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Short communication

Reduced serologic sensitivity to influenza A virus illness among inactivated influenza vaccinees

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ABSTRACT

We compared ≥4-fold increases in antibody titers by hemagglutination inhibition assay to RT-PCR results among 42 adults with PCR-confirmed influenza A virus illnesses. Serologic sensitivity was higher among unvaccinated (69%, 95% confidence interval [CI] = 48–90%) than vaccinated healthcare personnel (38%, 95% CI = 29–46%) in a 2010–11 prospective cohort.

Q3 Although serologic studies have been described as the "gold standard" approach to estimating influenza virus infection rates [1], the interpretation of increases in antibody titers after an influenza season has long been viewed as problematic. Relying on serologic outcomes may lead to spuriously high estimates of influenza vaccine effectiveness (VE) if vaccinees with breakthrough influenza infections are less likely to manifest substantial increases in antibody titers [2]. Yet, there is limited information on the extent of this presumed sensitivity gap [3–5], especially as it applies to evaluations of illness with influenza A(H3N2) virus [6]. With data from our prospective cohort study of healthcare personnel (HCP), we compared HCP who received 2010–11 inactivated trivalent influenza vaccine (IIV3) with unvaccinated HCP in order to estimate the sensitivity of a ≥4-fold increase in antibody titers (seroconversion) to influenza A virus using the hemagglutination inhibition (HI) assay compared to virus confirmation by real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay. As an exploratory analysis, we examined whether this serologic sensitivity is lower when pre-season antibodies are elevated or when RT-PCR results vary across swab types (i.e., suggesting low viral load).

1. Methods

HCP aged 18–65 years old providing direct patient care were enrolled at two medical centers, one in Oregon and one in Texas, USA. Details of our study methodology have been previously published, including: study recruitment (from September to November 2010) and participant characteristics [7,8]; collection of blood at enrollment, ~28 days post-vaccination (October to December 17, 2010), and ~7 months after enrollment (May and June 2011) [9]; the documentation of vaccination status from integrated medical and employee records [7,10,11]; active surveillance for febrile acute respiratory illness (FARI; fever, feverishness, or chills and cough) [12,13] from December 18 2010 to April 30 2011; collection of three separate specimens using nasal, oropharyngeal, and nasopharyngeal swabs and testing each using US CDC's RT-PCR procedures [14]. Serum was tested by HI using standard procedures against the IIV3 components A/California/7/2009 (H1N1) (i.e., A[H1N1]pdm09) and A/Perth/16/2009-like H3N2 virus [9,15]; all of the influenza A viruses from our study were characterized as vaccine-like [9,15],

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similar to national trends that season [16]. We define seroconversion as a ≥4-fold increase in geometric mean titers (GMT) to either A viruses from T1 (baseline) to T3 (post-season) for unvaccinated HCP and from T2 (~28 days post-vaccination) to T3 for vaccinees. Estimates of sensitivity and specificity were made using a generalized linear model for repeated measures [17] adjusting for study site; non-overlapping 95% confidence interval (CI) indicated a statistically significant difference.

2. Results

The median age of study participants was 43 years old and the majority were female, white, and non-Hispanic (see [7,9,10] and Supplemental Table). Our analyses focused on FARI episodes among 47 unvaccinated and 174 vaccinated HCP (Supplemental Figure).

We identified 12 and 30 RT-PCR-confirmed influenza A virus infections associated with FARI among unvaccinated and IIV3 vaccinated HCP, respectively (Table 1). Most (74%, 31/42) were influenza A(H3N2) virus illnesses. While 67% (8/12) of unvaccinated HCP with RT-PCR influenza A virus illness demonstrated seroconversion, only 37% (11/30) of vaccinated HCP did so. Thus, the sensitivity of seroconversion to RT-PCR-confirmed influenza A was significantly higher among unvaccinated (69%, 95% CI = 48–90%) than vaccinated HCP (38%, 95% CI = 29–46%); we observed similar significant differences for influenza A(H3N2) virus illnesses only (sensitivity 63% vs. 36%, see Table 1) and similar but non-significant differences for influenza A(H1N1)pdm09 virus illnesses (sensitivity 85% [95% CI = 55–100%] vs. 50% [95% CI = 39–60%]).

In an exploratory analysis, we noted that serologic sensitivity to RT-PCR-confirmed influenza A(H3N2) virus illness was lower when unvaccinated HCP had elevated antibodies to influenza A(H3N2) virus at T1 and vaccinees had elevated antibodies at post-vaccination T2 (Table 1). For example, sensitivity declined from 50% for vaccinees with undetectable antibodies to influenza A(H3N2) virus (GMT <5) at T2 to 15% for vaccinees at T2 (GMT ≥40), but CIs overlapped. The effect of pre-season GMT could not be distinguished from prior season (2009–10) vaccination status, since none of the unvaccinated HCP and 83% (19/23) of vaccinees had been vaccinated in the previous season.

Additionally, we noted that serologic sensitivity was higher when the RT-PCR cycle threshold (CT) results were lower (≤30 vs. 31–37) (Table 1), though CIs overlapped. This was especially notable among vaccinees (50% vs. 25%). Similarly, but only among vaccinees, serologic sensitivity declined from 47% for those with

Table 1
Sensitivity and specificity of ≥4-fold serologic seroconversion to RT-PCR influenza illness among healthcare personnel unvaccinated or vaccinated with inactivated trivalent influenza vaccine (IIV3) in 2010–11.

	Unvaccinated (*N* = 47)		IIV3 Vaccinated (*N* = 174)	
	Crude	Adjusted[a]	Crude	Adjusted[a]
Influenza A viruses				
Positivity criteria				
RT-PCR Positive (*N* = 42)[b]	12		30	
Serologic Positive ≥4-fold (*N* = 28)[c]	13		15	
Serologic (Sero.) positive as ≥4-fold change				
Sensitivity (Sero. Positives/RT-PCR Positives)	8/12 (67%)	69% (48–90%)	11/30 (37%)	38% (29–46%)[f]
False Negatives (Sero. Neg/RT-PCR Positive)	4/12 (33%)		19/30 (63%)	
Specificity (Sero. Negative/RT-PCR Negatives)	30/35 (86%)	87% (75–99%)	140/144 (97%)	97% (94–100%)
False Positives (Sero. Pos/RT-PCR Negatives)	5/35 (14%)		4/144 (3%)	
Sensitivity				
By RT-PCR cycle threshold (Ct) values with an oropharyngeal (OP) swab				
Low (≤30 Ct)	3/4 (75%)	75% (33–100%)	7/14 (50%)	50% (25–81%)
High (31–37 Ct)	5/8 (63%)	63% (24–100%)	4/16 (25%)	25% (6–43%)
By RT-PCR results for OP, nasal, and nasopharyngeal swabs				
Consistent swab results	3/6 (50%)	50% (14–86%)	9/19 (47%)	47% (25–70%)
Inconsistent swab results	5/6 (83%)	82% (54–100%)	2/11 (18%)	19% (8–30%)[f]
Influenza A(H3N2)				
Positivity criteria				
RT-PCR Positive (*N* = 31)	8		23	
Serologic Positive ≥4-fold (*N* = 22)	10		12	
Serologic positive as ≥4-fold change				
Sensitivity (Sero. Positives/RT-PCR Positives)	5/8 (63%)	65% (42–87%)	8/23 (35%)	36% (26–46%)[f]
Sensitivity				
By pre-season A(H3N2) GMT[d]				
GMT ≤ 5	4/5 (80%)	78% (46–100%)	3/6 (50%)	50% (23–78%)
GMT > 5	1/3 (33%)		40% (7–73%)	
GMT 10 ≤ 20	4/9 (44%)		44% (30–59%)	
GMT ≥ 40	1/8 (13%)		15% (3–27%)	
By Receipt of 2009–10 IIV3 vaccination[e]				
Not vaccinated prior season			1/4 (25%)	27% (0–54%)
Vaccinated prior season			7/19 (37%)	38% (28–48%)

[a] Adjusted models and 95% confidence intervals (reported from 0% to 100%) are estimated using a generalized linear model, with study site as the only adjusted covariate.
[b] Febrile acute respiratory illness (FARI; fever, feverishness, or chills and cough) and influenza A positive from real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay.
[c] Serologic positive is defined by ≥4-fold change in antibody titers from pre-season to post-season for either A(H1N1)pdm09 or A(H3N2), which among unvaccinated participants is a change from baseline to post-season titers and for vaccinated from ~30 days post-vaccination to post-season.
[d] Strata of geometric mean titers (GMT) for A/Perth/16/2009-like H3N2 virus at baseline for unvaccinated HCP and post-vaccination for vaccinees. Undetectable antibodies were represented as 5 GMT. Given low titers among unvaccinated HCP, strata considered those with and without detectable baseline titers. For vaccinees, we considered three strata including those with low and relatively high GMT.
[e] None of the 8 unvaccinated HCP with A(H3N2) PCR-confirmed illness had been vaccinated the previous season.
[f] 95% confidence intervals of estimates for unvaccinated vs. vaccinated HCP do not overlap.

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