 - 1.844)	0.830 (0.341 - 2.017)	0.851 (0.393 - 1.844)		
comorbi			0.883 (0.379 - 2.061)			0.922 (0.390 - 2.180)	0.935 (0.393 - 2.222)		1.215 (0.376 - 3.921)	0.671 (0.182 - 2.473)	1.259 (0.387 - 4.097)	0.672 (0.182 - 2.483)		
rpnv					0.996 (0.489 - 2.028)		0.897 (0.429 - 1.877)				0.696 (0.144 - 3.367)	0.978 (0.399 - 2.393)		
Observations	576	576	576	576	576	576	576	576	213	363	213	363	213	363

*** p<0.01, ** p<0.05, * p<0.1

Table 8 Univariate regression with ORV control

Variable	*Odds ratio*	*95% CI*		*LR Chi2*	*P*
Crude (vaccination)	0.37	0.19	0.73		
Age group (60 – 74)	1				(Reference)
>=75	0.65	0.34	1.25	1.65	0.1985
Sex	0.68	0.35	1.32	1.28	0.2572
Hospital (La Plana)	1				(Reference)
Arnau de Vilanova	1.87	0.71	4.93		
La Ribera	0.51	0.12	2.11		
San Juan	1.05	0.37	2.94		
General Elda	1.09	0.46	2.60	3.63	0.4578
Obesity	0.90	0.44	1.82	0.09	0.7609
Comorbidity	0.48	0.15	1.49	1.63	0.2021
Smoking status (Non-smoker)	1				(Reference)
Ex-smoker	1.62	0.79	3.32		
Current smoker	3.19	1.18	8.60	5.72	0.0573
GP visits (None)	1				(Reference)
1 visit	1.43	0.55	3.71		
2 or more visits	0.66	0.28	1.57	4.02	0.1249
Occupation (Skilled Non-Manual)	1				(Reference)
Skilled manual	0.64	0.20	2.08		
Unskilled	0.35	0.14	0.89	5.71	0.0575
Contact with kids	1.80	0.92	3.55	2.91	0.0881
Hospitalization within 12 months	0.89	0.46	1.72	0.13	0.7216
Vaccination 120days before symptoms	0.68	0.23	2.07	8.73	0.0127
Swab taking within 4days of symptom onset	0.99	0.51	1.91	0.00	0.976
Prior season flu vaccination	0.45	0.23	0.88	5.43	0.0198
Pneumococcal vaccination	0.56	0.25	1.22	2.22	0.1359
Pandemic flu vaccination	0.99	0.48	2.01	0.00	0.9703

Table 9 Different models with ORV control

	(Crude)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(With *a priori*)	(Full)	(Adjusted)	(*a priori* 60-74)	(*a priori* >=75)	(Full 60-74)	(Full >=65)	(Adjusted 60-74)	(Adjusted >=65)
vac	0.373***	0.379***	0.385***	0.388***	0.412**	0.373***	0.395***	0.394***	0.396**	0.405**	0.510*	0.395***	0.282**	0.590	0.479	0.951	0.314**	0.621
	(0.190 - 0.734)	(0.192 - 0.748)	(0.195 - 0.760)	(0.195 - 0.774)	(0.207 - 0.821)	(0.188 - 0.741)	(0.198 - 0.786)	(0.199 - 0.780)	(0.190 - 0.827)	(0.202 - 0.814)	(0.229 - 1.134)	(0.198 - 0.786)	(0.0878 - 0.905)	(0.212 - 1.640)	(0.115 - 2.004)	(0.229 - 3.957)	(0.105 - 0.936)	(0.219 - 1.762)
6.hospital				1.804						1.941	2.105		1.896	1.952	3.763	2.683		
				(0.666 - 4.889)						(0.706 - 5.336)	(0.675 - 6.570)		(0.357 - 10.06)	(0.517 - 7.367)	(0.382 - 37.10)	(0.441 - 16.32)		
11.hospital				0.597						0.631	0.620		0.320	1.326	0.513	1.267		
				(0.140 - 2.551)						(0.147 - 2.718)	(0.131 - 2.933)		(0.0295 - 3.483)	(0.186 - 9.477)	(0.0315 - 8.333)	(0.119 - 13.46)		
17.hospital				1.232						1.330	1.215		1.907	1.156	4.642	0.543		
				(0.426 - 3.559)						(0.453 - 3.908)	(0.363 - 4.061)		(0.308 - 11.80)	(0.270 - 4.953)	(0.399 - 54.01)	(0.0693 - 4.250)		
18.hospital				1.080						1.161	1.116		1.515	0.971	2.388	0.450		
				(0.444 - 2.629)						(0.470 - 2.865)	(0.400 - 3.114)		(0.388 - 5.919)	(0.256 - 3.676)	(0.429 - 13.29)	(0.0788 - 2.566)		
sex		0.715								0.729	1.387		0.710	0.766	0.856	24.10**		
		(0.364 - 1.406)								(0.365 - 1.459)	(0.506 - 3.808)		(0.217 - 2.327)	(0.303 - 1.939)	(0.179 - 4.104)	(1.172 - 495.6)		
comorbi			0.546							0.552	0.711		0.590	0.516	0.519	0.626		
			(0.168 - 1.774)							(0.162 - 1.884)	(0.181 - 2.796)		(0.0987 - 3.533)	(0.0793 - 3.359)	(0.0585 - 4.609)	(0.0583 - 6.721)		

1.Smoke				1.534 (0.736 - 3.200)						1.779 (0.625 - 5.060)				0.762 (0.141 - 4.131)	36.23** (1.683 - 780.0)		
2.Smoke				2.636* (0.949 - 7.321)						2.772 (0.732 - 10.50)				1.429 (0.168 - 12.17)	886.5*** (13.14 - 59,785)		
1.gp3					1.474 (0.552 - 3.933)					1.779 (0.593 - 5.341)				0.923 (0.158 - 5.397)	3.800 (0.578 - 24.96)		
2.gp3					0.679 (0.279 - 1.650)					0.891 (0.330 - 2.402)				1.145 (0.192 - 6.843)	0.405 (0.0824 - 1.993)		
2.Occu						0.729 (0.217 - 2.444)				0.567 (0.149 - 2.151)	0.729 (0.217 - 2.444)			0.110** (0.0150 - 0.804)	13.79* (0.661 - 287.7)	0.218* (0.0396 - 1.206)	8.668* (0.729 - 103.1)
3.Occu						0.399* (0.156 - 1.024)				0.396* (0.140 - 1.119)	0.399* (0.156 - 1.024)			0.366 (0.0712 - 1.886)	0.795 (0.143 - 4.404)	0.322 (0.0796 - 1.301)	0.572 (0.145 - 2.259)
kids							1.620 (0.807 - 3.253)			1.936 (0.878 - 4.265)				1.504 (0.328 - 6.909)	5.851** (1.422 - 24.09)		
rpnv								0.842 (0.355 - 1.999)		0.910 (0.352 - 2.354)				0.324 (0.0395 - 2.657)	0.879 (0.201 - 3.851)		
Observations	156	156	156	156	156	156	156	156	156	156	156	65	91	65	91	65	91

*** p<0.01, ** p<0.05, * p<0.1

Table 10 Univariate regression with PAN control

Variable	*Odds ratio*	*95% CI*		*LR Chi2*	*P*
Crude (vaccination)	0.59	0.34	1.03		
Age group (60 – 74)	1				(Reference)
>=75	0.49	0.28	0.85	6.49	0.0108
Sex	0.90	0.52	1.58	0.13	0.7182
Hospital (La Plana)	1				(Reference)
Arnau de Vilanova	1.84	0.84	4.03		
La Ribera	0.43	0.12	1.55		
San Juan	3.65	1.43	9.37		
General Elda	1.13	0.54	2.36	13.12	0.0107
Obesity	0.94	0.51	1.71	0.05	0.8272
Comorbidity	0.90	0.38	2.09	0.06	0.8017
Smoking status (Non-smoker)	1				(Reference)
Ex-smoker	1.23	0.67	2.27		
Current smoker	2.40	1.11	5.18	4.63	0.0988
GP visits (None)	1				(Reference)
1 visit	0.92	0.42	2.01		
2 or more visits	0.55	0.26	1.14	3.92	0.1405
Occupation (Skilled Non-Manual)	1				(Reference)
Skilled manual	0.93	0.38	2.28		
Unskilled	0.58	0.29	1.16	3.02	0.2214
Contact with kids	1.31	0.75	2.28	0.89	0.3461
Hospitalization within 12 months	0.78	0.45	1.38	0.73	0.3944
Vaccination 120days before symptoms	1.75	0.68	4.48	4.95	0.0843
Swab taking within 4days of symptom onset	1.03	0.59	1.79	0.01	0.919
Prior season flu vaccination	0.53	0.30	0.92	4.98	0.0257
Pneumococcal vaccination	0.94	0.47	1.88	0.03	0.8524
Pandemic flu vaccination	0.66	0.36	1.20	1.92	0.1655

Table 11 Different VE models with PAN control

	(Crude)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(With *a priori*)	(Full)	(Adjusted)	(*a priori* 60-74)	(*a priori* >=75)	(Full 60-74)	(Full >=65)	(Adjusted 60-74)	(Adjusted >=65)
vac	0.592* (0.341 - 1.027)	0.628 (0.358 - 1.101)	0.582* (0.334 - 1.013)	0.593* (0.341 - 1.032)	0.623* (0.356 - 1.092)	0.604* (0.347 - 1.050)	0.600* (0.345 - 1.046)	0.647 (0.352 - 1.190)	0.618* (0.351 - 1.090)	0.736 (0.391 - 1.387)	0.628 (0.358 - 1.101)	0.325** (0.129 - 0.819)	1.167 (0.507 - 2.685)	0.210** (0.063 6 - 0.691)	1.701 (0.679 - 4.265)	0.339** (0.137 - 0.842)	1.157 (0.507 - 2.640)
6.hospital		1.785 (0.812 - 3.924)							1.792 (0.814 - 3.943)	1.646 (0.729 - 3.714)	1.785 (0.812 - 3.924)	1.239 (0.368 - 4.173)	2.290 (0.801 - 6.545)	1.418 (0.396 - 5.080)	2.269 (0.719 - 7.165)	1.226 (0.365 - 4.116)	2.254 (0.791 - 6.423)
11.hospital		0.458 (0.127 - 1.645)							0.464 (0.129 - 1.668)	0.362 (0.097 5 - 1.344)	0.458 (0.127 - 1.645)	0.329 (0.037 0 - 2.929)	0.551 (0.111 - 2.723)	0.366 (0.036 3 - 3.680)	0.314 (0.058 8 - 1.681)	0.339 (0.038 4 - 2.999)	0.539 (0.109 - 2.655)
17.hospital		3.687** (1.432 - 9.495)							3.741** (1.449 - 9.656)	2.787** (0.993 - 7.816)	3.687** (1.432 - 9.495)	2.310 (0.555 - 9.605)	6.740** (1.784 - 25.47)	2.484 (0.537 - 11.49)	4.065** (0.924 - 17.89)	2.248 (0.543 - 9.297)	6.480** (1.742 - 24.11)
18.hospital		1.148 (0.550 - 2.398)							1.149 (0.550 - 2.401)	0.989 (0.447 - 2.190)	1.148 (0.550 - 2.398)	1.542 (0.522 - 4.551)	0.933 (0.324 - 2.690)	1.723 (0.511 - 5.813)	0.644 (0.199 - 2.077)	1.532 (0.520 - 4.508)	0.918 (0.319 - 2.641)
sex			0.850 (0.483 - 1.498)						0.840 (0.473 - 1.493)	1.087 (0.468 - 2.525)		0.866 (0.350 - 2.141)	0.888 (0.403 - 1.959)	0.819 (0.218 - 3.079)	1.688 (0.496 - 5.748)		
comorbi				0.964					1.017	1.107		1.392	0.738	1.303	1.109		

			(0.410 - 2.264)						(0.426 - 2.428)	(0.445 - 2.752)		(0.427 - 4.543)	(0.197 - 2.774)	(0.356 - 4.766)	(0.269 - 4.577)		
1.Smoke				1.278 (0.690 - 2.367)						1.177 (0.496 - 2.796)				0.703 (0.180 - 2.745)	2.183 (0.628 - 7.590)		
2.Smoke				2.233** (1.028 - 4.849)						1.929 (0.712 - 5.230)				0.811 (0.189 - 3.483)	4.429* (0.899 - 21.82)		
1.gp3					0.936 (0.428 - 2.049)					1.277 (0.544 - 2.998)				0.650 (0.176 - 2.396)	2.019 (0.570 - 7.148)		
2.gp3					0.561 (0.267 - 1.178)					0.709 (0.317 - 1.587)				0.464 (0.138 - 1.558)	0.813 (0.251 - 2.641)		
2.Occu						0.989 (0.399 - 2.449)				0.919 (0.354 - 2.388)				0.751 (0.189 - 2.986)	1.898 (0.425 - 8.467)		
3.Occu						0.608 (0.303 - 1.218)				0.784 (0.374 - 1.642)				0.838 (0.285 - 2.461)	1.023 (0.305 - 3.436)		
rpfv							0.802 (0.414 - 1.556)			0.854 (0.421 - 1.732)				2.371 (0.724 - 7.762)	0.415* (0.155 - 1.112)		
Observations	478	478	478	478	478	478	478	478	478	478	478	176	302	176	302	176	302

*** p<0.01, ** p<0.05, * p<0.1

Table 12 Results of univariate regression with TND control (rVE)

Variable	*Odds ratio*	*95% CI*		*LR Chi2*	*P*
Hospital (La Plana)	1				Reference
Arnau de Vilanova	1.791	0.520	6.165		
La Ribera	0.990	0.238	4.122		
San Juan	4.040	1.201	13.593		
General Elda	1.307	0.438	3.902	5.69	0.2235
Age group (60 – 74)	1				Reference
>=75	1.041	0.456	2.378	0.01	0.9237
sex	1.347	0.621	2.925	0.56	0.4529
Obesity	1.091	0.477	2.495	0.04	0.8374
Comorbidity	1.170	0.263	5.197	0.04	0.8334
Smoking (Non-smoker)	1				Reference
Ex-smoker	0.992	0.431	2.285		
Current smoker	2.006	0.606	6.641	1.28	0.526
GP visits in last 3 months (None)	1				Reference
1 visit	0.839	0.281	2.504		
2 or more visits	0.520	0.188	1.438	2.01	0.3664
Occupation (Skilled non-manual)	1				Reference
Skilled manual	3.279	0.628	17.123		
Unskilled	1.983	0.448	8.772	2.28	0.319
Contact with kids	0.971	0.434	2.172	0.01	0.9429
Hospitalization within 12 months	0.959	0.697	1.318	0.2	0.6587
Vaccination 120days before symptoms	1.479	0.581	3.764	0.72	0.3968
Swab taking within 4days of symptom onset	0.926	0.424	2.020	0.04	0.8465
Prior season flu vaccination	0.935	0.268	3.266	0.01	0.9172
Pneumococcal vaccination	1.678	0.757	3.720	1.56	0.211
Pandemic flu vaccination	0.820	0.378	1.778	0.25	0.6152

Table 13 Different rVE models with TND control

	(Crude)	(2)	(3)	(4)	(5)	(6)	(7)	(*a priori*)	(Full)
vac2	0.628 (0.285 - 1.381)	0.624 (0.283 - 1.377)	0.632 (0.287 - 1.392)	0.644 (0.292 - 1.421)	0.628 (0.285 - 1.382)	0.659 (0.298 - 1.459)	0.624 (0.283 - 1.376)	0.646 (0.291 - 1.435)	0.681 (0.304 - 1.524)
agegp 2		1.081 (0.471 - 2.478)						0.992 (0.418 - 2.356)	0.924 (0.384 - 2.220)
sex			1.332 (0.613 - 2.898)					1.304 (0.585 - 2.904)	1.299 (0.582 - 2.898)
vac12 0				1.418 (0.555 - 3.623)				1.396 (0.544 - 3.583)	1.353 (0.525 - 3.490)
comor bi					1.160 (0.260 - 5.166)			1.116 (0.246 - 5.059)	1.031 (0.225 - 4.725)
rpnv						1.599 (0.717 - 3.566)			1.564 (0.685 - 3.567)
r2009s fv							0.881 (0.251 - 3.097)	0.908 (0.255 - 3.238)	0.937 (0.261 - 3.364)
Obser vation s	355	355	355	355	355	355	355	355	355

*** p<0.01, ** p<0.05, * p<0.1

Table 14 Univariate regression with ORV control (rVE)

Variable	*Odds ratio*	*95% CI*		*LR Chi2*	*P*
Hospital (La Plana)	1				Reference
Arnau de Vilanova	1.67	0.41	6.82		
La Ribera	1.25	0.25	6.26		
San Juan	1.54	0.41	5.82		
General Elda	1.40	0.41	4.81	0.67	0.955
Age group (60 – 74)	1				Reference
>=75	1.03	0.40	2.64	0.00	0.9456
sex	0.92	0.38	2.21	0.04	0.8478
Obesity	0.91	0.36	2.31	0.04	0.8389
Comorbidity	0.79	0.14	4.57	0.07	0.7928
Smoking (Non-smoker)	1				Reference
Ex-smoker	1.45	0.56	3.73		
Current smoker	2.52	0.59	10.81	1.71	0.4247
GP visits in last 3 months (None)	1				Reference
1 visit	1.13	0.31	4.03		
2 or more visits	0.62	0.19	1.98	1.52	0.4673
Occupation (Skilled non-manual)	1				Reference
Skilled manual	3.37	0.52	21.73		
Unskilled	1.70	0.34	8.55	1.98	0.3713
Contact with kids	1.49	0.59	3.80	0.69	0.4058
Hospitalization within 12 months	0.97	0.40	2.38	0.00	0.9479
Vaccination 120days before symptoms	0.68	0.23	2.07	0.44	0.5067
Swab taking within 4days of symptom onset	0.94	0.39	2.29	0.02	0.897
Prior season flu vaccination	1.23	0.31	4.92	0.09	0.7674
Pneumococcal vaccination	1.03	0.42	2.53	0.00	0.9478
Pandemic flu vaccination	1.47	0.60	3.56	0.71	0.3983

Table 15 Different rVE models with ORV control

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
vac2	0.814 (0.333 - 1.988)	0.809 (0.329 - 1.988)	0.806 (0.329 - 1.977)	0.810 (0.331 - 1.981)	0.788 (0.320 - 1.940)	0.829 (0.335 - 2.053)	0.766 (0.295 - 1.986)
agegp2		1.061 (0.413 - 2.728)					1.197 (0.410 - 3.495)
sex			0.900 (0.372 - 2.177)				0.903 (0.350 - 2.331)
comorbi				0.775 (0.133 - 4.507)			0.763 (0.126 - 4.617)
vac120					0.663 (0.217 - 2.026)		0.637 (0.201 - 2.018)
r2009sfv						1.169 (0.285 - 4.784)	1.096 (0.248 - 4.843)
1.gp3							
2.gp3							
Constant	0.436*** (0.247 - 0.770)	0.396 (0.0766 - 2.045)	0.511 (0.120 - 2.173)	0.555 (0.0960 - 3.202)	0.617 (0.206 - 1.851)	0.377 (0.0900 - 1.580)	0.659 (0.0397 - 10.94)
Observations	98	98	98	98	98	98	98

Table 16 Univariate regression with PAN control (rVE)

Variable	*Odds ratio*	*95% CI*		*LR Chi2*	*P*
Hospital (La Plana)	1				Reference
Arnau de Vilanova	1.82	0.52	6.36		
La Ribera	0.94	0.22	3.95		
San Juan	6.75	1.87	24.37		
General Elda	1.29	0.43	3.87	9.31	0.0539
Age group (60 – 74)	1				Reference
>=75	1.04	0.45	2.40	0.01	0.9206
sex	1.50	0.69	3.29	1.02	0.312
Obesity	1.15	0.50	2.66	0.10	0.7461
Comorbidity	1.28	0.28	5.73	0.11	0.7417
Smoking (Non-smoker)	1				Reference
Ex-smoker	0.90	0.39	2.08		
Current smoker	1.88	0.56	6.33	1.25	0.5342
GP visits in last 3 months (None)	1				Reference
1 visit	0.77	0.25	2.34		
2 or more visits	0.49	0.17	1.39	2.07	0.3552
Occupatioin (Skilled non-manual)	1				Reference
Skilled manual	3.26	0.62	17.21		
Unskilled	2.07	0.46	9.21	2.20	0.3713
Contact with kids	0.87	0.39	1.97	0.11	0.7404
Hospitalization within 12 months	0.84	0.38	1.86	0.19	0.6619
Vaccination 120days before symptoms	1.75	0.68	4.48	1.48	0.2241
Swab taking within 4days of symptom onset	0.92	0.42	2.03	0.04	0.8383
Prior season flu vaccination	0.86	0.24	3.05	0.05	0.8164
Pneumoccal vaccination	1.95	0.87	4.38	2.50	0.1138
Pandemic flu vaccination	0.70	0.32	1.53	0.79	0.3744

Table 17 Different rVE models with PAN control

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
vac2	0.585 (0.264 - 1.297)	0.580 (0.261 - 1.291)	0.590 (0.265 - 1.311)	0.585 (0.264 - 1.298)	0.597 (0.269 - 1.328)	0.581 (0.261 - 1.291)	0.625 (0.279 - 1.398)	0.601 (0.268 - 1.348)
agegp2		1.100 (0.475 - 2.549)						0.965 (0.402 - 2.318)
sex			1.485 (0.676 - 3.265)					1.463 (0.652 - 3.283)
comorbi				1.274 (0.283 - 5.735)				1.226 (0.266 - 5.641)
vac120					1.705 (0.664 - 4.375)			1.684 (0.652 - 4.351)
r2009sfv						0.817 (0.228 - 2.926)		0.839 (0.232 - 3.033)
rpnv							1.840 (0.813 - 4.164)	
Constant	0.139*** (0.0839 - 0.231)	0.131*** (0.0625 - 0.275)	0.118*** (0.0631 - 0.220)	0.112*** (0.0253 - 0.492)	0.0933*** (0.0379 - 0.230)	0.168*** (0.0473 - 0.595)	0.111*** (0.0602 - 0.206)	0.0565*** (0.00487 - 0.654)
Observations	285	285	285	285	285	285	285	285

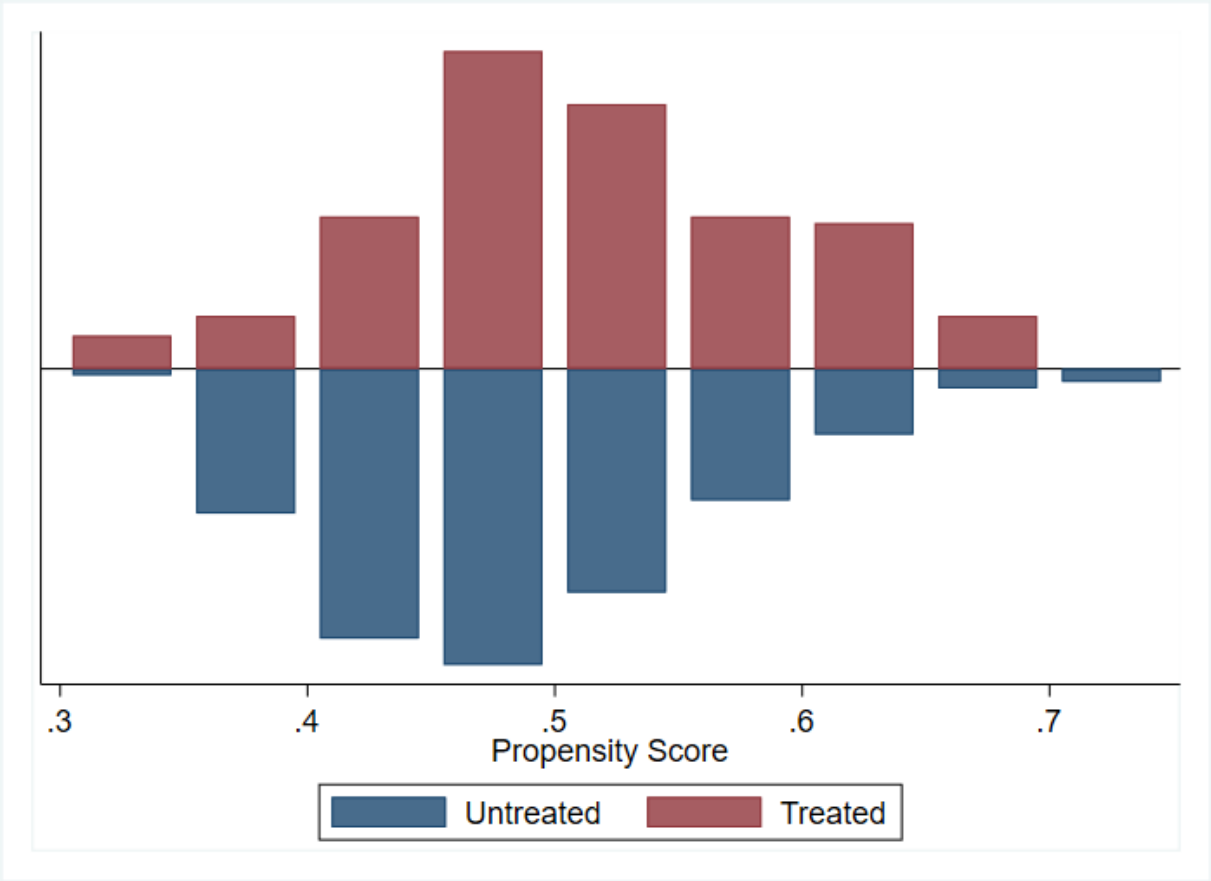

Figure 12 Balance of propensity score(rVE)

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