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Effects of imperfect test sensitivity and specificity on observational studies of influenza vaccine effectiveness

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ABSTRACT

Background: The recently developed test-negative design is now standard for observational studies of influenza vaccine effectiveness (VE). It is unclear how influenza test misclassification biases test-negative VE estimates relative to VE estimates from traditional cohort or case-control studies.
Methods: We simulated populations whose members may develop acute respiratory illness (ARI) due to influenza and to non-influenza pathogens. In these simulations, vaccination reduces the risk of influenza but not of non-influenza ARI. Influenza test sensitivity and specificity, risks of influenza and non-influenza ARI, and VE were varied across the simulations. In each simulation, we estimated influenza VE using a cohort design, a case-control design, and a test-negative design.
Results: In the absence of influenza test misclassification, all three designs accurately estimated influenza VE. In the presence of misclassification, all three designs underestimated VE. Bias in VE estimates was slightly greater in the test-negative design than in cohort or case-control designs. Assuming the use of highly sensitive and specific reverse-transcriptase polymerase chain reaction tests for influenza, bias in the test-negative studies was trivial across a wide range of realistic values for VE.
Discussion: Although influenza test misclassification causes more bias in test-negative studies than in traditional cohort or case-control studies, the difference is trivial for realistic combinations of attack rates, test sensitivity/specificity, and VE.

1. Introduction

In recent years, the so-called "test-negative" design has become the standard approach for observational studies of influenza vaccine effectiveness (VE) [1–3]. In a test-negative design, the study population comprises patients who present to an outpatient clinic or hospital with acute respiratory illness (ARI) and who are tested for influenza infection [4]. VE is defined as one minus the ratio of the risk of influenza among the vaccinated to the corresponding risk among the unvaccinated. In case-control and test-negative studies, VE is estimated as one minus the odds ratio of influenza for vaccinated vs. unvaccinated. Relative to some other observational designs, the test-negative design offers the advantage of reduced confounding from differences in healthcare-seeking behavior between vaccinated and unvaccinated persons [5].

Misclassification of influenza leads to biased VE estimates, regardless of the study design. Assuming the misclassification is not differential by vaccination status, misclassification will introduce bias that will tend to underestimate VE. The degree of bias due to misclassification has been believed to be low in test-negative studies, primarily due to a 2007 paper by Orenstein and colleagues [6]. In that paper, the authors concluded that case-control and test-negative studies were less biased than cohort studies in populations subject to similar amounts of misclassification of influenza. That paper, however, had an important flaw. The authors based their calculations on a cumulative design [7] for the case-control and test-negative studies, in which the controls are sampled from those who did not get influenza during the follow-up period. Controls in a case-control study correspond to the denominator information in a cohort study. The cumulative sampling strategy excludes those who get influenza from the sampled denominators, biasing study results away from the null [7]; this bias is small for rare disease but is larger if the disease is common. In the paper by Orenstein et al, the bias toward the null in VE stemming from misclassification of disease was countered in the test-negative and case-control design by a bias away from the null due to the cumulative design

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Table 1
Parameter values for simulations.

Parameter	Description	Values	
		All ages	Young children
VE_{true}	Vaccine effectiveness	50%	70%
IP_{flu}	Incidence proportion of ARI* due to influenza	5%	15%
IP_{other}	Incidence proportion of ARI due to other pathogens	10%	30%
Sens (RT)	Rapid test sensitivity	80%	
Spec (RT)	Rapid test specificity	90%	
Sens (PCR)	RT-PCR sensitivity	95%	
Spec (PCR)	RT-PCR specificity	97%	

* ARI = acute respiratory illness.

sampling strategy. With no misclassification, a properly designed case-control study would give expected results equivalent to those from a cohort study, but the cumulative design considered by Orenstein et al. would not. Thus, the work of Orenstein et al. does not allow a valid conclusion about the effects of test sensitivity and specificity on VE estimates in test-negative studies.

In this paper, we compare the effects of imperfect test sensitivity and specificity on VE estimates from cohort, case-control, and test-negative studies. We correct the problem in the paper by Orenstein et al. by using simulations based on sampling controls from the full population at risk (sometimes referred to as "case-cohort sampling") [7] rather than a cumulative design for the case-control and test-negative studies.

2. Methods

To focus on the effects of imperfect sensitivity and specificity, we assumed that other sources of bias are absent. Specifically, we assumed that there is no confounding, no selection bias, and no misclassification of exposure (vaccination) status. We simulated populations at risk for two outcomes: medically attended influenza infection and medically attended infection with other pathogens. We assumed infection with influenza to be independent from infection with other pathogens. We also assumed that subjects could only be infected once with influenza, but could be infected multiple times with non-influenza pathogens. Our simulation involves five parameters (Table 1):

VE = influenza vaccine effectiveness against medically attended influenza

IP_{flu} = incidence proportion (risk) of influenza ARI in unvaccinated persons

IP_{other} = incidence proportion of ARI due to non-influenza pathogens

sens = sensitivity of influenza test

spec = specificity of influenza test

For consistency with Orenstein et al., we performed one set of "young children" simulations, in which we assumed that $IP_{flu} = 15\%$, $IP_{other} = 30\%$, and VE = 70%, based on expected incidence and VE in children 6–24 months of age [6]. We also performed a set of "all ages" simulations assuming $IP_{flu} = 5\%$, $IP_{other} = 10\%$, and VE = 50%, which are more realistic values for the population of all ages that is a frequent target of test-negative VE studies. Following Orenstein et al., we assumed influenza test sensitivity to be 0.8 and specificity to be 0.9. These values were based on the use of rapid antigen tests for detecting influenza. In practice, nearly all modern studies use reverse-transcriptase polymerase chain reaction (RT-PCR) assays for influenza testing, which are both more sensitive and more specific than rapid antigen tests [8–10]. We therefore repeated our analyses using sensitivity and specificity parameters based on RT-PCR (Table 1).

We ran a series of 1000 simulations to compare the study designs. In each, we simulated a population of 50,000 subjects, which gives study sizes roughly equal to those in existing observational VE studies [1,3,11]. We assumed that 40% of subjects received influenza vaccine at the start of follow-up. Within the follow-up period, subjects could be infected (up to once) with influenza, with risk equal to IP_{flu}, and infected (up to once) with a non-influenza pathogen, with risk equal to IP_{other}. We assumed that risk of influenza is independent of the risk of non-influenza ARI, as analyses of the effects of hypothetical non-independence on test-negative VE estimates have been performed previously [12]. After running the simulation to determine the simulated disease events, we randomly allowed these simulated events to be misclassified according to the rapid test and RT-PCR values for sensitivity and specificity.

We estimated VE using three separate designs, first using the correctly classified outcomes. In the cohort design, we calculated the risk of influenza infection in the vaccinated and the unvaccinated. We then estimated $\widehat{VE}_{cohort}$ as $(1 - RR)$, where RR is the risk ratio. In the case-control design, for each detected influenza case, we randomly sampled three controls from the total study population. We estimated $\widehat{VE}_{cc}$ as $(1 - OR_{cc})$, where OR_{cc} is the odds ratio from the case-control study, which estimates the risk ratio [7]. For the test-negative design, we included all ARI events testing positive for influenza as cases. All ARI events testing negative for influenza were selected as a comparison group. We estimated $\widehat{VE}_{tn}$ as $(1 - OR_{tn})$, where OR_{tn} is the odds ratio from the test-negative study. We calculated 95% confidence limits from the 2.5th and 97.5th percentiles of the simulations.

After estimating VE in the simulated population based on the true disease status, we repeated the analysis using the rapid test misclassified outcomes, and again using the RT-PCR misclassified outcomes. We calculated the bias of each design from each simulation as a percent: $\text{Bias} = [(\widehat{VE}/VE) - 1] \times 100\%$. For each design at each level of misclassification, we calculated the mean bias with 95% confidence limits.

We further assessed the independent effects of influenza test sensitivity and test specificity on VE estimates in the test-negative design, using the "all ages" scenario. For this, we ran 1000 simulations assuming $IP_{flu} = 5\%$, $IP_{other} = 10\%$, and VE = 50%. In each simulated population, we calculated VE_{tn} at a range of test sensitivities (from 0.8 to 1.0, keeping specificity at 1.0) and at a range of specificities (from 0.8 to 1.0, holding sensitivity at 1.0). Finally, we assessed the degree to which bias in the cohort and test-negative designs varies with varying VE. For this, we ran 1000 simulations, assuming PCR sensitivity and specificity, $IP_{flu} = 5\%$, and $IP_{other} = 10\%$, while varying VE between 10 and 70%.

Finally, we conducted sensitivity analyses of the "young children" and "all ages" scenarios, where we allowed subjects to have multiple influenza and non-influenza ARI events during follow-up. Instead of incidence proportions, the number of events per person was randomly sampled from a Poisson distribution with mean equal to IP_{flu} (for influenza ARI) and IP_{other} (for non-influenza ARI). Results were trivially different from the main analyses for all study designs in both scenarios (less than one percentage point difference in estimated VE at PCR levels of misclassification) and are not further presented here. Analyses were conducted using SAS Version 9.3 (SAS Institute, Cary NC) and R Version 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

In the absence of misclassification, all three designs accurately estimated VE in the "young children" scenario, with mean $\widehat{VE}$ across the simulations of 70% (Table 2). In the presence of misclassification

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