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Influenza vaccine effectiveness: potential of the test-negative design. A systematic review

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SUMMARY

Background—The test-negative design is a variant of the case control study being increasingly used to study influenza vaccine effectiveness. In these studies, patients with influenza-like illness are tested for influenza. Vaccine coverage is compared between those testing positive versus those testing negative to estimate vaccine effectiveness.

Objectives—We reviewed features in the design, analysis and reporting of 85 published test-negative studies.

Data sources—Studies were identified from PubMed, reference lists and email updates.

Study eligibility—All studies using the test-negative design reporting end-of-season estimates were included.

Study appraisal—Design features that may affect the validity and comparability of reported estimates were reviewed, including setting, study period, source population, case definition, exposure and outcome ascertainment and statistical model.

Results—There was considerable variation in the analytic approach, with 68 unique statistical models identified among the studies.

Conclusion—Harmonisation of analytic approaches may improve the potential for pooling vaccine effectiveness estimates.

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Keywords

influenza; vaccine effectiveness; public health; case control; test-negative study

INTRODUCTION

There are three circulating influenza types/subtypes in humans—influenza type A with subtypes H1N1 and H3N2, and influenza type B—and most influenza vaccines include a component against each of these. The predominant circulating type/subtype changes from year to year, which can influence the severity and duration of the season. Moreover, within each type/subtype the virus undergoes frequent antigenic changes to escape neutralization by the immune system. As a result, the vaccine's strain composition is regularly updated. When updating the strain composition of influenza vaccines, manufacturers must demonstrate immunogenicity of the updated vaccine, but there is no need to demonstrate efficacy or effectiveness [1, 2]. Randomized controlled trials are expensive and time-consuming, and infeasible in some populations, while observational data can be used to monitor variation in vaccine effectiveness (VE) from year to year and in different groups [3].

In recent years, there has been a dramatic increase in the number of published estimates of influenza VE in the community. This is in large part due to the increasing use of a new convenient study design termed the test-negative design (TND). The name is derived from the method of comparison. In this study design, patients presenting to their general practice (GP) clinic or hospital with influenza-like illness (ILI) are enrolled if they meet a certain clinical case definition and fulfil other inclusion criteria. All cases are then swabbed and tested for influenza virus. Influenza vaccine coverage is compared between those testing positive versus those testing negative for influenza to estimate VE (see Box 1). Therefore, unlike the classic case-control study design, there is no control group in a prospective test-negative study because all enrolled patients meet the same case definition. The design can be applied to ILI surveillance data, laboratory data or health management data systems, so long as there is a record of an influenza test and vaccination status is collected or can be ascertained through linkage to other datasets. Thus it can easily be applied, either retrospectively or prospectively.

The TND is intended to minimise confounding by healthcare seeking behaviour [4]. This design appears to be robust in theory [5–7], and gives unbiased estimates when nested in a randomized controlled trial with the consequent minimization of confounding [8]. However, there can be considerable variation in the VE estimates reported by different TND studies. Some of this variation may be due to differences in the influenza viruses circulating, vaccines used, age-specific vaccine coverage, population structure, health care systems and sample size—facets of a study that are difficult to control and which can be location-specific. However, some variation will also likely be due to differences in the study design and statistical model used. Although these studies follow the same basic design, subtle differences may in fact lead to variation in estimates. The objective of this review is to identify methodological variations in published TND studies of influenza VE, and discuss the potential of the TND to monitor influenza VE.

METHODS

Search strategy

Studies reporting influenza VE estimates were initially retrieved from PubMed on 8 November 2013. We searched for articles using the following terms:

1. “influenza” OR “flu”
2. “vaccine effectiveness OR “VE”
3. “test-negative” OR “test negative” OR “case-control” or “case control”
4. One, two & three.

The reference lists of retrieved articles were reviewed to identify any additional studies. Articles were also identified, on an ongoing basis, from influenza email alerts from the Centre for Infectious Disease Research and Policy [9]. Additional PubMed searches were conducted on 28 July 2014 and 4 September 2014. Searches were limited to articles in English only.

Study selection

The titles of all papers identified were independently screened by two authors (SG Sullivan and BJ Cowling). Abstracts of potentially relevant papers were reviewed for eligibility. We excluded articles which reported interim or mid-year analyses as these studies tend to be rapid reports and may not be conducted with the same level of care used in a final analysis. Similarly, we excluded abstracts as they typically do not provide a detailed methods section. We also excluded studies which involved a reanalysis of data, unless part of this reanalysis included previously unpublished data (e.g. data for an entire season not previously published). Studies reporting effectiveness estimates for any type of influenza vaccine (trivalent inactivated, live-attenuated, monovalent, adjuvant/non-adjuvant or otherwise unspecified) were considered.

Data retrieval

The methods of each article were carefully reviewed for the inclusion and treatment of design features and variables used in the calculation of VE estimates. These were broadly broken down into the following:

1. Setting
2. Source population
3. Case definition
4. Exposure definition
5. Outcome definition: duration of symptoms, swab site, test used to confirm influenza infection
6. Study period/season
7. Other exclusions

8. Statistical model
9. Variables included in the model to estimate VE

The rationale for focusing on each of these variables is summarized in Table 1. A standardised form was used to record each data item. If the methods referred to a previous paper, the methods reported in the previous paper were recorded. When reporting which studies chose a certain strategy, specific studies are listed only when there were fewer than 10.

Assessment of heterogeneity and asymmetry

Statistical heterogeneity among studies was assessed using the I^2 statistic and Cochran's Q . The I^2 statistic can be interpreted as total variability in a single study's estimate of the mean of a random-effects distribution that is due to heterogeneity [10]. It measures the overlap of confidence intervals and is independent of the number of studies included in a review. I^2 values of less than 30% are commonly accepted to indicate limited statistical heterogeneity [11]. In contrast, Cochran's Q test measures the weighted sum of squared differences between individual study effects and the pooled effect across studies and is therefore dependent on the number of studies included. It follows a chi-squared distribution with small p-values indicative of a lack of homogeneity.

Publication bias was assessed by plotting funnel plots to visually assess asymmetry and calculating the Egger test statistic. This test performs a linear regression of the odds ratios on their standard errors, weighting by $1/(\text{variance of the odds ratio})$. However, it can suffer from false-positive test results when using odds ratios.

RESULTS

Search results are described in the flow diagram in Figure 1. We identified 1192 articles on influenza vaccine effectiveness in our PubMed search. An initial review of titles suggested 171 articles were potentially eligible for inclusion in the review. Review of abstracts excluded a further 80 which did not use the TND, reported interim estimates or reanalysed published data. The full text was retrieved and reviewed for 91 articles, after which a further 27 were excluded. An additional 21 articles were added, identified from the reference lists of included articles, two additional searches on PubMed and from CIDRAP. This left 85 articles reporting final VE estimates for 111 seasons, summarised in table 1 [12–96]. The number of articles published each year is shown in Figure 2. The first article we identified using the TND for influenza VE originated from British Columbia, Canada in 2005 and reported results for the 2003/2004 season [73].

Setting

Studies were reported from four continents—North America, Europe, Australia and Asia. In total, 28 countries were represented, although for some countries data were presented as part of pooled analyses (i.e. Romania, Poland, Hungary [49–52, 93], Scotland, Wales and Northern Ireland [13, 38, 45] and Russia [68]). Established surveillance networks appear to exist in most of these countries. No studies were identified from South America, Africa, or

the majority of Asian countries. Patient recruitment was through primary care, such as a GP or hospital outpatient clinic or emergency department (n=41), hospital inpatients (n=17) or both inpatients and outpatients (n=26), some which used comprehensive healthcare networks. One study was among healthcare workers [42].

Source population

The majority of studies made VE estimates for patients of all ages (n=48; one excluded children <10y [85]), with the remainder targeted at adults (<15y; n=12; 2 restricted to adults 50y [27, 87]), children (n=9 [19, 26, 29, 31, 47, 59, 62, 81, 94]), elderly (n=4 [51, 53, 72, 95]), children and adults aged 50+ (n=1 [84]), pregnant women (n=1 [89]), military (n=2 [28, 40]), healthcare workers (n=1 [42]) or people at high-risk or in a target group for vaccination (n=7 [14, 18, 20, 25, 43, 44, 52]).

Samples sizes used to generate VE estimates ranged from just 34 in a single season [66] to 7016 across 4 seasons [40] (mean: 738; sd: 561). In some cases, the final sample size reported was probably not that used in the estimation of VE due to missing data.

ILI case definition

The ILI case definition used to select patients varied enormously among (and sometimes within) studies. In 10 studies it was not defined at all. Sixty studies included fever in their case definition and 28 of these appeared to require it. However, a temperature-based definition of fever was specified in only 14 studies and ranged from 37.1°C to 39°C.

Exposure

The papers reviewed included VE estimates against trivalent inactivated vaccines (n=59; including 6 papers which did not specify the vaccine tested but where only TIV is available), both inactivated and live-attenuated vaccines (n=4 [28, 97, 84, 90]), monovalent pandemic vaccines (n=10), and both monovalent and trivalent vaccines (n=12). No studies included a separate estimate for the quadrivalent vaccine. Information on the manufacturer or the number of different vaccines on the market was generally not provided and was therefore not assessed. Vaccination status was provided by self-report, GP record or registry. The impact of this potential source of error is examined in Box 2.

Seroconversion after vaccination is required before the vaccine can be expected to have any effect. Studies in healthy young adults have identified protective serum antibody titres in 90% of participants within 2 weeks of vaccination [98]. Thus, classification of patients presenting within 2 weeks of vaccination as vaccinated may represent a source of exposure misclassification. In most analyses (n=67), patients were classified as vaccinated if 14 or more days had elapsed between vaccination and either symptom onset or presentation – four of these actually used 13 days [31, 37, 68, 92], and one used 15 days [66]. Seventeen papers did not apply any restriction or did not specify that they did. Two studies which assessed a pandemic vaccine used shorter criteria of 7 [66] and 10 days [39]. Thirty-six studies failed to further specify how patients presenting within 14 days (or the cut-off used) were classified, 20 specified them as unvaccinated, 10 excluded these patients, 3 included a partially vaccinated category [14, 56, 62]. Six studies examined the impact of the cut off used in a

sensitivity analysis [34, 40, 41, 53, 77, 87]. As seen in Table 3, this source of misclassification of vaccination status generally had a minimal impact on estimates, except in the Singaporean study where estimates made using the stricter criteria were significantly higher [40]. This may be a function of the numbers of patients who fell into these categories, although this information was not provided.

Study periods

Few studies reported data from before 2009 (Figure 2), while data from 2009 were more frequently reported than from other years (Figure 3). However data from more recent years may not yet have been published. Most studies limited their analyses to influenza “seasons” i.e. periods of peak influenza activity, as data from this period should provide the best information on vaccine effectiveness. The means by which a season is defined can have a substantial impact on the precision of estimates [99]. Twenty-eight studies failed to define their season, 18 used consecutive weeks with cases, with a further 11 using weeks with cases occurring a certain period after the vaccination campaign. Eighteen based their season on evidence for circulation, either by health department or diagnostic laboratory criteria, and nine used all data from the surveillance period, which was usually November–April in the Northern Hemisphere and April–November in the southern hemisphere, but in three studies from tropical regions year-round data were used [26, 40, 55].

Outcome

Respiratory specimens were collected in all studies. Sixteen studies collected nasopharyngeal swabs exclusively (for adults), and a further 22 accepted either nasopharyngeal or another type of sample (e.g. nasal, throat, nasal wash). Twenty studies did not specify the swab site. Twenty-seven used nasal and/or throat swabs. Eight studies reported the type of swab used, i.e. flocked [31, 67, 100, 91, 94, 95], cellular foam [82] or flocked/cotton [47]. The type of swab and swab site used to collect the sample may affect the quality of the sample collected and therefore sensitivity of the testing procedure [101, 102], although the specificity is likely to be high [103, 104] and so swab quality may only minimally bias VE estimates (see Box 3).

In challenge studies and in natural settings, the average duration of virus shedding in infected persons is around 5–7 days, and highest around the time of illness onset [105, 106]. Thus, many studies limited the recruitment of patients to those presenting soon after illness onset to reduce the likelihood of a false negative test result (reduced sensitivity). Swabs were taken from patients presenting within 29 days of symptom onset ($n=3$ [38, 63, 107]), 10 days ($n=2$ [108, 88]), 9 days ($n=2$ [18, 25]), 8 days ($n=1$ [89]), 7 days ($n=29$), 5 days ($n=1$ [109]) 4 days ($n=10$), 3 days ($n=7$ [27, 28, 40, 44, 46, 47, 72]). Twenty-six studies did not restrict or did not specify how they restricted patients. Twenty-three studies examined how estimates changed when using different restriction criteria (see Table 4). Given that this restriction is only likely to affect the sensitivity of influenza detection, we would expect these restrictions to minimally bias VE estimates with bias towards the null (i.e. increased VE estimates; see Box 3). Indeed 12 studies observed no or minimal change to estimates ($< 5\%$). Seven observed an increased VE estimate when using stricter criteria, one [24] quite dramatic (> 20 percentage points). Three studies observed a decreased VE estimate, and in

all cases this was dramatic [21, 36, 73]. Applying stricter criteria reduced precision and may have exacerbated sparse data bias.

Twelve studies attempted to control for misclassification associated with shedding by including the days since symptoms onset as a covariate, either as a linear term (n=2 [31, 96]) or stratified (n=4 [35, 58, 77, 78]) and the remainder did not clearly specify how the variable was included [18, 97, 66, 85, 86, 89]. This may represent over-adjustment as the duration of symptoms is not a confounder that is a common cause of exposure and outcome [110, 111].

Reverse-transcription polymerase chain reaction (RT-PCR) is currently the most sensitive and specific method of detection of influenza viruses [112]. Nearly all the studies reviewed used RT-PCR to detect influenza, either solely (n=52) or they accepted a diagnosis by either RT-PCR, viral culture, immunofluorescence or ELISA (n=19). (Where a study listed “PCR” as the method of detection it was assumed this was RT-PCR.) Three studies indicated the use of rapid test kits [25, 85, 86]. Fifty-eight studies specified that the RT-PCR was real-time, although it is probable that all studies used a real-time assay. This assay is highly specific and sensitive but its ability to detect virus depends on the quantity of virus in a sample; the less virus, the more cycles required to identify influenza product. The US Centers for Disease Control and Prevention has suggested using 37 cycles as the limit for classifying a sample as positive, as positive results detected using a higher number of cycles may be false positives [113] and therefore harm specificity (see Box 3). Only one of the TNDs reviewed specified the cycle time cut off used in laboratory testing [83].

All studies compared patients who tested positive for influenza with patients who tested negative for influenza. However, in one study, the comparison group comprised patients who tested negative for influenza and positive for a non-influenza respiratory virus [67]. In addition, seven studies included sensitivity analyses comparing influenza-positive patients with non-influenza respiratory virus-positive patients as well as pan-negative patients [19, 26, 47, 60, 82, 84, 109].

Statistical model

Twenty-six studies estimated VE against all influenza only, while 27 reported no overall estimate and instead reported estimates by type/subtype (20 during the 2009/2010 pandemic). VE was estimated using logistic regression, although two studies reported crude estimates only [15, 94]. The studies adjusted for between one and 11 variables; however, five studies [23, 66, 87, 88, 91] calculated a propensity score for vaccination, the model for which may have included considerably more variables. A total of 68 different combinations of variables were used, the most common being adjustment for age and calendar time (n=5 [14, 33, 34, 38, 48]). The variables included were treated in various ways. For example, in those five studies in which only age and calendar time were adjusted, four different categories for age group were used and calendar time was included as week, month or “phase”. Such seemingly simple variations could change the VE point estimate considerably, particularly if using finer categories results in a positive prediction problem and the subsequent loss of observations [114]. Moreover, these fine adjustments also mean that nearly every paper used a different, unique model in its principal analysis.

The variable most commonly included in adjusted estimates was age (Table 5). Age is accepted as a confounder of VE estimates because vaccine coverage and the risk of illness caused by influenza virus infection can both vary substantially by age. Five studies did not include some kind of adjustment for age either because they used a stepwise model building approach [36, 40, 62, 92] or restricted the age group studied [72]. Age was usually specified as a categorical variable (n=67), the choices of which varied considerably, but was also used as a linear term (n=9 [12, 29, 47, 59, 80, 81, 108, 87, 88, 95]), cubic spline (n=2 [60, 97]), a matching variable (n=3 [14, 64]) and in one instance as a quadratic term [26].

The variable next most often adjusted was a variable to describe the calendar time of either onset or swab collection/recruitment (n=64). This variable was described as month (n=21), week (n=24) or some other “period” relative to the start [87] or peak of the season (n=3 [27, 31, 91]) or variously defined (n=10 [16, 20–22, 31, 39, 40, 48, 58, 89]). It was often unclear whether the variable was specified as a categorical or linear term. Ten studies matched on calendar time, specified as week [14, 26, 64, 74], two-week period [23, 24, 29], or date [39, 80, 81]. Seven multi-season studies included a variable denoting the year [12, 19, 35, 46, 108, 87, 89].

Most analyses also included some measure of the high risk status of a patient (n=62 studies), defined as a high risk condition, chronic condition, comorbidity, or eligibility for free vaccination. The presence of certain conditions increases a person’s risk of severe influenza and is often an eligibility criterion for free vaccination. Thus, this is another important confounder of the vaccination-influenza effect. However, a list of conditions included in this category was often not provided (n=32), and when provided, the range of conditions varied enormously, and sometimes included smoking, pregnancy, health-worker and/or indigenous status depending on whether the variable was designed to capture all patients at high risk of infection (i.e. in a target group for vaccination) or to capture only patients at high risk due to an illness. Measures of illness were sometimes complemented with frequency of hospital (n=10) or GP (n=13) visits, or a measure of functional status (n=6 [31, 51, 97, 70, 72, 91]). In one paper only one condition, obesity [36], was adjusted for and in another the data were restricted to those in a target group for vaccination with no further adjustment [44].

Other variables included in the analyses included sex (n=40), recruitment location (e.g. site, scheme, province, region, community, rural/urban, n=28), healthcare setting (e.g. inpatient/outpatient, long-term care, n=5 [21, 22, 58, 91, 95]), interval between disease onset and swab (linear term or categorised, n=12), previous vaccination (e.g. seasonal & monovalent or multiple seasonal, n=14), receipt of the pneumococcal vaccine (n=2 [20, 72]), antiviral treatment (n=1 [56]), race/ethnicity (n=8 [19, 42, 47, 97, 87–90]), insurance status (n=4 [29, 37, 81, 90]), socioeconomic status (n=5 [42, 53, 56, 57, 91]), number of children in the household (n=1 [20]), whether or not the illness was medical (n=1 [89]), overseas travel (n=1 [42]), enrolment outside active recruitment (n=1 [31]), and preterm birth (n=2 [19, 47]). Only one study explored any interactions, namely the product of vaccination history and military camp [40].

No study attempted to examine or control for correlations among patients recruited from the same practice or recruitment site. Nine studies matched test-positive to test-negative

patients. All matched on calendar time. In addition, three of these studies also matched on age [14, 39, 64], two on geographic location [14, 29, 64, 81], visit setting [29] and sex [39]. Only five of these studies used conditional logistic regression [23, 24, 29, 39, 81].

Heterogeneity and publication bias assessment

Forrest plots were constructed for overall estimates (Figure 4) and by type/subtype (online supplement Figures S1 – S5). Visual inspection of the estimates and confidence intervals suggested considerable heterogeneity in overall estimates, as some confidence intervals were completely exclusive; e.g. Treanor et al [90] reported an overall estimate with confidence limits of (54, 66) for the US in 2010, while for the same season Skowronski et al [77] reported an estimate with confidence limits of (17, 52) for Canada and Eick-Cost et al. [28] reported confidence limits of (–6, 53) for US military personnel. The Cochran's Q test also suggested heterogeneity with $p=0.001$. In contrast, the I^2 suggested only moderate heterogeneity with $I^2=39\%$. However, summary estimates were not calculated given the considerable variability in methodology described above and visible heterogeneity among VE estimates [10].

Funnel plot analysis suggested some asymmetry, with some smaller studies (those with higher standard errors) tending to report lower odds ratios (higher VE estimates) (see Figure S6). The Egger test for asymmetry suggested no small-study effects ($p=0.09$). These observations suggest limited publication bias.

DISCUSSION

We reviewed 85 published test-negative studies to determine the similarities and differences in methodological approaches to the TND. All studies recruited patients with respiratory symptoms either defined as ILI or acute respiratory infection, although there was considerable variation and overlap in the case definitions. Confirmation of influenza infection was generally by RT-PCR, which is a highly specific test. Vaccine status was classified in different ways, and misclassification bias could have affected some studies, particularly where vaccination status is obtained by self-report. The patient population also varied, with estimates made for the general population, specific age groups and specific patient groups. In addition, the reviewed studies differed considerably in the statistical model used for the principal analysis. Not surprisingly, there is often considerable variation among VE estimates within a season, sometimes with mutually exclusive confidence intervals, e.g. the US [90] and Korea [53] in 2011 (Figure 4). For a single surveillance system, subtle changes to the restrictions placed on the data (e.g. the period of surveillance used) and the variables included in the statistical model and how they are treated (e.g. categorical, continuous or spline) can impact VE estimates. For example, VE estimates for 2011 from Victoria were initially reported as 56% (95% CI: –2, 81) [32] but in various sensitivity analyses have since been reported at 48% (95% CI: 0, 73) [115] to as low as 31% (95% CI: –64, 71) [99].

Since February 2013, influenza VE estimates generated from surveillance data using the TND have been presented at the WHO's twice-annual strain selection meeting [115]. Ultimately, it is expected that these data will be able to influence decisions made at those

meetings, by providing additional support to continue or change a strain recommendation. However, tremendous variation in the estimates presented could undermine their credibility. While it is understood that VE may be influenced by location-specific circumstances, such as vaccination policy and circulating strains, variations due to the study design can, in principle, be avoided. The I-MOVE collaborative network has already made great progress towards this goal by developing a shared protocol for use in Europe [116]. A useful addendum might be the inclusion of a standard analytical model using multivariable logistic regression to be reported in sensitivity analyses and for the vaccine meeting. While it may be impossible to derive a single protocol that is appropriate in all settings, our results highlight some points of general agreement that could form the basis of a minimum common model. First, there seems to be a general consensus that estimates must adjust for age and high risk status/eligibility for free vaccination as these are obvious and potentially important confounders. How best to specify these variables, particularly age needs further discussion. For example, given the non-linear relationship of age with influenza risk, it may be appropriate to use splines; conversely, given the age thresholds used for vaccination policy, there is also justification for using categories. Similarly, most studies will at best only be able to include a single covariate to denote the presence of any high risk condition; a single variable for each condition would lead to over-parameterization of the model. Most studies also adjusted for calendar time, but it is still unclear how best to do this [6, 117]. For example, it is not ideal to use fine categories, such as calendar week in smaller studies, as this could result in the loss of observations due to perfect prediction. Here again, splines may be more appropriate as they can allow for a non-linear relationship, with knots placed at times where the relationship is expected to change. Pooled estimates may also need to include a fixed/random effect for location. Restriction of patients to only those presenting a short time after onset of illness or within 14 days of vaccination may have limited impact on reducing disease misclassification and could harm precision if it results in the loss of cases, particularly vaccinated cases.

In this review we noted inconsistencies in the information provided (or not), particularly with reference to definitions for the study period, exposure and outcome. VE can only be estimated for periods during which influenza is circulating [6], but the methods used to define this period vary by locality and may be more difficult to identify in tropical regions [99]. In the minimum common model proposed here, we suggest development of an agreed definition of the season to be used across VE studies, such as that used by I-MOVE [49]. We further recommend that influenza VE studies clearly define the exposure status of patients and indicate whether vaccination status was determined by self-report, from medical records or a registry as this can provide some insight into the level of exposure misclassification. Similarly, the methods for identifying influenza virus should continue to describe the type of swab used to collect respiratory samples, the swab site, the method of detection and, if real-time RT-PCR, the threshold for positivity to enable judgement of imperfect specificity. With the availability of online appendices, such detailed information can easily be provided. Indeed, Canadian researchers have been taking advantage of this option for a number of years [77, 78].

In this field of study, there is intense focus on the point estimates derived, often without adequate consideration of the confidence limits. However, for many of the TND studies

reviewed here, the sample size was limited, which can result in unstable and imprecise estimates. Very few of the studies reviewed provided sample sizes with their tables of estimates, and in some cases it was clear that the final sample size reported in the text was not the same sample used by the regression (e.g. observations would have been dropped due to missing data, perfect prediction, etc.). This is especially true if there are few exposed cases, something which may become apparent when stricter restrictions are applied in sensitivity analyses. It is useful if tables of VE estimates include the sample size used to make each estimate, including the numbers of test-positive and test-negative patients and the numbers of each who are vaccinated. Displaying the data in this way also reveals when estimation should be avoided (e.g. when there is only 1 exposed case) or when shrinkage estimation could be used to stabilize estimates [118]. Furthermore, because 95% Wald confidence limits perform badly when data are sparse, it may be prudent to report profile likelihood limits [119].

While a number of studies have explored the potential for bias in the TND [5–7], a comprehensive theoretical framework has not been presented. However, this should not preclude the continued use of the TND. The case-control study was used for decades [120] before the theoretical framework was comprehensively described by Miettinen [121]. We now know that in the most common type of case control study with incidence density sampling, the exposure odds ratio directly estimates the incidence rate ratio without the need for the rare disease assumption [122, 123]. The TND has some similarities to the “indirect cohort” method used to evaluate VE for pneumococcal disease [124, 125]. An important next step is to describe the theoretical basis for the application of the TND and the analysis of resulting data. From this review it is evident that adjusted estimates include covariates which are not confounders and which may represent over or unnecessary adjustment. Visualising the underlying causal model (such as by using directed acyclic graphs) can inform the statistical model used [126], and would be helpful.

In the presence of consistency in the model used to estimate VE, variation across years is still expected as both the vaccine and predominant type/subtype may change. Thus, this review did not attempt to synthesize the results of these studies in order to provide some overall estimates of influenza VE. By highlighting variations in study design and reporting we hope to motivate further discussion on an agreed international protocol for conducting influenza VE studies, one which includes a minimum common model for estimation and guidelines for variable specification and the presentation of data.

EXPERT COMMENTARY

The test-negative study design is the most efficient approach currently available for routine assessment of influenza VE. In our review, we found that there was a general consensus in the basic design of test-negative studies, whether conducted in outpatient or inpatient settings. Studies generally used RT-PCR assays on respiratory specimens collected from patients with acute respiratory illness, and compared vaccination history in patients testing positive for influenza virus versus in those testing negative for influenza virus. However there was considerable variation in the approach to analysis of data from test-negative

studies. Estimates of vaccine effectiveness from test-negative studies were heterogeneous, even from the same season.

5-YEAR VIEW

The key research groups publishing studies in this areas have already held several meetings to discuss harmonisation of study design practices, e.g. [127, 128], so it is likely that within the coming years we will see standardisation of approaches in these studies. In the context of a uniform protocol, the reporting of VE estimates will perhaps will be routinely disseminated in a similar style to ILI surveillance data, for example via organizational websites or regular reports, rather than as full-length research articles.

A central theme of these meetings has been on the validity of pooling and many desire to see pooling of data across regions of the world. Indeed, Europe is already doing this, Australia and New Zealand have started as is Latin America. Through incorporation of VE estimates into vaccine strain selection meeting and increased collaboration of epidemiologists with virologists and immunologists, we should improve our understanding of how routine VE assessment can contribute to policy decisions such as strain selection, targeted vaccination programmes and mathematical models.

As sample sizes of individual or pooled datasets increases, it will be possible to make more precise estimates of VE by age group or against different strains. There would also be interest in comparing VE of different vaccine types (e.g. live attenuated vaccine versus split virion inactivated vaccine), and even in comparing vaccines of the same type with different manufacturers and brands.

Although unlikely within the coming five years, the introduction of universal vaccines in the medium term may mean the end of TND studies for influenza VE. A central assumption of the TND is that viral shedding is not affected by vaccination status and this appears to hold with current vaccines [5, 129]. In contrast, vaccines that target T-cells are likely to reduce shedding among the vaccinated and therefore violate this assumption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY ISSUES

- The test-negative design is growing in popularity as a feasible and efficient approach to estimation of influenza vaccination effectiveness.
- These studies often take advantage of existing surveillance systems that routinely monitor influenza-like illness in hospitals or the community. However, specific research studies have also been conducted using this design.
- Although all these studies used the same basic design, there is tremendous variation in restriction criteria and the specification of variables used in the logistic regression models used to obtain estimates.
- We show how some of the design features commonly employed to reduce bias may have little impact on estimates. For example, restricting patients to only those presenting soon after illness onset may only minimally reduce estimates. In contrast, many studies appear to rely on patient recall or GP records to ascertain vaccination status, and this may be a greater source of measurement error.
- Because of the unusual emphasis placed on variations of the odds ratio estimate in these types of studies it may be prudent for a common minimal study design to be employed to facilitate comparison across studies.

Box 1**Estimating influenza vaccine effectiveness from the TND**

A selection of patients seeking care with ILI are swabbed and tested for influenza. Vaccination status is recorded, as well as additional patient data. The odds of vaccination among the test-positives is compared with the negatives by adjusted logistic regression, from which a VE estimate can be calculated.

Box 2**Misclassification of vaccination status**

Misclassification of vaccination status may arise because of poor patient recall, inability to verify vaccination status from medical records or incorrect or incomplete medical records. In most of the studies reviewed here, vaccination status was by self-report or GP report, with registries available for 13 studies. Studies of patient recall have placed the sensitivity of vaccination status recall around 95% and specificity around 90% [130, 131], but as low as 70% in certain populations [132]. Below table shows observed vaccine effectiveness (VE) estimates for fictitious data with a true odds ratio = 0.41 (VE = 59%) under varying levels of sensitivity and specificity of self-reported vaccination status using formulas published by Greenland [133]. At 95% sensitivity and 90% specificity (shaded grey) the observed VE estimate is more than 15 percentage points higher than the true VE (75% versus 59%). Thus, studies which use patient recall to ascertain vaccination status may overestimate VE. This is likely to be a stronger source of bias than misclassification due to unclear degree of protection among patients presenting soon after vaccination. Moreover, if patient report differs by influenza status, the direction of the error is not guaranteed to be away from the null (off-diagonal values).

Table B2-2

Corrected VE% estimates under various assumptions
about the sensitivity and specificity of vaccination
status among test-positives and test-negatives

Test-positive			Test-negatives					
Se	Sp	Se	1	0.95	0.9	1	0.95	0.9
		Sp	1	1	0.95	0.95	0.9	0.9
1	1		59.1	62.3	59.5	52.2	46.9	51.3
0.95	1		56.5	59.8	56.9	49.1	43.5	48.2
0.9	0.95		66.5	69.1	66.9	60.9	56.6	60.2
1	0.95		70.6	72.9	70.9	65.7	61.8	65.0
0.95	0.9		80.9	82.4	81.1	77.7	75.3	77.3
0.9	0.9		79.6	81.2	79.8	76.2	73.5	75.7

Box 3**Sensitivity analyses of misclassification of disease status**

Many studies have employed strategies to ensure the detection of cases, such as applying criteria for the inclusion based on the interval between symptom onset and presentation. Such strategies improve the sensitivity of case detection, but may reduce bias only minimally. This is demonstrated in the below table. Using the methods of Copeland [134], the table shows how the risk ratio (RR) is biased in the presence of varying levels of non-differential sensitivity and specificity for detection of influenza virus. Based on a meta-analysis of clinical trials, we constructed a hypothetical cohort with a true RR=0.41 [135]. The RT-PCR assay that is commonly used in these studies has been shown to have near-perfect specificity [136, 137], so values for specificity are probably high, at 0.95–0.99. Based on viral shedding data, we suggest that sensitivity may be 86% on day 1, 97% on day 2 but only 50% by day 4 [105]. The table shows what the observed VE estimates would be under these values of sensitivity and specificity when misclassification is non-differential by vaccination status. As seen in the table, with near-perfect specificity (0.99%) even very low sensitivity has minimal impact on estimates. In contrast, a less specific test (e.g. virus culture) with specificity of 0.90 would result in VE estimates that are more than 10 percentage points below the true value.

Table B3-1

Observed VE under different values for the sensitivity and specificity of the test for influenza status. The true RR for these data was 0.41 (VE=59%).

		Specificity				
		1	0.99	0.97	0.95	0.9
Sensitivity	1	49.0%	47.6%	45.0%	42.6%	37.2%
	0.97	49.0%	47.6%	44.9%	42.4%	36.9%
	0.86	49.0%	47.4%	44.4%	41.7%	35.6%
	0.74	49.0%	47.2%	43.7%	40.6%	33.9%
	0.5	49.0%	46.3%	41.4%	37.2%	28.6%

Figure 1.
Identification of eligible articles

Figure 2. Number of published test-negative studies by year of publication. The bar for 2014 includes nineteen studies published between January and August 2014.

Figure 3. Seasons for which estimates had been published as of August 2014. Influenza seasons are labelled according to the year in which they started, i.e. 2003 includes the 2003 southern hemisphere season as well as the 2003–04 northern hemisphere season.

Figure 4.

Vaccine effectiveness (VE) estimates reported in the principal analyses, by year of study. Influenza seasons are labelled according to the year in which they started. E.g. 2003 includes the 2003 southern hemisphere season as well as the 2003/2004 northern hemisphere season. Studies for the 2009 season largely reported estimates against A(H1N1)pdm09 only and are therefore not included in this figure.

Table 1

Study design features reviewed and rationale

Group	Feature	Description	Rationale
Setting	Setting	Hospital, GP	Affects generalizability
Source population	Eligibility criteria	Restriction to certain populations/age groups	Affects generalizability
	Sample size	Sample size used in VE estimates	Affects precision
	ILI/ARI definition	Conditions included in the definition; whether fever was measured/reported and temperature used	Affects positive:negative ratio
Study period	Study period	Criteria used to define the 'season'	Affects positive:negative ratio, can lead to sparse data bias
Exposure	Exposure definition	Classification of patients according to how long after vaccination they present	Misclassification of exposure
	Ascertainment	Self-report, GP-report, registry	Misclassification of exposure
	Vaccine used	Trivalent or monovalent; inactivated or live-attenuated; adjuvanted	Affects generalizability
Outcome	Type of swab and site	Type of swab used (e.g. flocked, cotton); site of swabbing (e.g. nose, throat)	Misclassification of outcome
	Test	RT-PCR, viral culture, immunofluorescence or rapid test	Misclassification of outcome
	Duration of symptoms	Restriction of patients presenting soon after symptom onset who are more likely to be shedding virus	Misclassification of outcome
Model	Adjusted VE model (main analysis)	Appropriateness of regression model used (e.g. conditional logistic regression if used matching)	Validity
	Covariates	Number of variables included in the adjusted model (main analysis); how they were specified (e.g. categorised/linear/ spline); variations in the definition of key confounders/predictors (e.g. age, time, comorbidities); inclusion of non-confounders	Affects comparability, precision, and efficiency; can lead to sparse data bias/perfect prediction problems; may result in over/unnecessary adjustment, residual confounding, misclassification of a confounder
	Test-negative definition	Whether test-negative for influenza or all respiratory viruses, or negative for influenza but positive for another respiratory virus	Misclassification of outcome
VE estimate	VE estimate	Estimates reported in the abstract (if multiple), overall and by type/subtype	Affects interpretation

Table 2

Studies included in this review.

Author	Year of publication	Year of study	Vaccine	Type of patients	Target group	Ref
Amour	2012	2004–2009	TIV	inpatients	hospitalised adults 18 years+	[12]
Andrews	2011	2009/2010	AMIV(AS03)	inpatients & outpatients	high-risk patients	[14]
Andrews	2014	2012/13	TIV	outpatients	all ages	[13]
Barret	2012	2010/2011	TIV	outpatients	all ages	[15]
Bateman	2013	2010/2011	TIV	inpatients & outpatients	all ages	[16]
		2010/2011	MIV, MLV	inpatients & outpatients	all ages	
Belongia	2009	2004/2005	TIV	inpatients & outpatients	children 6–23 months, 65+ or in a target group	[17]
		2005/2006	TIV	inpatients & outpatients	children 6–23 months, 50 years+ or in a target group	
		2006/2007	TIV	inpatients & outpatients	children 6–59 months, 50 years+ or in a target group	
Belongia	2011	2007/2008	TIV	inpatients & outpatients	all ages	[18]
Blyth	2014	2008–2012	TIV	inpatients & outpatients	children 6–59 months	[19]
Castilla	2012	2010/2011	TIV	inpatients & outpatients	target group for vaccination	[20]
		2010/2011	MIV	inpatients & outpatients	target group for vaccination	
Castilla	2011	2009/2010	MIV/AMIV (AS03/MSS9)	inpatients & outpatients	all ages	[22]
Castilla	2013	2011/2012	TIV	inpatients & outpatients	all ages	[21]
Cheng	2013	2010–2011	MIV/TIV	inpatients	adults 18 years+	[23]
Cheng	2011	2010	MIV/TIV	inpatients	adults 18 years+	[24]
Coleman	2011	2004/2005	TIV	inpatients & outpatients	children 6–23 months, 65+ or in a target group, active surveillance	[25]
		2004/2005	TIV	inpatients & outpatients	children 6–23 months, 65+ or in a target group, clinical testing	
		2005/2006	TIV	inpatients & outpatients	children 6–23 months, 50 years+ or in a target group, active surveillance	
		2005/2006	TIV	inpatients & outpatients	children 6–23 months, 50 years+ or in a target group, clinical testing	

Author	Year of publication	Year of study	Vaccine	Type of patients	Target group	Ref
Cowling	2014	2006/2007	TIV	inpatients & outpatients	children 6–59 months, 50 years+ or in a target group, active surveillance	[26]
		2006/2007	TIV	inpatients & outpatients	children 6–59 months, 50 years+ or in a target group, clinical testing	
		2007/2008	TIV	inpatients & outpatients	children 6–59 months, 50 years+ or in a target group, active surveillance	
		2007/2008	TIV	inpatients & outpatients	children 6–59 months, 50 years+ or in a target group, clinical testing	
Dawood	2014	2009/2010	TIV/MIV	inpatients	children 6 months–17 years	[27]
		2010/2011	TIV/MIV	inpatients	children 6 months–17 years	
		2011/2012	TIV	inpatients	children 6 months–17 years	
		2012/2013	TIV	inpatients	children 6 months–17 years	
Eick-Cost	2012	2010–2011	TIV	inpatients	Adults, 50 years	[28]
		2010/2011	TIV, LAIV	outpatients	Military	
		2003/2004	TIV	inpatients	children <5 years	
		2004/2005	TIV	inpatients	children <5 years	
England	2013	2010/2011	TIV	outpatients	all ages	[30]
		2010/2011	MIV/AMIV	outpatients	all ages	
		2010–2012	TIV	inpatients (ICU)	children, 6 months–17 years	
		2010	TIV	outpatients	all ages	
Ferdinands	2011	2010	MIV	outpatients	all ages	[33]
		2007	TIV	outpatients	all ages	
		2008	TIV	outpatients	all ages	
		2011	TIV	outpatients	all ages	
Fielding	2011	2005/2006	TIV	outpatients	all ages	[34]
		2006/2007	TIV	outpatients	all ages	
		2007/2008	TIV	outpatients	all ages	
		2012/2013	TIV	outpatients	all ages	
Felding	2010	2005/2006	TIV	outpatients	all ages	[32]
		2006/2007	TIV	outpatients	all ages	
		2007/2008	TIV	outpatients	all ages	
		2012/2013	TIV	outpatients	all ages	
Gefenaite	2011	2009/2010	MIV/AMIV	inpatients	adults 18 years+	[36]
		2009/2010	MIV	inpatients & outpatients	all ages	
		2009/2010	MIV/AMIV	outpatients	all ages	
		2009/2010	TIV	outpatients	all ages	
Griffin	2011	2009/2010	MIV/AMIV	inpatients	adults 18 years+	[37]
		2009/2010	MIV	inpatients & outpatients	all ages	
		2009/2010	MIV/AMIV	outpatients	all ages	
		2009/2010	TIV	outpatients	all ages	
Hardeid	2011	2009/2010	MIV/AMIV	inpatients	adults 18 years+	[38]
		2009/2010	MIV	inpatients & outpatients	all ages	
		2009/2010	MIV/AMIV	outpatients	all ages	
		2009/2010	TIV	outpatients	all ages	

Author	Year of publication	Year of study	Vaccine	Type of patients	Target group	Ref
Hellenbrand	2012	2009/2010	AMIV(AS03)	inpatients	adults 18–65 years	[39]
Ho	2014	2010	MIV	inpatients & outpatients	military, 19 years+	[40]
		2011–2013	TIV	inpatients & outpatients	military, 19 years+	
Janjua	2012	2007/2008	TIV	outpatients	all ages	[41]
Jefferies	2011	2009	TIV	healthcare workers	healthcare workers, 20–65 years	[42]
Jiménez-Jorge	2012	2010/2011	TIV	inpatients & outpatients	target group for vaccination	[44]
Jiménez-Jorge	2013	2011/2012	TIV	outpatients	target group for vaccination	[43]
Kafatos	2013	2010/2011	TIV	outpatients	all ages	[45]
Kelly	2009	2003	TIV	outpatients	all ages	[46]
		2004	TIV	outpatients	all ages	
		2005	TIV	outpatients	all ages	
		2006	TIV	outpatients	all ages	
		2007	TIV	outpatients	all ages	
Kelly	2011	2008	TIV	inpatients & outpatients	children <5 years	[47]
Kelly	2011	2009	TIV	outpatients	all ages	[48]
Kissling	2011	2010/2011	TIV	outpatients	all ages	[50]
Kissling	2009	2008/2009	TIV	outpatients	elderly 65 years+	[51]
Kissling	2013	2011/2012	TIV	outpatients	target group for vaccination	[52]
Kissling	2014	2012/2013	TIV	outpatients	all ages	[49]
Kwong	2013	2010/2011	TIV	inpatients	elderly >65 years	[53]
Larrauri	2011	2009	TIV	outpatients	all ages	[54]
Lo	2013	2011/2012	TIV	outpatients	all ages	[55]
Mahmud	2011	2009/2010	MIV/AMIV	inpatients & outpatients	all ages	[56]
Mahmud	2012	2009	TIV	inpatients & outpatients	all ages	[57]
Martínez-Baz	2013	2010/2011	TIV	outpatients	all ages	[58]
Menniti-Ippolito	2014	2011–2013	TIV	inpatients & outpatients	children, 6 months–17 years	[59]
Ohmit	2014	2011/2012	TIV, LAIV	inpatients & outpatients	all ages	[61]
Örtqvist	2012	2009	MIV(AS03)	inpatients	children <18 years	[62]
Nunes	2014	2012/2013	TIV			[60]

Author	Year of publication	Year of study	Vaccine	Type of patients	Target group	Ref
Pebody	2011	2009	TIV	inpatients & outpatients	all ages	[64]
Pebody	2012	2010/2011	MIV	outpatients	all ages	[65]
		2010/2011	TIV	outpatients	all ages	
		2010/2011	TIV&MIV	outpatients	all ages	
Pebody	2013	2011/2012	TIV	outpatients	all ages	[63]
Puig-Barberà	2010	2009/2010	TIV	inpatients	all ages	[66]
		2009/2010	TIV	inpatients	all ages	
		2009/2010	AMIV(AS03/MS59)	inpatients	all ages	
Puig-Barberà	2012	2010/2011	TIV	inpatients	adults 18 years+	[67]
Puig-Barberà	2014	2012/2013	TIV	inpatients	adults, 18 years+	[68]
Rondy,	2013	2011/2012		inpatients	adults 18 years+	[69]
Savulescu	2010	2008/2009	TIV	outpatients	elderly 65 years+	[72]
Savulescu	2011	2009/2010	MIV	outpatients	all ages	[70]
		2009/2010	MIV	outpatients	all ages	
Savulescu	2014	2003–2011	TIV	outpatients	all ages	[71]
Skowronski	2005	2004/2005	TIV	outpatients	all ages	[73]
Skowronski	2007	2005/2006	TIV	outpatients	all ages	[79]
Skowronski	2009	2006/2007	TIV	outpatients	all ages	[75]
Skowronski	2010	2008/2009	TIV	inpatients & outpatients	all ages	[74]
		2009	TIV	inpatients & outpatients	all ages	
		2009	TIV	inpatients & outpatients	all ages	
Skowronski	2011	2009	AMIV(AS03)	outpatients	all ages	[76]
Skowronski	2012	2010/2011	TIV	outpatients	all ages	[77]
Skowronski	2014	2011/2012	TIV	outpatients	all ages	[78]
Song	2011	2009/2010	MIV/AMIV(MS-59)	inpatients & outpatients	all ages	[80]
		2009/2010	TIV	inpatients & outpatients	all ages	
Staat	2011	2005–2007	TIV	inpatients & outpatients	children <5 years	[81]
		2005/2006	TIV	inpatients & outpatients	children <5 years	
		2006/2007	TIV	inpatients & outpatients	children <5 years	
Sullivan	2013	2012	TIV	inpatients & outpatients	all ages	[83]
Sullivan	2014	2012	TIV	outpatients	all ages	[82]

Author	Year of publication	Year of study	Vaccine	Type of patients	Target group	Ref
Sundaram	2013	2004/2005–2009/2010	TIV, LAIV	inpatients & outpatients	children <5 years	[84]
Suzuki	2013	2004/2005–2009/2011	TIV, LAIV	inpatients & outpatients	adults 50 years+	[86]
Suzuki	2014	2010/2011	TIV	outpatients	adults >15 years	[85]
Talbot	2011	2011/2012	TIV	outpatients	patients >10 years	[87]
		2006/2007–2008/2009	TIV	inpatients	50 years+	
		2006/2007	TIV	inpatients	50 years+	
		2007/2008	TIV	inpatients	50 years+	
		2008/2009	TIV	inpatients	50 years+	
Talbot	2013	2011/2012	TIV	inpatients	adults 18 years+	[88]
Thompson	2014	2010/2011–2011/2012	TIV	inpatients & outpatients	pregnant women	[89]
Treanor	2012	2010/2011	TIV, LAIV	inpatients & outpatients	all ages	[90]
Turner	2014	2012	TIV	inpatients & outpatients	all ages	[91]
Uphoff	2011	2009/2010	AMIV(AS03)	outpatients	age <14 years	[92]
		2009/2010	AMIV(AS03)	outpatients	age 14 years+	
Valenciano	2011	2009/2010	TIV	outpatients	all ages	[93]
Van Buynder	2010	2009	AMIV(AS03)	inpatients & outpatients	children <9 years	[94]
Van Buynder	2013	2011/2012	ATIV	outpatients	elderly 65 years+	[95]
		2011/2012	TIV	outpatients	elderly 65 years+	
Yang	2014	2012/13	TIV	outpatients	all ages	[96]

Table 3

VE estimates and 95% confidence limits presented in sensitivity analyses testing the classification of patients presenting within 14 days of vaccination

Study	<14D=Vaccinated	<14D=Unvaccinated	<14D=excluded	Ref
Fielding 2011	"variations of 0% to 7% around the VE point estimates"	59(25,78) in 2007 9(−96,58) in 2008	"Variations of 0–7% around the VE point estimates"	[34]
Ho 2014	MIV: 80(70,87) Period 3: 83(73,90) Period 4: 77(59,87)	MIV: 97(92,99) Period 3: 81(66,89) Period 4: 93(86,97)		[40]
Janjua 2012	60(45,71)	"5–10% lower"	"between UV and V"	[41]
Kwong 2013	42(29,53)	41 (27,52)		[53]
Skowronski 2012	39 (20,53)	35 (14,50)	37(17,52)	[77]
Talbot 2011	61(17,82) (nb. 7–14d=V)	61(17,82)		[87]

V: vaccinated; UV: unvaccinated

Table 4

VE estimates and 95% confidence limits (CL) presented in sensitivity analyses which have examined the effect of excluding participants based on the number of days between symptom onset and presentation.

Study	n(positive)	Reported VE% (95%CL)	Restrictions considered VE% (95%CL)	Direction of change in estimate using more restrictive criteria	Ref
Belongia 2011	855	7d: 37(22,49)	3d: 44(27,58)	↑	[17]
Castilla 2013	411	UR: 31(−21,60)	4d: 9(−38,63)	↓*	[21]
Cheng 2011	199	UR: 49(13,70)	4d: 69(30,86)	↑*	[24]
Fleming 2010	282	<30d: 65(51,74)	4d: 65(48,76)	--	[35]
Gefenaite 2014	111	7d: 86(19,97)	4d: 55(−235,94)	↓*	[36]
Hardelid 2011	1746	UR: 72(21,−90)	7d: 70(15,−89)	--	[38]
Hellenbrand 2012	24	<7d: 46(−376,100)	4d: 50(−344,100)	--	[39]
Martinez-Baz	303	UR: 67 (24; 86)	4d: 65 (16,85)	--	[58]
Nunes	152	UR: 69(21,87)	4d: 71(22,89)	--	[60]
Ohmit 2013	681	7d: 47(36,56)	4d: 46 (33,57)	--	[61]
Pebody 2011	949	NS: −6 (−43,22)	2d: 4(−84,50) 5d: 6(−53,45)	↑	[64]
Pebody 2012	1492	<30: 56(42,66)	<7d: 63(48,73)	--	
Pebody 2013	377	<29d: 23(−10,47)	7d: 23 (−15,50)	--	[63]
Rondy 2013 †	349	4d: 35(−1.8,45)	7d: 18(−4.4,35)	↑	[69]
Savulescu 2011	331	7d: 65(−221,96)	4d: 72(−290,99)	↑	[70]
Skowronski 2005	47	UR: 45(−44,79)	48h: −17(−319,67)	↓*	[73]
Skowronski 2014	447	7d: 59(43,70)	4d: 55(36,69)	--	[78]
Thompson 2014	1028	8d: 44(5,67)	4d: 39(−26,71)	--	[89]
Yang 2014	695	NS: 52(12,74)	3d: 52(12,74)	--	[96]
Studies with no specific estimates provided in sensitivity analyses:					
Bateman 2013	177	7d: 63(37,78)	3d: "similar"	--	[16]
Fielding 2011 †	1369	4d: 79(33,93)	UR: "variations ... of 0%–3%"	--	[33]
Fielding 2011 †	194	4d: 59(25,78)	UR: "reduction of ... between 7% and 15% in 2007 and between 5% and 35% in 2008.	↑	[34]
Fielding 2012 †	155	4d: 56(−2,81)	UR: "reduced ... VE estimates from 2% to 14%."	↑	[32]

UR: unrestricted (includes studies where restrictions were not specified)

* indicates studies which saw a change in estimate of ≥ 20 percentage points

† indicates studies which used the stricter criteria for their main estimate

Table 5

Summary of selected variables in principal analyses.

Variable	N(studies)
Vaccination status	
Trivalent vaccines (n=75 studies) ¹	
14 days = vaccinated	58
Unrestricted of unspecified	17
Classification of patients vaccinated <14 days before onset	
<14 days = excluded	8
<14 days = unvaccinated	18
<14 days = unspecified	38
Shedding – exclusions based on presentation X days after onset	
>3 days	7
>4 or 5 days	11
>7 or 8 days	30
Other number of days	8
No restriction	26
Included in model as a variable (linear or dichotomous)	12
Definition of season	
Weeks with positive cases	18
Weeks with cases 2 weeks after vaccination campaign	11
Evidence of circulation	18
Surveillance period	9
Not defined	28
Common covariate adjustments	
Age	80
Categorical term	67
Linear term (includes splines)	11
Matching/strata term	3
Squared	1
Calendar time adjusted	64
Week	20
Month	21
Linear spline	2
Time relative to peak	34
Period (variously defined)	8
Matched on	10
Date	3
Week	4

Variable	N(studies)
Vaccination status	
2-week interval	3
Season (studies which pooled data only)	7
Adjustment for high risk status / comorbidities	62
Defined	30
No. hospital visits	10
No. GP visits	13
Smoking status	10
Pregnancy status	6
Functional/health status	6
Obesity	2
Healthcare worker	1
Sex	40
Study site/location/state/province	28
Healthcare setting (e.g. inpatient v outpatient)	5
Prior vaccination	14
Pneumococcal vaccination	2
Race/ethnicity	8
Socioeconomic status	5
Insurance status	4
Preterm birth	2
Number of children in household	1
Two or more cohabitants in household	1
Overseas travel	1
Enrolment outside active recruitment	1
Antiviral treatment	1
Whether or not the illness was medical	1
Reported contact with a person suspected of having influenza	1

¹ where 14 also includes studies which used cut offs of 13 or 15 days.

Note, not all variables ever adjusted for are listed as this does not include all the variables included in propensity score models.