MAJOR ARTICLE

Influenza Vaccination Is Not Associated With Detection of Noninfluenza Respiratory Viruses in Seasonal Studies of Influenza Vaccine Effectiveness

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Background. The test-negative control study design is the basis for observational studies of influenza vaccine effectiveness (VE). Recent studies have suggested that influenza vaccination increases the risk of noninfluenza respiratory virus infection. Such an effect could create bias in VE studies using influenza-negative controls. We investigated the association between influenza infection, vaccination, and detection of other respiratory viruses among children <5 years old and adults ≥50 years old with acute respiratory illness who participated in seasonal studies of influenza vaccine effectiveness.

Methods. Nasal/nasopharyngeal samples collected from 2004–2005 through 2009–2010 were tested for 19 respiratory virus targets using a multiplex reverse-transcription polymerase chain reaction (RT-PCR) platform. Vaccination status was determined using a validated registry. Adjusted odds ratios for influenza and vaccination status were calculated using three different control groups: influenza-negative, other respiratory virus positive, and pan-negative.

Results. Influenza was detected in 12% of 2010 children and 20% of 1738 adults. Noninfluenza respiratory viruses were detected in 70% of children and 38% of adults without influenza. The proportion vaccinated did not vary between virus-positive controls and pan-negative controls in children ($P = .62$) or adults ($P = .33$). Influenza infection was associated with reduced odds of vaccination, but adjusted odds ratios differed by no more than 0.02 when the analysis used influenza-negative or virus-positive controls.

Conclusions. Influenza vaccination was not associated with detection of noninfluenza respiratory viruses. Use of influenza-negative controls did not generate a biased estimate of vaccine effectiveness due to an effect of vaccination on other respiratory virus infections.

Keywords. influenza; vaccination; nonspecific immunity; vaccine effectiveness.

The case vs test-negative control study design is the basis for observational studies of influenza vaccine effectiveness (VE) [1–6]. Cases and controls are recruited at the time of presentation of acute respiratory illness (ARI) in clinic and hospital settings. Individuals presenting with ARI who test positive for influenza are considered cases, whereas those who test negative for influenza are considered controls. This study design is convenient to implement and inherently accounts for potential confounding due to differences in healthcare-seeking behavior between vaccinated and unvaccinated individuals [1–3].

It has recently been suggested that influenza vaccination may increase the risk of non-influenza respiratory virus infection by decreasing temporary nonspecific immunity [7, 8]. One proposed mechanism involves activation of the innate immune response following

Received 5 April 2013; accepted 23 May 2013; electronically published 6 June 2013.

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Clinical Infectious Diseases 2013;57(6):789–93

DOI: 10.1093/cid/cit379

influenza infection, leading to a temporary reduction in the risk of infection with a different respiratory virus. By reducing the risk of influenza infection, the influenza vaccine could paradoxically create an increased risk of infection with other noninfluenza respiratory viruses. If this phenomenon occurs, it could lead to biased estimates of influenza vaccine effectiveness in studies using laboratory-confirmed influenza cases and influenza-negative controls. In this scenario, the risk of noninfluenza viral illness would be higher in vaccinated than unvaccinated individuals, and an 'influenza-negative' control group would therefore have a higher proportion of vaccinated individuals compared to the source population. This could theoretically contribute to overestimation of true VE (ie, bias away from the null); therefore, a key assumption of the test-negative control design of influenza vaccine effectiveness studies is that the proportion of noninfluenza viral illness does not differ by influenza vaccination status [9].

The goals of this study were to determine if influenza vaccination is associated with detection of noninfluenza respiratory viruses and to determine if vaccine effectiveness estimates differ when different control groups are used in the analysis. To achieve these goals, we analyzed available data from members of a community cohort who saw a physician for acute respiratory illness and consented to participate in a study of influenza vaccine effectiveness over 6 influenza seasons. The vaccine effectiveness study enrolled individuals of all ages (with some variation by season), but this analysis was restricted to children <5 years old and adults ≥50 years old. For participants in these age groups, multiplex reverse transcription polymerase chain reaction (RT-PCR) testing was subsequently performed to detect other respiratory viruses, providing an opportunity to investigate the relationship between influenza vaccination and infection with other viral pathogens. Young children and older adults are among the most vulnerable individuals to influenza infection and complications [10, 11] and calculating influenza vaccine effectiveness in these groups is therefore of high importance [12].

METHODS

Participants and Setting

The Marshfield Clinic Research Foundation has conducted seasonal studies of influenza vaccine effectiveness in a Wisconsin population cohort since the 2004–2005 season. The details of the seasonal studies have been reported elsewhere [2, 3]. Briefly, patients with ARI were recruited during each influenza season in primary care clinics, urgent care, emergency department, and an acute care hospital. Symptom eligibility criteria varied by season but included fever/feverishness or cough during most seasons. Individuals with illness duration ≥10 days (2004–2005 through 2006–2007) or >7 days (2007–2008 through 2009–2010) were excluded to minimize false negative RT-PCR results. After obtaining informed consent, a nasal swab (children <12 years old) or a nasopharyngeal swab (adolescents and adults) was obtained and placed in viral transport media for influenza testing. Symptoms and onset date were assessed during the enrollment interview. Real-time RT-PCR was performed each season to identify influenza cases. After testing was complete, aliquots of samples in viral transport media were frozen at −80°C.

This study was reviewed and approved by the Marshfield Clinic Institutional Review Board. During each season, all study participants (or parents) provided informed consent for influenza testing. Multiplex RT-PCR testing to detect additional viruses was subsequently approved by the IRB with a waiver of informed consent.

Laboratory

Archived samples were tested for the presence of respiratory virus nucleic acid using a multiplex respiratory virus panel (GenMark Dx eSensor Respiratory Viral Panel). This multiplex panel tested for respiratory syncytial virus (RSV) A and B, human rhinovirus, human metapneumovirus, parainfluenza viruses 1–4, influenza A and B (including subtypes of influenza A), coronaviruses OC43, NL63, HKU1, and 229E, and adenoviruses B and E. Nucleic acid was extracted from the swabs using the Roche MagnaPure 2.0 system and was then amplified using RT-PCR with target-specific primers. Target-specific signals were determined by voltammetry, a process that generates electrical signals from ferrocene-labeled signal probes. The multiplex assay has been validated for influenza and RSV A and B against singleplex assays approved by the Centers for Disease Control and Prevention (CDC) and found to have 97%, 93%, and 98% sensitivity and 97%, 99%, and 99% specificity for influenza A, influenza B, and RSV, respectively (unpublished data).

Analytic Approach

Cases were defined as laboratory-confirmed influenza based on a positive real-time RT-PCR test for influenza A (H3N2), A (H1N1), A (H1N1)pdm09, or type B. Three different control groups were constructed for analytical purposes. Control Group 1 included all individuals testing negative for influenza (influenza-negative controls); group 2 included individuals negative for influenza but positive for at least one other respiratory virus in the multiplex panel (virus-positive controls); and group 3 included individuals negative for all respiratory viruses in the multiplex panel (pan-negative controls). The first control group is the standard definition used in test-negative case-control designs, whereas the second and third control groups represent subsets of the first group.

Vaccination status and dates were determined by a real-time, internet-based registry used by all immunization providers serving the local population (www.recin.org). Cases and controls were considered vaccinated if seasonal trivalent inactivated influenza vaccine (TIV) or live attenuated influenza vaccine (LAIV) was received at least 14 days before ARI symptom onset. Children who were partially vaccinated according to the Advisory Committee on Immunization Practices (ACIP) definition (ie, received 1 of 2 recommended doses) were excluded [13].

Table 1. Clinical and Demographic Characteristics of Respiratory Virus-Positive and Pan-Negative Control Groups, Reported Separately for Children <5 Years Old and Adults ≥50 Years Old

Characteristic	Virus-positive Controls, No. (%) or mean ± SD	Pan-negative Controls, No. (%) or mean ± SD	*P* Value
Children (6 mo – < 5 y)			
Age	1.8 ± 1.3	2.0 ± 1.3	.22
Female gender	646 (47.9)	203 (49.5)	.56
Vaccinated	782 (58.0)	232 (56.6)	.62
Interval from symptom onset to swab date, days	3.2 ± 1.9	3.0 ± 2.0	.06
High-risk health condition	152 (12.0)	36 (8.7)	.07
Season			
2004–2005	136 (10.1)	45 (11.0)	<.0001
2005–2006	41 (3.0)	24 (5.9)	
2006–2007	226 (16.8)	112 (27.3)	
2007–2008	226 (16.8)	49 (12.0)	
2008–2009	334 (24.8)	109 (26.6)	
2009–2010	386 (28.6)	71 (17.3)	
Adults, ≥50 y			
Age	65.2 ± 10.8	64.5 ± 10.9	.24
Female gender	391 (61.0)	457 (60.6)	.88
Vaccinated	439 (68.5)	498 (66.1)	.33
Interval between symptom onset and swab date, days	4.0 ± 1.8	3.8 ± 2.2	.20
High-risk health condition	301 (47.0)	396 (52.5)	.04
Season			
2004–2005	68 (10.6)	99 (13.1)	.0003
2005–2006	42 (6.6)	97 (12.9)	
2006–2007	99 (15.4)	101 (13.4)	
2007–2008	87 (13.6)	113 (15.0)	
2008–2009	145 (22.6)	129 (17.1)	
2009–2010	200 (31.2)	215 (28.5)	

All participants in both groups had a negative reverse transcription polymerase chain reaction for influenza.

Abbreviation: SD, standard deviation.

Separate analyses were conducted for children <5 years old and adults ≥50 years old, and only the first enrollment was included for each individual in a given season. Vaccination status was compared for virus-positive controls and pan-negative controls using logistic regression. Odds ratios describing the association between vaccination and influenza infection were determined separately for each of the 3 case-control groups by logistic regression. Logistic regression models included influenza status as the outcome and vaccination status as the primary exposure variable with adjustment for potential confounders, including gender, influenza season, age (continuous), presence of a chronic disease conferring increased risk for influenza complications [14], and interval in days from symptom onset to swab collection. SAS 9.2 (SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

Over 6 influenza seasons, 1616 children and 1568 adults contributed 2010 and 1738 unique observations, respectively. Influenza was detected in 251 (12.5%) children and 343 (19.7%) adults. Among those without influenza, a respiratory virus was detected in 1411 (70.2%) of children and 659 (37.9%) of adults. In children without influenza, the most commonly detected single viruses were RSV (n = 435), human rhinovirus (n = 298), human metapneumovirus (n = 150), and parainfluenza (n = 113); 271 (13.5%) of swabs from children were positive for ≥2 noninfluenza respiratory viruses. In children, the most common coinfection was RSV with rhinovirus (n = 73). In adults without influenza, the most commonly detected single viruses were RSV (n = 170), human rhinovirus (n = 126), human metapneumovirus (n = 125), and coronavirus (n = 122); 47 (2.7%) of swabs from adults were positive for ≥2 noninfluenza respiratory viruses. In adults, the most common coinfection was RSV with coronavirus (n = 8).

The virus-positive control group and pan-negative control group did not differ with regard to age, gender, vaccination status, or interval from symptom onset to swab in either children or adults (Table 1). The proportion of children with a high-risk health condition did not differ between control groups; there were more adults with a high-risk health condition in the pan-negative control group than in the virus-positive control group (Table 1). In univariate analyses, no association was found between influenza vaccination and single virus detection of RSV, adenovirus, human metapneumovirus, human rhinovirus, or coronavirus. Single infection with parainfluenza virus was less common in vaccinated children (4.6%) than vaccinated children (6.7%; $P = .03$). Among adults, there was a significant association in the opposite direction: parainfluenza was detected in 4.6% of vaccinated and 2.6% of unvaccinated adults ($P = .04$).

For both children and adults, the adjusted odds of influenza infection were significantly lower in the vaccinated group relative

Table 2. Unadjusted and Adjusted Odds Ratios for Vaccination in Influenza Cases vs Controls Using Three Different Control Groups: All Influenza Negative (Group 1), Noninfluenza Virus Positive (Group 2), and Pan-negative for all Viruses Tested in the Multiplex Panel (Group 3)[a]

	Cases vs All Controls (Group 1)			Cases vs Noninfluenza Virus-Positive Controls (Group 2)			Cases vs Pan-Negative Controls (Group 3)		
Age Group	uOR	aOR	95% CI	uOR	aOR	95% CI	uOR	aOR	95% CI
Children	0.55	0.52	.39–.69	0.54	0.50	.37–.67	0.57	0.56	.39–.80
Adults	0.61	0.56	.42–.75	0.58	0.57	.40–.82	0.65	0.53	.37–.74

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; uOR, unadjusted odds ratio.

[a] All models adjusted for gender, influenza season, age (in years), presence of high-risk health condition, and interval (days) from symptom onset to swab collection.

to any of the 3 control groups, indicating significant vaccine effectiveness (Table 2). Among adults, the adjusted odds ratio (aOR) ranged from 0.53 to 0.58; among children the aOR ranged from 0.50 to 0.56 depending on which control group was used. In both children and adults, the aOR using 'influenza-negative controls' (ie, the current standard for VE studies) varied by no more than 0.02 compared to the aOR using other virus-positive controls, and all aOR confidence intervals overlapped. The adjusted odds of being vaccinated did not differ significantly in the pan-negative control group vs the other virus-positive control group, either in children ($P = .96$) or in adults ($P = .89$). Results were similar when the analysis was repeated after excluding 14 children who received LAIV. There were no adults in this analysis who received LAIV.

DISCUSSION

Detection of a noninfluenza respiratory virus by multiplex RT-PCR was not associated with influenza vaccination status over a period of six influenza seasons. In both children and adults, the virus-positive control group and the pan-negative control group were similar in terms of age distribution, gender, and influenza vaccination status. There was no association between influenza vaccination and detection of RSV, adenovirus, human metapneumovirus, human rhinovirus, or coronavirus. Only parainfluenza infection was significantly associated with influenza vaccination, but the association was in opposite directions for children and adults. The *P* values were not adjusted for multiple comparisons, and the inconsistency between children and adults suggest that this association is the result of a type 1 error.

To assess the potential impact of noninfluenza respiratory viruses in vaccine effectiveness studies, we calculated the aOR for influenza vaccination in cases and controls using 3 different control groups. Among children, the aORs were 0.52 and 0.50, respectively, using influenza-negative controls and virus-positive controls. This corresponds to an absolute difference of 2% in the vaccine effectiveness estimate, with broadly overlapping confidence intervals. Nearly all vaccinated children in this analysis received TIV, and the results were similar when LAIV recipients were excluded. Among adults ≥50 years old, the adjusted odds ratios were nearly identical (0.56 and 0.57, respectively) when using influenza-negative controls and virus-positive controls, with overlapping confidence intervals.

Other studies have found evidence of an association between influenza vaccination and infection with a non-influenza respiratory virus. In 2008, a study of influenza vaccine effectiveness in Australian children 6 to 59 months old found that influenza vaccination was associated with detection of other respiratory viruses [15]. Nasal swabs from 289 children were tested by RT-PCR for influenza, rhinoviruses, RSV, parainfluenza virus 1–3, human metapneumovirus, and enteroviruses. Adjusted vaccine effectiveness was 58% (95% CI, 9–81) using influenza-negative controls and 68% (95% CI, 26–86) using other virus-positive controls. The authors noted the higher point estimate of vaccine effectiveness using virus-positive controls, although there was substantial overlap in the confidence intervals for these 2 point estimates. The authors speculated that the difference between point estimates could be due to false negative RT-PCR tests in some children (ie, pan-negative controls) due to inadequate sample collection. They also mentioned the possibility that influenza vaccination could increase the risk of infection with other viruses, but they viewed this as biologically implausible.

The relationship between influenza vaccination and infection with noninfluenza viruses was also investigated in a clinical trial of TIV in children [7]. In that study, TIV recipients (n = 69) had a 4-fold increase in risk of infection with noninfluenza respiratory viruses, compared to placebo recipients (n = 46); the incidence of seasonal influenza did not differ significantly between the TIV and placebo groups. The authors acknowledged that the association with noninfluenza viruses could be spurious, but they suggested that 'receipt of TIV could increase influenza immunity at the expense of reduced immunity to noninfluenza respiratory viruses, by some unknown biological mechanism' [7]. They identified temporary nonspecific immunity as a possible explanation for this observation. According to this proposed mechanism, unvaccinated children were more likely to acquire influenza infection, and the

resulting innate immune response provided temporary, nonspecific protection against infection with other respiratory viruses. This was described further in a letter that raised concerns about bias in studies of vaccine effectiveness that use RT-PCR confirmed cases and influenza-negative controls [8].

These 2 studies had relatively small sample sizes and were limited to a single influenza season. However, one of the studies was a randomized, placebo-controlled trial, a study design that is less susceptible to bias and confounding compared to observational studies. We were unable to replicate the previously reported association in children or adults with a larger sample size over multiple influenza seasons. However, we cannot rule out the possibility that vaccination may alter susceptibility to non-influenza viruses in some circumstances. There is limited biological evidence to support an effect of nonspecific immunity across virus families or species and limited evidence to support the role of vaccination in such immunity. Cross-reactivity of the T-cell response has been described before for different subtypes of influenza A [16–18], although cross-reactivity generated by the innate immune system likely lasts only 10–14 days [19] and may not apply to interactions between viruses of different families or species. Other researchers have also suggested that outbreaks of different respiratory viruses may interact or interfere with each other on a population level [20–22]. This mechanism may help to explain effects not seen in our analysis but which have been reported elsewhere [7, 8].

In conclusion, our findings do not support the hypothesis that influenza vaccination is associated with an increased risk of infection with noninfluenza respiratory virus. Further research may aid in determining the biological plausibility of temporary nonspecific immunity generated by infection with specific respiratory viruses. Finally, we found no difference in vaccine effectiveness estimates using test-negative vs other virus-positive control groups. The results of this analysis strongly support the validity of case vs test-negative control study designs that are currently used in multiple countries to estimate influenza vaccine effectiveness in the outpatient setting.

Notes

Acknowledgments. We gratefully acknowledge the staff of data managers, research coordinators, interviewers, laboratory technicians, and others who contributed to the vaccine effectiveness study and this analysis. We also thank Alicia Fry, Mark Thompson, and Frank Sifakis for manuscript review and feedback.

Financial support. MedImmune, LLC, provided funding for multiplex reverse-transcription polymerase chain reaction testing.

Potential Conflicts of Interest. M. E. S., J. K. M., and E. A. B. receive research funding from MedImmune, LLC. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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