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Review

Effect of recent seasonal influenza vaccination on serum antibody responses to candidate pandemic influenza A/H5N1 vaccines: A meta-analysis

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ABSTRACT

Recent studies have suggested that among those receiving seasonal influenza vaccine (SIV), reduced immunogenicity is observed in recently vaccinated (RV; within the past season or 2) persons when compared with those not recently vaccinated (NRV). We performed a meta-analysis to assess the effect of recent immunization with SIV on serum H5 hemagglutination inhibition (HAI) antibody responses after influenza A/H5N1 vaccination using data from a series of randomized controlled trials. The primary outcome was seroconversion measured by HAI assays following receipt of 2 doses of H5N1 vaccine. The geometric mean titer (GMT) of serum HAI antibody after vaccination was the secondary outcome. Analyses were performed using propensity score (PS) matching. The PS for each individual in the meta-analysis cohort was calculated using logistic regression and covariates included age, gender, race, antigen dose, adjuvant, statin use and vaccine manufacturer. 2015 subjects enrolled in 7 clinical trials were eligible for inclusion in the meta-analysis cohort; among these, 915 (45%) were RV. 901 RV subjects were matched (1:1) with replacement to a subject who was NRV. Subjects who received SIV within the previous season were significantly less likely to seroconvert following H5N1 vaccination (adjusted odds ratio 0.76; 95%CI 0.60–0.96; p = 0.024), and the GMT was 18% higher among NRV subjects (GM ratio of HAI antibody 1.18; 95%CI 1.04–1.33; p = 0.008). Further work is needed to better define the effects of, and mechanisms contributing to, reduced immune responses to H5N1 vaccine among RV subjects.

Contents

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1. Background

Annual immunization with seasonal influenza vaccines (SIVs) is currently recommended for persons ≥6 months of age in the United States [1]. Recent studies have suggested that those receiving prior SIV develop reduced vaccine responses and/or vaccine effectiveness (VE) when compared with those being immunized with SIV for the first time [for a review, see Ref. [2]]. However, considerable variability exists in these studies with different seasons and different influenza virus subtypes.

The results of prospective clinical trials of candidate pandemic influenza vaccines have indicated that subjects who received SIV in recent seasons have diminished immune responses to the novel hemagglutinins (HAs) contained in experimental H5N1 and H7N9 vaccines [3–5]. Diminished responses to 2009 H1N1pdm vaccine candidates also were observed among adults who recently had received SIV [6–8], although many adults had detectable antibody to the vaccine virus strain prior to immunization. In contrast, most of the H5 and H7 vaccinees have no detectable antibody to the H5 or H7 HA before vaccination. Our meta-analyses of individual subject data from a series of randomized controlled trials (RCTs) evaluates the effect of recent immunization with SIV on serum antibody responses following immunization of healthy adults with candidate H5N1 vaccines. To mimic the characteristics of a randomized controlled trial and to control for possible confounding, we use propensity score matching.

2. Material and methods

2.1. Study design

Between 2005 and 2012, the Division of Microbiology and Infectious Diseases (DMID)/National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH) sponsored 19 RCTs of the safety and immunogenicity of inactivated influenza A/H5N1 (A/H5N1) vaccines at up to 14 DMID-sponsored Vaccine and Treatment Evaluation Unit (VTEU) sites (A list of all 19 RCTs is provided with Supplementary Materials). Data management for all trials was conducted by a single DMID-sponsored Statistical and Data Coordinating Center (Emmes, Rockville, MD) and the serologic assays were conducted by a single central laboratory (Southern Research, Birmingham, AL). Taking advantage of the uniformity of databases and trial methodologies, this investigation was designed as an individual patient data (IPD) meta-analysis based on the subject-specific data, thus permitting adjustment for the same set of confounders across multiple studies [9–11]. This meta-analysis combines individual subject data from multiple RCTs to explore the relationship between recent receipt of SIV (within the past season or 2) and the humoral immune response to A/H5N1 vaccination.

2.2. Inclusion criteria for the meta-analysis cohort

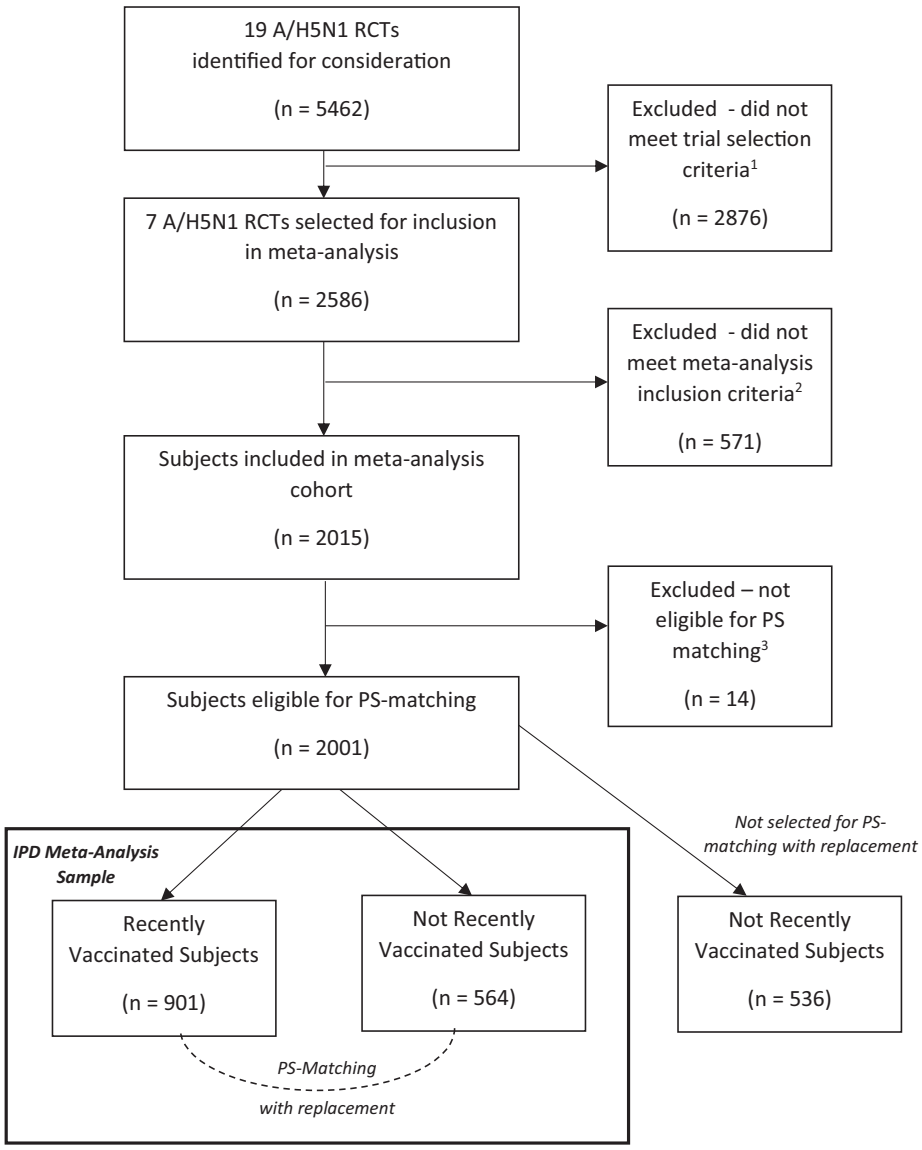
To obtain a relatively homogeneous cohort and to exclude possible confounders, we first selected 7 of the 19 RCTs for inclusion in the meta-analysis (Fig. 1). Studies were included if they had at least one study arm in which subjects received vaccines derived from influenza A/Vietnam/1203/2004 (H5N1), and enrolled generally healthy subjects ages 18–64 years old who were A/H5N1-naïve and not previously exposed to other experimental influenza A/H5 vaccines. Enrollment criteria were similar across the selected RCTs, and are provided as supplementary material. From the 7 selected RCTs, we further excluded individual subjects who did not receive two A/Vietnam/1203/2004 (H5N1) vaccinations given 21–28 days apart [12] using the same vaccine regimen at each dose (i.e., both vaccinations administered by the same route- intradermally (ID) or intramuscularly (IM) in the deltoid; both contained the same HA dosage; and both formulated with the same adjuvant - aluminum hydroxide or MF59 – or without adjuvant), or who did not have immunogenicity blood samples collected within 24 days of the planned collection window.

2.3. Outcome measures

The immune response to the vaccine antigen was measured by serum hemagglutination inhibition (HAI) assays using venous blood samples collected prior to the first dose of vaccine and approximately 21–28 days after the second vaccination. Serum HAI assays were performed using horse erythrocytes and standard techniques [13] and testing was done by a single laboratory. A binary indicator of seroconversion, defined as a pre-vaccination HAI titer <10 and a post vaccination HAI titer ≥40 or a pre-vaccination HAI titer ≥10 and a minimum four-fold rise in post-vaccination HAI antibody titer [14], was used as the primary outcome. Log-transformed HAI titer was used as a secondary outcome.

2.4. Covariates

The main covariate (exposure) of interest in this meta-analysis was recent receipt of SIV (recently vaccinated, or RV). Recent was defined as receipt in the prior season (this was the only information available for protocols 04-062, 04-063, 05-0015, 05-0127, 06-0052, 06-0089) or in at least one of the past 2 prior seasons (information for both prior seasons was available only for protocol 07-0019). Information regarding prior influenza vaccination was extracted from self-reported medical history records recorded by study personnel for each subject at the time of first A/H5N1 vaccination. Additional covariates in statistical modeling were demographic characteristics (age, gender, race), vaccine dosage, presence of adjuvant ('yes' or 'no' binary variable), H5N1 vaccine manufacturer, vaccine administration route (ID/IM), comorbid con-



1. Details of 19 RCTs included in supplemental materials
2. Summaries of subjects excluded from each of the 7 selected protocols provided in Table 1
3. Additional details of Propensity Score (PS) Matching algorithm provided in supplemental materials

Fig. 1. Selection of subjects for inclusion in the IPD meta-analysis.

ditions, seasonal allergies, statin use and study site. Presence of adjuvant was included in the analyses as a binary variable (1 = yes adjuvanted with aluminum hydroxide or MF59, 0 = no adjuvant). Comorbid conditions were extracted from self-reported medical history records collected at the time of enrollment in the RCTs and were included as a binary variable (0 = no comorbidity, 1 = has at least one comorbidity). The comorbidities included were cardiovascular disease, chronic lung disease, diabetes, hyperlipidemia, hypertension, and other. The "other" comorbidities referred to cancer history, liver, kidney, blood, metabolic other than diabetes or hyperlipidemia, autoimmune, or chronic musculoskeletal or neurological disorders. The medical history information collected at the time of enrollment was reviewed and categorized by 2 clinicians (WAK and RLA) who were unaware of the subjects' vaccine group assignment and history of recent receipt of SIV. Seasonal allergies were included as a binary variable (0 = no seasonal allergies, 1 = yes seasonal allergies). Of note, the use of medication reported at the time of enrollment in the RCT was not included in the analysis because it was highly correlated with presence of comorbidities (data not shown). However, the use of statins at enrollment was assessed [15].

2.5. Statistical analyses

Similar to an RCT setting, the two "treatment" groups can be defined as the group of subjects who were RV with SIV and the group of subjects who were not recently vaccinated (NRV). The subjects used in this meta-analysis received SIV in one or both of the two seasons prior to study enrollment based on their own preferences; therefore, it is possible that there are influential differences between persons who chose to receive SIV in the years prior to H5N1 study vaccine enrollment and those who did not. To make the two "treatment" groups more comparable, we performed analyses based on propensity score matching [11,16]. The statistical modeling used for final inference was defined a priori in an analysis plan and based on the recommended one-step

approach for individual subject meta-analysis that fits one statistical model for all subjects across the entire meta-analysis cohort while accounting for clustering by study, rather than a two-step approach that fits a statistical model for each study and then pools results for final inference using standard meta-analytic techniques [9]. All statistical analyses were performed using SAS 9.3 or higher.

2.6. The propensity score model

The Propensity Score (PS) is the conditional probability of recently receiving SIV, given the measured covariates. The PS for each individual in the meta-analysis cohort was calculated using logistic regression and covariates (age, gender, race, antigen dose, adjuvant, vaccine manufacturer, site, comorbidities, seasonal allergies and use of statins) believed to be potential or true confounders, and assumed not to be influenced by SIV. When developing the PS model, it is preferable that all included covariates be measured before the receipt of SIV [17,18]; however, comorbidities and seasonal allergies were measured at the time of enrollment in each RCT, after receiving SIV. We assumed that these records represent the status of comorbidity and seasonal allergies before receiving SIV and note this as a limitation of our study. In addition, we controlled for unmeasured confounding by site by including site in the PS model as a fixed effect [19]. We determined common support by excluding subjects with extreme low or high PS values using methods detailed by Caliendo & Kopeinig [20]. Similar to a randomized controlled trial, the excluded subjects (i.e., subjects with extreme PS values) represent subjects who did not meet the inclusion/exclusion criteria for the meta-analysis cohort, whereas the remaining subjects (i.e., common support) represent subjects eligible for enrollment/randomization.

2.7. Propensity-score matching

RV subjects in the common support were matched with subjects who were NRV using PSs. For the primary analyses, the matching was performed one-to-one with replacement. Matching without replacement may lead to more subjects being discarded, reducing power and generalization [21]; therefore, we considered this a sensitivity analysis. We used the nearest neighbor matching algorithm with a specified caliper distance of 0.2 of the standard deviation of the logit of the PS [15,21], which has been reported to eliminate up to 99% of the bias due to the measured confounders. Covariate balance after matching between RV and NRV subjects was evaluated by assessing the standardized differences and ensuring none were larger than 0.1 [22].

2.8. Analysis of the primary outcome

The primary outcome analysis explored the association between recent SIV and a binary indicator of seroconversion following receipt of 2 doses of H5N1 vaccine. Using the PS-matched subjects (with replacement), we fit a mixed effects logistic regression with recent receipt of SIV as the main covariate of interest and PS included as a continuous covariate. The PS-matched subjects and enrolling RCT may be viewed as clusters, and thus random effects for the match ID and protocol number were included to account for correlation within these clusters [11]. A sensitivity analysis was performed by fitting the same models but considering propensity scores matching without replacement.

2.9. Analysis of the secondary outcome

The secondary outcome analysis explored the association between recent SIV and a continuous level of reciprocal HAI titer following receipt of 2 doses of H5N1 vaccine. The statistical modelling follows the same steps as the analyses of the primary outcome, adapted for a continuous outcome with a normal distribution on a log scale.

3. Results

Two thousand and fifteen subjects from 7 clinical trials were eligible for inclusion in the meta-analysis cohort (Table 1; Refs. [12,23–28]); 915 (45%) of these subjects were RV with SIV (within the past one or two seasons). Among the 915 RV subjects, 874 (96%) were vaccinated within the past one season. Characteristics of those who were included are summarized in Table 2. Most were white (78%), female (57%) and had no underlying comorbidities (84%). The average age was 34 years (range: 18–64 years). Of note, there were a number of significant differences between RV and NRV subjects. Specifically, RV subjects were more likely to be female, to be white, to be on statins, to have 1 or more comorbidities, to report a history of seasonal allergies, and to be older, as well as to have received adjuvanted study vaccine ($p < 0.05$ for each).

Table 1
Number of subjects randomized in each protocol and included in the meta-analysis

Protocol Number/Registration [Ref.]	Trial start-stop dates	Total Subjects Randomized, N	Subjects included in Meta-analysis cohort, n (%)	Subjects excluded from Meta-analysis cohort, n (%)	Most common reason for exclusion	Subjects included in the Meta-analyses cohort that were previously vaccinated, n (%)
04-062 NCT00280033 [26]	3/6/2006–11/20/2006	394	360 (91)	34 (9)	Subjects were randomized to the placebo arm	233 (64%)
04-063 NCT00115986 [27]	3/28/2005–1/25/2006	452	389 (86)	63 (14)	Subjects were randomized to the placebo arm	165 (42%)
05-0015 NCT00310206 [28]	7/11/2005–10/25/2006	100	100 (100)	NA	NA	28 (28%)
05-0127 NCT00296634 [29]	3/7/2006–12/21/2006	600	592 (99)	8 (1)	Missing follow-up assay results	263 (44%)
06-0052 NCT00382980 [30]	10/16/2006–9/21/2007	308	245 (80)	63 (20)	Subjects were randomized to the placebo arm	84 (34%)
6-0089 NCT00439335 [31]	3/14/2007–2/5/2008	227	227 (100)	NA	NA	94 (41%)
07-0019 NCT00703053 [15]	9/8/2008–11/12/2009	505	102 (20)	403 (80)	Subjects received a different vaccine Strain (H5 A/Indo) or only one dose vaccination was received per protocol	48 (47%)
All Protocols	3/28/2005–11/12/2009	2586	2015 (78)	571 (22)		915 (45%)

Table 2
Summary of covariates overall and by recent seasonal influenza vaccination

Covariate*	Overall (N = 2015)		Not recently vaccinated (N = 1100)		Recently vaccinated (N = 915)		p-value**
	n	*(%)*	*n*	*(%)*	*n*	*(%)*	
Gender							<0.001
Female	1157	(57.4)	594	(54.0)	563	(61.5)	
Male	858	(42.6)	506	(46.0)	352	(38.5)	
Race							<0.001
Other	444	(22.0)	296	(26.9)	148	(16.2)	
White	1571	(78.0)	804	(73.1)	767	(83.8)	
Adjuvant							0.037
None	1436	(71.3)	805	(73.2)	631	(69.0)	
Any[1]	579	(28.7)	295	(26.8)	284	(31.0)	
Route							0.001
Intradermal	163	(8.1)	109	(9.9)	54	(5.9)	
Intramuscular	1852	(91.9)	991	(90.1)	861	(94.1)	
Statin Use							<0.001
No	1946	(96.6)	1076	(97.8)	870	(95.1)	
Yes	69	(3.4)	24	(2.2)	45	(4.9)	
Comorbidities							<0.001
None	1686	(83.7)	967	(87.9)	719	(78.6)	
Any[2]	329	(16.3)	133	(12.1)	196	(21.4)	
Seasonal Allergies							0.007
No	1329	(66)	754	(68.5)	575	(62.8)	
Yes	686	(34)	346	(31.5)	340	(37.2)	
Antigen Dose (mcg)							0.236
3	25	(1.2)	18	(1.6)	7	(0.8)	
3.75	121	(6.0)	62	(5.6)	59	(6.5)	
7.5	369	(18.3)	200	(18.2)	169	(18.5)	
9	25	(1.2)	18	(1.6)	7	(0.8)	
15	434	(21.5)	245	(22.3)	189	(20.7)	
30	346	(17.2)	176	(16.0)	170	(18.6)	
45	493	(24.5)	270	(24.6)	223	(24.4)	
90	202	(10.0)	111	(10.1)	91	(9.9)	
Age							<0.001
Mean ± sd	34.4 ± 11.0		31.8 ± 9.9		37.4 ± 11.5		
Range	[18,64]		[18,64]		[18,64]		

* Vaccine Manufacturer and Study site are also included in the PS model as categorical variables. For simplicity, results for these covariates are summarized in the Supplementary Materials.
** p-value corresponds to a chi-square test for categorical variables and to a *t*-test test with unequal variances for the continuous variables.
[1] Any adjuvant includes aluminum hydroxide or MF59.
[2] Any comorbidity includes cardiovascular disease, lung disease, diabetes, hyperlipidemia, hypertension, and "other". The "other" comorbidities referred to cancer history, liver, kidney, blood, metabolic other than diabetes or hyperlipidemia, autoimmune, or chronic musculoskeletal or neurological disorders.

Of all subjects included, 14 subjects were considered "ineligible" for PS-matching (as having extremely high or low PS values [20]) and were therefore excluded from the meta-analyses. The remaining 2001 subjects representing the common support were considered "eligible," and only these were used for matching. The "ineligible" subjects were more likely to be older, female, with at least one comorbidity, and to have a record of statin use compared to subjects who were considered "eligible". These results are detailed in the Supplementary Materials. All 901 eligible RV subjects were matched with replacement to a NRV subject, resulting in a total sample size of 1802 records. The 901 matched NRV subjects were comprised of 564 unique individuals; most were matched with no more than 3 RV subjects, but several NRV subjects were matched with as many as 11 RV subjects. The distribution of the PSs before and after matching is presented in Fig. 2. Statin use and study site were not balanced after matching [21] between RV and NRV subjects. These covariates were included in the primary outcome model to further adjust for possible covariate imbalance.

Fig. 3 presents odds ratios (OR) and 95% confidence intervals (CI) for the primary outcome (seroconversion). Individual subject data were first analyzed separately for each protocol for comparison with the analyses over the entire meta-analyses cohort. The latter analyses were used for final inference, as described in the *Statistical Analyses* section. All but two trials (06-0052 and 07-0019) suggested lower immune response to H5N1 for subjects who were RV with SIV; however, no statistically significant effect of recent SIV was observed in individual studies. When individual subject data were analyzed over the entire meta-analysis cohort, subjects who were RV with SIV were significantly less likely to seroconvert in response to H5N1 vaccination than those who were NRV. Reduction in antibody responses was observed among persons who received SIV within the previous season; insufficient data were available to conclude a reduction among persons who received SIV within the previous 2 seasons. The adjusted logistic regression over the entire meta-analysis cohort after matching with replacement suggests that the odds of seroconverting at approximately one month after second vaccination with A/H5N1 was 24% lower for RV subjects versus subjects who were NRV (adjusted OR = 0.76; 95%CI 0.60–0.96; p = 0.024).

The sensitivity analysis of the primary outcome provides similar inference. After matching without replacement, only 712 (79%) out of 901 RV subjects were matched to subjects who were NRV, resulting in total sample of 1424 subjects. Using this sample, the odds of achieving seroconversion after vaccination with A/H5N1 was 32% lower for RV subjects versus NRV subjects (adjusted

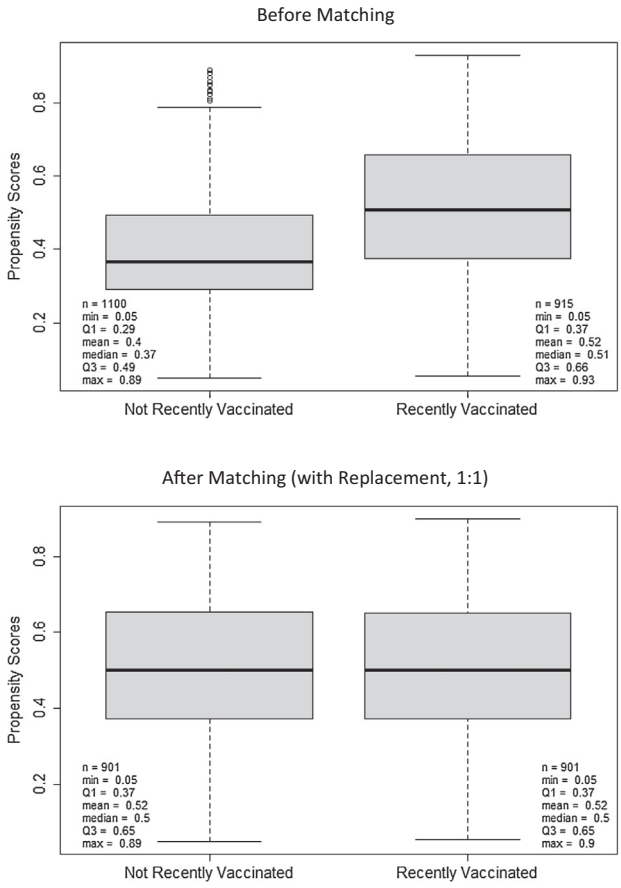


Fig. 2. Box-plots of the propensity scores by recent seasonal influenza vaccination.

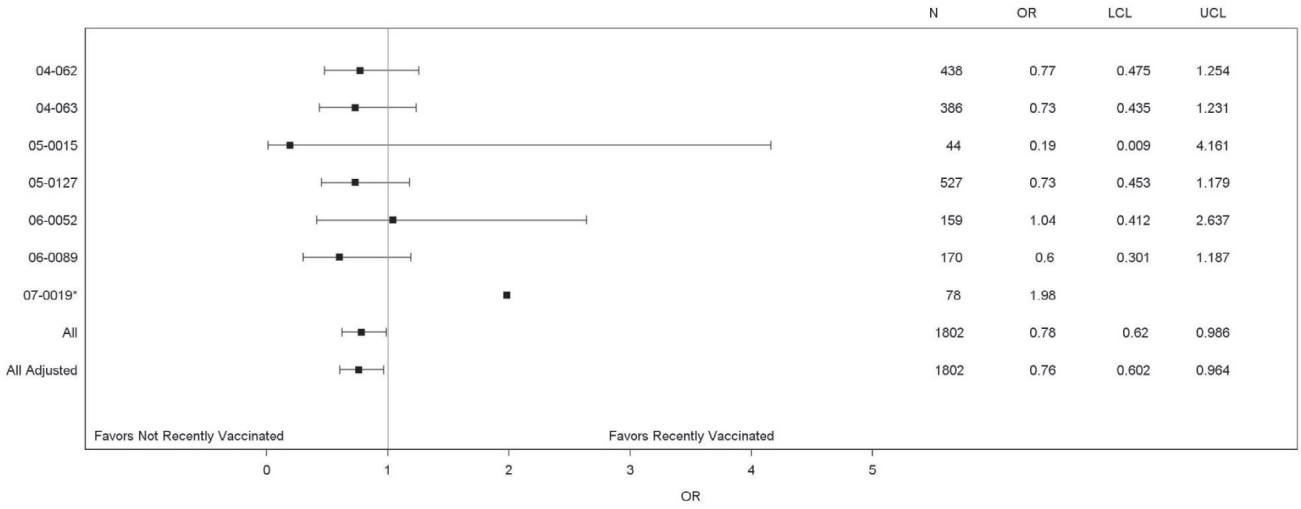
OR = 0.68; 95% CI 0.53–0.88; p = 0.003). Results are presented in the Supplementary Materials.

Table 3 presents results for the analyses of the secondary outcome (HAI titer). The adjusted linear mixed model over the entire meta-analysis cohort shows a geometric mean ratio (GMR) of 1.18 (95%CI: 1.04, 1.33; p = 0.008), which suggests that the geometric mean titer (GMT) for subjects NRV is 18% higher compared to RV subjects.

4. Discussion

Currently, there is no consensus as to whether recent vaccination with SIV modulates the immune response to subsequent immunization with SIV, other experimental influenza vaccines, or to influenza infection itself [29,30]. The results of this meta-analysis suggest that recent immunization with SIV is associated with a significant reduction in serum antibody responses following immunization with experimental influenza A/H5N1 vaccines in healthy younger adults despite receiving two doses of vaccine. The odds of seroconversion were 24% lower for subjects who were RV with SIV when compared to subjects who were NRV. Similarly, the H5N1 HAI GMT was 18% higher for NRV subjects versus RV subjects. The analyses of the primary and secondary outcomes yielded consistent results: the immune response to H5N1 vaccination was significantly lower for RV subjects compared to those NRV.

Several reports have suggested that recent immunization with SIV results in reduced antibody responses upon revaccination. However, one randomized clinical trial conducted in middle-aged adults concluded that there was no consistent reduction in strain-specific immunogenicity over a 5-year period [31], although reduced immunogenicity among repeatedly vaccinated subjects was observed during the last year of the trial. Notably, whole virus vaccines were used rather than split virus vaccines. Results of a



N= total sample size including recently vaccinated subjects and their matched controls (with replacement, 1:1); OR = Odds Ratios; LCL = 95% lower confidence limit; UCL = 95% upper confidence limit; the y-axis identifies the protocol data that were used to run the logistic regression.

Adjusted suggests that the logistic regression to get the OR was adjusted for possible baseline imbalance (statins use and study site).

*Note that the model for protocol 07-0019 did not converge

Fig. 3. Primary outcome analyses: Logistic regression models to determine the effect of recent seasonal influenza vaccination on seroconversion in response to A/H5N1 vaccination, stratified by protocol and across all protocols after propensity score matching (with replacement).

Table 3
Secondary outcome analyses: GMTs and GMRs for not recently vaccinated versus recently vaccinated subjects for seasonal influenza, stratified by protocol and across all protocols after propensity score matching (with replacement, 1:1).

Protocol	Not recently vaccinated		Recently vaccinated		GMR (GMT0/GMT1)	95%CI	p-value
	n	GMT0	n	GMT1			
04-062 (N = 438)	219	12.80	219	10.70	1.20	(0.94, 1.52)	0.146
04-063 (N = 386)	221	14.00	165	11.83	1.18	(0.91, 1.54)	0.206
05-0015 (N = 44)	16	14.14	28	10.64	1.33	(0.62, 2.84)	0.453
05-0127 (N = 527)	264	9.38	263	8.49	1.10	(0.90, 1.35)	0.340
06-0052 (N = 159)	75	9.03	84	8.62	1.05	(0.73, 1.51)	0.799
06-0089 (N = 170)	76	23.50	94	14.46	1.63	(0.98, 2.70)	0.061
07-0019 (N = 78)	30	15.87	48	21.86	0.73	(0.34, 1.55)	0.403
All protocols (N = 1802)*	901	13.21	901	11.40	1.16	(1.03, 1.31)	0.017
All protocols, adjusted (N = 1802)**	901	13.61	901	11.56	1.18	(1.04, 1.33)	0.008

n = sample size; GMT = geometric mean titers; GMT0 = GMT for NRV subjects; GMT1 = GMT for RV subjects; GMR = geometric mean ratios.

* The mixed model consists of recent influenza vaccination and propensity score as fixed effects, and protocol as a random effect. Parameter estimates are based on restricted maximum likelihood.

** The mixed model consists of recent influenza vaccination and propensity score as fixed effects, and possible imbalanced covariates after matching (site) which are included as fixed effects. Statin was removed since its effect was estimated to be zero. Protocol is included as a random effect. Parameter estimates are based on restricted maximum likelihood. Note that match was removed from the mixed models since its variance components were estimated to be zero.

recent meta-analysis also concluded that evidence for reduced VE among participants who were immunized annually was not supported, but that confidence in the evidence was low [32]. One hypothesis for the apparent reduction in vaccine effectiveness among people who have been recently vaccinated was posited by Smith et al., in which prior vaccination with a vaccine that contains the same or antigenically very similar strain as the current vaccine and is given during a season when the circulating virus is significantly different results in vaccine interference (antigenic distance hypothesis [33]). In this case, the antigenic distance between the vaccine and epidemic strain's HAs (based on HAI responses) accounts for reduced effectiveness mediated by negative interference in repeat vaccinees when compared with persons being immunized for the first time. However, this hypothesis may not account for reduced immunogenicity following immunization with vaccines containing novel HAs (e.g., H5N1), against which most people lack detectable HAI antibody.

The effects of repeated vaccinations have been studied in murine models of infection and vaccination. In these studies, reduced HA-specific antibody responses are often accompanied by reduced HA-specific T helper cell responses. Richards et al. explored the effect of repeated influenza vaccination on CD4 T cell responses. They demonstrated that a commercial split virus IIV3 elicited CD4 T cell responses to several antigens other than the HA, including neuraminidase, nucleoprotein, matrix protein and nonstructural protein 1. They postulated that repeated immunization results in a 'refocusing' of immune responses on antigens other than the HA that are contained in the vaccines [34]. Nayak et al. addressed the effect of competition between memory and naïve CD4 T cells on the specificity of CD4 T cell and B cell responses in a murine model of sequential influenza infection [35]. Their results indicated that there were marked decreases in CD4 T cell responses to nonconserved HA epitopes after secondary infection; this was associated with significant reductions in antibody responses to the HA. This group subsequently demonstrated that this negative effect on antibody responses could be reversed by selectively expanding HA-specific CD4 T cell memory before the secondary infection [36]. They concluded that immunization regimens that elicit broadly cross-reactive HA-specific memory CD4 T cells might promote the production of HA-specific antibodies, even upon exposure to pandemic strains for which there is little B cell memory. Of interest is the observation that prior immunization of healthy adults with SIV was associated with lower antibody responses following immunization with a purified recombinant HA [37]. In this case, no other internal influenza antigens could account for diminished responses; however, preferential responses to conserved HA epitopes may have contributed to reduced responses to the nonconserved HA epitopes.

Bodewes et al. also have shown that vaccination with SIV inhibits the induction of heterosubtypic immunity induced by infection [38]. In this study, immunization of ferrets with an inactivated H3N2 vaccine reduced protective heterosubtypic immune responses elicited by H3N2 infection in ferrets following challenge with H5N1 virus. The same group subsequently demonstrated that annual vaccination of children with cystic fibrosis is associated with reduction in the development of virus-specific CD8 T cell responses [39], likely the result of protection of vaccinated children against infection. Kedl et al. demonstrated that there is competition between CD8 T cells for antigen presenting cells in a non-influenza model [40]. These results were extended by Willis et al., who showed that competition between CD8-positive T cell populations occurs early in the immune response [41]. Hence, reductions in both antibody and cell-mediated immune responses may occur following immunization with SIVs. In one recent study, adult subjects who were vaccinated with SIV each of the preceding 5 years were shown to have reduced antibody secreting cells, T follicular helper cells and HAI antibody titer increases after vaccination when compared with subjects who had not been vaccinated in any of the past 5 years (Boone and Mulligan; manuscript in preparation). Whether novel adjuvants would be able to overcome the negative effects of previous immunization is incompletely explored. Notably, relatively few vaccinees in the current meta-analysis received MF59 adjuvant, which has been shown to partially overcome the inhibitory effect of prior SIV receipt on responses to an H7N9 vaccine [5]. Inclusion of a potent adjuvant in vaccines for control of pandemic influenza could potentially offset the negative effects of prior immunization with SIVs. Further work is needed to better define effects on humoral and cell-mediated immune responses using better standardized assays.

While animal model-based data demonstrate mechanisms that could account for impairment of HA-specific antibody responses in previously vaccinated animals, the mechanisms that could explain this phenomenon have not been thoroughly explored in human studies. Clinical trials among subjects who are randomized to receive SIV or placebo for several seasons are necessary to confirm results from clinical trials in which subjects have not been randomized to receive SIV in recent seasons, such as the trials included in this meta-analysis. Detailed immunologic analyses could potentially point to mechanisms responsible for the appar-

ent attenuation of HA-specific responses among RV subjects. Thus, the major limitation of the current study is that subjects were not randomized to SIV or placebo in the 2 seasons preceding immunization with an H5N1 vaccine; therefore, the potential for unidentified confounders exists. However, because annual immunization against influenza is recommended for most US citizens, such a trial would be impractical/unethical to conduct in the US. Other limitations include self-reporting of SIV receipt, and lack of more remote history of influenza vaccination and history of influenza infection are additional limitations. With regard to the former, several studies have reassuringly confirmed that self-reporting of receipt of SIV is a sensitive method of ascertaining immunization status when compared with documentation in the medical record [42–44]. Finally, we considered relatively strict criteria for selecting studies for inclusion on the analysis, which may limit the generalizability of results beyond healthy adults or the specific influenza strain considered. Additional studies expanding the population to consider children or older adults or considering vaccine with other influenza vaccine strains are warranted.

In conclusion, this IPD meta-analysis of 7 clinical trials of the immunogenicity of H5N1 vaccines using a propensity score matching approach, in contrast to previous studies using multivariable logistic regression with stepwise selection procedure for covariates, demonstrated a reduction in antibody responses to the H5 HA among persons who received SIV within the previous season. This meta-analysis took advantage of a homogenous database in terms of study design, implementation, laboratory methods used to measure the outcome and inclusion/exclusion criteria used to select the participants in each study. In contrast to earlier studies, the analyses of influenza antibody responses was based on propensity scores in an attempt to adjust for confounders. Further randomized studies in humans will be needed to confirm the effect, and to determine the immunologic mechanisms responsible for the reductions in HA-specific responses. Influenza vaccine studies will benefit greatly from improved long-term knowledge of prior vaccinations, the types of vaccine antigens and adjuvants used, and prior influenza exposure history. Vaccines containing both T and B cell epitopes, administered in combination with specific adjuvants to boost antigen presentation to T cells, may be best for eliciting broad-based protective immune responses.

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Disclaimer

The views expressed here do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

Conflict of interest statements

None: RLA, ARB, RBB, DIB, JDC, KME, HH, LAJ, WAK, CMM, MJM, SP, SMP, PS, JJT, DCV, AW, MCW, KMZ.

CLD: Served as a consultant for Genentech on an influenza project.

GAP is Chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories. He offers consultative advice on vaccine development to Merck & Co. Inc., Avianax, Adjuvance, Alopexx, Sanofi Pasteur, GlaxoSmithKline, and Emergent Biosolutions. He holds four patents related to vaccinia and measles peptide research.

EBW has received funding from Merck and Novavax to conduct clinical research studies; none influenza.

Appendix A. Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.04.066.

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