Antibody to Influenza Virus Neuraminidase: An Independent Correlate of Protection

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(See the editorial commentary by Eckard and Webby on pages 1180–1.)

Background. Laboratory correlates of influenza vaccine protection can best be identified by examining people who are infected despite vaccination. While the importance of antibody to viral hemagglutinin (HA) has long been recognized, the level of protection contributed independently by antibody to viral neuraminidase (NA) has not been determined.

Methods. Sera from a controlled trial of the efficacies of inactivated influenza vaccine (IIV) and live attenuated influenza vaccine (LAIV) were tested by hemagglutination inhibition (HAI) assay, microneutralization (MN) assay, and a newly standardized lectin-based neuraminidase inhibition (NAI) assay.

Results. The NAI assay detected a vaccine response in 37% of IIV recipients, compared with 77% and 67% of participants in whom responses were detected by the HAI and MN assays, respectively. For LAIV recipients, the NAI, HAI, and MN assays detected responses in 6%, 21%, and 17%, respectively. In IIV recipients, as NAI assay titers rose, the frequency of infection fell, similar to patterns seen with HAI and MN assays. HAI and MN assay titers were highly correlated, but NAI assay titers exhibited less of a correlation. Analyses suggested an independent role for NAI antibody in protection, which was similar in the IIV, LAIV, and placebo groups.

Conclusions. While NAI antibody is not produced to a large extent in response to current IIV, it appears to have an independent role in protection. As new influenza vaccines are developed, NA content should be considered.

Clinical Trials Registration. NCT00538512.

Keywords. influenza; serologic assays; hemagglutinin; neuraminidase; clinical trial; influenza vaccine; vaccine response; immune correlates.

The critical role of antibody to viral hemagglutinin (HA) in producing protection against influenza was recognized as early as the first demonstration that an inactivated influenza vaccine (IIV) was efficacious [1]. Data from vaccination and viral challenge studies were subsequently used to determine levels of antibody, as measured by the hemagglutination inhibition (HAI) assay, that produced protection [2–4]. These estimated levels became so accepted that they became standards for evaluation and, at times, licensing of new influenza vaccines [5]. The virus neutralization assay has more recently been added as another test to estimate protection, recognizing that it is measuring mainly antibody to HA [6, 7]. Yet, it was clear even from the early studies that those who develop laboratory-confirmed influenza despite being vaccinated can sometimes have high levels of antibodies to HA [8]. Thus, there have been efforts to identify additional correlates of protection.

Antibody to the other influenza virus surface antigen, neuraminidase (NA), has long been recognized as a possible contributor to protection; antibody to NA does not block viral infectivity but reduces viral replication [9]. However, because of the dominant role of antibody to HA in protection and the fact that infection or vaccination may often produce antibodies to both antigens, the precise contribution of antibody to NA has been hard to determine. An additional problem was

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that the antibody assay was challenging to perform and required use of toxic reagents, thus limiting the number of specimens that were tested [10]. Limited data on the independent role of antibody to NA in protection has come from special situations, such as the 1968 pandemic, when the HA but not the NA of the circulating influenza A virus changed. The differences in patterns of spread of the pandemic virus in various parts of the world were attributed to antibody to NA in the population, as supported by specific studies of antibody titers in individuals with and those without infection [11].

With the increasing realization of the potential importance of antibody to NA to protection, there has recently been a collaborative effort to standardize a lectin-based neuraminidase inhibition (NAI) assay to minimize variation in results [12]. We have previously reported on how HAI antibody correlated with protection from laboratory-confirmed influenza, using data from a clinical trial of IIV and live attenuated influenza vaccine (LAIV), and again demonstrated that most of the relatively few people who were infected after receipt of IIV had high antibody titers [13]. Here we extend these studies to include NAI antibody, to determine its value as a correlate of protection. We also compare results from the HAI and NAI assays with those estimated by the microneutralization (MN) assay, now often considered the gold standard, to examine correlation of values between tests.

MATERIAL AND METHODS

Data and Specimens

Data and sera used here were from healthy volunteers aged 18–49 years who were participating in a randomized, placebo-controlled trial examining the efficacies of IIV (Fluzone, Sanofi-Pasteur) and LAIV (FluMist, MedImmune) during the 2007–2008 influenza season [14]. The study was approved by the institutional review board at the University of Michigan Medical School. Written informed consent was obtained from all participants before enrollment. Blood specimens for serologic studies were collected immediately before receipt of the assigned intervention, approximately 30 days later, and at the end of the influenza season (approximately 4 months later). Symptomatic acute respiratory illnesses were laboratory confirmed as influenza by virus identification in real-time polymerase chain reaction (PCR) assays. Sera from a subset of all enrolled subjects representing all 3 intervention groups (IIV, LAIV, and placebo) were selected to be tested by the HAI assay. This subset included all subjects with laboratory-confirmed influenza and a randomly selected sample of the remaining participants who provided all 3 blood specimens. Sera from 728 (37%) of 1952 subjects enrolled during the 2007–2008 influenza season were tested in the HAI assay. Sera from 497 subjects with HAI data were selected for processing in the NAI assay; excluded subjects included, in part, those with laboratory-confirmed influenza A(H1N1) or B and those with serologic evidence of influenza virus infection (≥4-fold increase in HAI titer between postvaccination and postseason sera) not confirmed by PCR [8]. HAI and NAI data from these 497 subjects (25% of all participants) were included in this analysis, including 103 subjects with PCR-confirmed influenza A(H3N2) (ie, cases) and 394 subjects without laboratory-confirmed influenza (ie, noncases). Because the MN assay is labor-intensive, a smaller subset of sera from 171 subjects was processed in that assay; selected sera included 81 cases and 90 noncases.

Laboratory Assays

The HAI assay detects antibodies that block the binding of the HA receptor binding site to glycan receptors on red blood cells, thus inhibiting hemagglutination; it is considered a surrogate assay for the detection of neutralizing antibodies that block receptor binding [15]. The HAI assays were performed in the respiratory virus research laboratory at the University of Michigan School of Public Health. Prior to HAI testing, all sera were treated overnight with receptor destroying enzyme and heat inactivated to prevent nonspecific inhibition; sera were also adsorbed with red blood cells to remove nonspecific agglutinins. Serial 2-fold dilutions (with an initial dilution of 1:8) were prepared for each set of 3 sera (before vaccination, approximately 30 days after vaccination, and after the influenza season) in 96-well microtiter plates followed by incubation with standardized concentrations (4 HA units per 25 μL) of monovalent IIV subunit material (Sanofi-Pasteur) representing the 2007–2008 A(H3N2) vaccine virus strain (A/Wisconsin/67/05). Turkey red blood cells were added to wells and allowed to settle. The strain-specific HAI antibody titers at each time point for each individual were calculated as the reciprocal (eg, 128) of the highest dilution of sera (eg, 1:128) that inhibited hemagglutination. HAI titers below the limits of detection (ie, <8) were denoted as half of the threshold detection value (ie, 4); titers greater than the upper test value (ie, 4096) were denoted as having twice that value (ie, 8192).

The MN assay directly measures functional virus neutralization by detecting antibodies to viral HA that inhibit virus entry and block virus replication [15, 16]. The MN assays were performed in the Influenza Division research laboratory at the Center for Disease Control and Prevention (CDC). Sera were heat inactivated and serial 2-fold dilutions (with an initial dilution of 1:10) of each set of sera triplets were incubated with virus representing the 2007–2008 A (H3N2) vaccine virus strain in 96-well microtiter plates. Following a reaction time, log-phase growth Madin Darby canine kidney cells were added to wells and incubated. Neutralization was indicated by absence of detectable virus (or viral antigen) using an enzyme-linked immunosorbent assay indicator system [15]; MN titers were calculated as the reciprocal of the highest dilution that neutralized virus infectivity. MN titers below the limits of detection (ie,

<10) were denoted as half the threshold value (ie, 5); titers greater than the upper test value (ie, 5120) were denoted as twice that value (ie, 10 240).

The NAI assay, also known as the enzyme-linked lectin assay, measures antibodies that inhibit the enzymatic activity of viral NA [12, 17]. This assay was also performed in the CDC Influenza Division laboratory and used a reassortant influenza virus with a mismatched HA (H6 subtype), to avoid interference by HA-specific antibodies, and with the NA antigen representing the 2007–2008 A(H3N2) vaccine virus strain (kindly provided by M. Eichelberger, Food and Drug Administration). Sera were heat inactivated, and serial 2-fold dilutions (with an initial dilution of 1:10) of sera triplets were incubated with virus and then added to 96-well microtiter plates coated with fetuin. Following incubation, peroxidase-labeled peanut agglutinin (the lectin) and, later, peroxidase substrate were added to detect enzymatic cleavage of fetuin by viral NA, and the reaction optical density was measured with a microplate reader. The percentage inhibition of NA enzymatic activity at each serum dilution was calculated by comparison with values from virus control wells (virus but no serum); end point NAI titers were calculated as the reciprocal of the highest dilution with at least 50% inhibition. As with HAI and MN titers, NAI titers below threshold and above test limits were denoted as half and twice those values, respectively.

Study Objectives and Statistical Analyses

Our objectives here were to (1) assess the usefulness of each assay in identifying serologic immune responses to vaccination and influenza virus infection, (2) examine postvaccination titers as measured in each assay as correlates of protection against influenza A (H3N2) infection, and (3) examine the correlation of values measured in each assay and the independent value of antibody to HA and to NA in preventing influenza A(H3N2) infection.

Serologic immune response to vaccination was defined as a postvaccination titer of 40 (32 for HAI), given a prevaccination titer of <10 (<8 for HAI), or as a ≥4-fold increase in titer between prevaccination and postvaccination sera, given a prevaccination titer of ≥10 (≥8 for HAI). Serologic immune response to infection was defined as a ≥4-fold increase in titer between postvaccination and postseason sera. For each assay and each intervention, the frequency distributions of subjects, by postvaccination titer, were plotted as histograms with the *x*-axis on a log scale. The proportions of cases at each titer were plotted and overlaid on the histograms. Because the overall ratio of cases to noncases was set by the strategy used to select subjects for testing, the proportion of influenza cases at each assay titer were higher than they would be if the entire study population was tested and included. However, because the selection of noncases for testing was essentially random, it was assumed that if the entire study population were tested, the proportion of influenza cases would be lower but would follow the same pattern, by titer.

Individual HAI, MN, and NAI antibody titers at each time point were transformed to binary logarithms; original values were divided by half the threshold value of detection (4, for HAI values; 5, for MN and NAI values) to set the starting point of the log scale to zero prior to transformation. Mean $\log_2$ titers were calculated and compared by case/noncase status for each intervention at each time point (before vaccination, after vaccination, and after the influenza season), using Wilcoxon rank sum tests. The correlation of $\log_2$ titers between each assay were assessed for each time point, by intervention, using Spearman rank correlation coefficients (ρ).

The independent effectiveness of HAI and NAI titers as correlates of protection against laboratory-confirmed influenza was estimated in logistic regression models with PCR-confirmed influenza A(H3N2) infection as the outcome and postvaccination HAI and NAI $\log_2$ titers as continuous predictors. To determine whether the effectiveness of HAI and NAI titers differed by intervention, IIV and LAIV dummy variables were included in the model as main effect terms and as interaction terms with postvaccination HAI and NAI $\log_2$ titers (4 total interaction terms). Protective effectiveness was calculated as [(1 − odds ratio) × 100] and were interpreted as the percentage change in the odds of influenza A(H3N2) infection associated with a 1 $\log_2$ increase in titer of 1 antibody assay (HAI or NAI), holding the titer of the other assay constant.

All statistical analyses were carried out using SAS software (release 9.2; SAS Institute); a *P* value of <.05 or a positive lower bound of a 95% confidence interval (CI) were considered to indicate statistical significance.

RESULTS

Antibody Responses to Vaccination and Infection

Results from examination of immune responses to vaccination and infection, by intervention and assay, are presented in Table 1. Among IIV recipients, response to vaccination was demonstrated in 77% and 67% of subjects, based on the HAI and MN assays, respectively, but was found in only 37% of subjects, based on the NAI assay. Among IIV recipients with an HAI response to vaccination, 41% also exhibited an NAI response. In contrast, among LAIV recipients, response to vaccination was detected in 21%, 17%, and 6% of subjects, based on the HAI, MN, and NAI assays, respectively. Among LAIV recipients with an HAI assay–confirmed response to vaccination, 15% also exhibited a response detected by the NAI assay. Serologic confirmation of PCR-confirmed influenza virus infection was frequently demonstrated among placebo recipients, based on all assays, with the HAI assay confirming 97% of infections. As demonstrated previously [13], serologic confirmation of infection by the HAI assay among IIV recipients was infrequent (18%); however, 27% of cases among IIV recipients were serologically confirmed by the MN assay and 41% were confirmed

Table 1. Immune Responses to Vaccination and Infection, by Intervention, as Measured by Hemagglutination Inhibition (HAI), Microneutralization (MN), and Neuraminidase Inhibition (NAI) Assays

Variable	Placebo Recipients, No. (%) (n = 92)	IIV Recipients, No. (%) (n = 178)	LAIV Recipients, No. (%) (n = 227)
Vaccine-associated immune response[a]			
HAI assay (n = 497)	2/92 (2.2)	137/178 (77.0)	48/227 (21.2)
MN assay (n = 171)	2/59 (3.4)	35/52 (67.3)	10/60 (16.7)
NAI assay (n = 497)	5/92 (5.4)	65/178 (36.5)	14/227 (6.2)
Infection-associated immune response among cases[b,c]			
HAI assay (n = 103)	28/29 (96.5)	4/22 (18.2)	40/52 (76.9)
MN assay (n = 81)	26/29 (89.7)	6/22 (27.3)	20/30 (66.7)
NAI assay (n = 103)	22/29 (75.9)	9/22 (40.9)	33/52 (63.5)

Abbreviations: IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine.

[a] Response was defined as a ≥4-fold rise in antibody titers measured by HAI, MN, or NAI assays between sera collected at the prevaccination visit and those collected at the postvaccination visit.

[b] Response was defined as a ≥4-fold rise in antibody titers measured by HAI, MN, or NAI assays between sera collected at the postvaccination visit and those collected at the postseason visit.

[c] Influenza A(H3N2) infections (ie, cases) were laboratory confirmed as influenza by virus identification in real-time polymerase chain reaction assays. Influenza A (H3N2) infections (ie, cases) were identified in 29 of 92 placebo, 22 of 178 IIV, and 52 of 227 LAIV recipients.

by the NAI assay. Serologic confirmation of infection in LAIV recipients was more frequently observed than in IIV recipients, but the frequency of confirmation among LAIV recipients was similar with each of the 3 assays.

Antibody Titers in Cases and Noncases

Mean $\log_2$ titers measured by each assay at each time point, by intervention group, for PCR-confirmed cases and noncases are presented in Figure 1*A*–*I*. Results from all 3 assays and for all 3 intervention groups indicated that both prevaccination and postvaccination values were lower and frequently statistically significant for subjects who ultimately became cases, compared with noncases. Large postvaccination mean titer increases were demonstrated for IIV recipients in both assays that targeted the viral HA (ie, the HAI and MN assays); in contrast, only modest increases in postvaccination values were noted for LAIV recipients for the same tests. Postvaccination titer increases measured in the NAI assay were lower than corresponding HAI titers for both vaccine interventions. In postseason specimens, the expected rise in mean titers, reflecting infection, was demonstrated in all 3 assays for cases who had received LAIV or placebo, with statistically significant differences in postseason values for cases and noncases noted. The NAI assay detected statistically significant differences in postseason values for IIV recipients who were infected despite vaccination, compared with noncases; however, as reported previously, no statistically significant differences were demonstrated by the assays targeting the viral HA [8, 13].

Postvaccination Titers and Protection

Figure 2*A*–*I* presents the numerical distributions of postvaccination titers, as determined by each assay and the percentage of influenza A(H3N2)–positive cases within each titer value, for each intervention group. Overall, the distributions of titer values were wide, based on the HAI and MN assays, but the distribution of titers detected by the NAI assay were lower and had a more limited range; titers were higher for recipients of IIV, relative to those for LAIV and placebo recipients. In recipients of placebo, except for 1 case in an individual with a titer of 2048, a relationship between increasing HAI titers and decreasing frequencies of influenza virus positivity was clearly seen; similar relationships were demonstrated with increasing values measured in the MN and NAI assays. Results for IIV and LAIV recipients indicated a declining frequency of influenza virus positivity with increasing values for all assays, but this finding was most clearly noted with the NAI assay; failure of vaccine was identified in those with higher titers based on the HAI and MN assays.

The association of postvaccination titers detected by HAI and NAI assays with influenza A(H3N2) infection status is visually depicted in Figure 3. Consistent with results presented in Figures 1 and 2, a higher proportion of those with low $\log_2$ HAI or NAI assay titers were cases; however, cases were still identified even among those with high $\log_2$ HAI and NAI assay titers.

Correlation and Independence of Antibody Titers to HA and NA

Figure 4 examines the correlation of $\log_2$ titers measured in the 3 assays at prevaccination, postvaccination, and postseason time points for each intervention. Significant positive correlations between titers measured in each assay were identified at each time point for each intervention group. HAI and MN assay values were highly correlated for each time point and intervention, with ρ ranging from 0.78 to 0.88. In contrast, findings from NAI assays were similarly correlated with findings

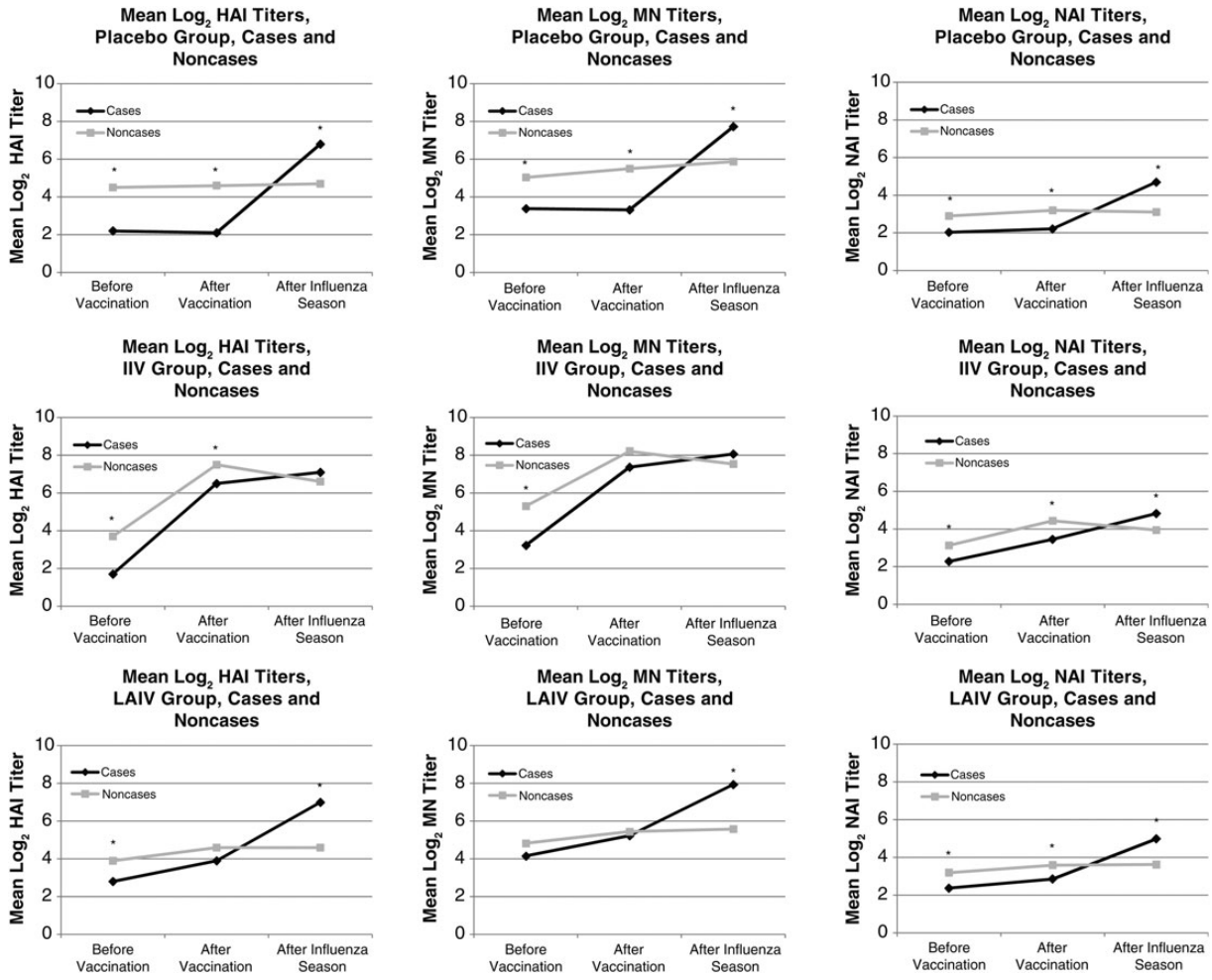

Figure 1. Mean $\log_2$ titers detected by hemagglutination inhibition (HAI), microneutralization (MN), and neuraminidase inhibition (NAI) assays among influenza A(H3N2)–infected cases and noncases at prevaccination, postvaccination, and postseason time points, by intervention group. *$P < .05$. Abbreviations: IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine.

from both HAI and MN assays, but these correlations were lower (ρ range, 0.31–0.61) than those between HAI and MN assay results, suggesting some degree of independence between values.

Table 2 presents results from examination of the independent efficacy of antibody titers, as measured in HAI and NAI assays, in preventing influenza A(H3N2) infection. Overall, a 1 $\log_2$ (2-fold) increase in HAI titer, holding the NAI assay titer constant, was associated with a 14% (95% CI, 5%–22%) decrease in the odds of infection. Similarly, a 2-fold increase in NAI titer, holding HAI assay titer constant, was associated with a 29% (95% CI, 16%–41%) decrease in the odds of infection. Examination of the interaction between interventions indicated that there were no statistically significant differences in the effect of a 2-fold increase in either HAI or NAI antibody titers among the placebo, IIV, or LAIV intervention groups.

DISCUSSION

Although it has been recognized for years that antibody to viral NA plays a role in the protection against influenza, little attention has been paid to the NA component in discussions of improved influenza vaccines. In fact, the viral NA has recently been termed "the forgotten antigen" [18]. Part of the problem has been the availability of little comparative data on the distinct role of antibody to NA in protection, given the major role of antibody to HA. In addition, the vaccine-induced antibody response to NA may be variable, owing to the lack of standardization of antigen content in current vaccines [19]. In the current study, a ≥4-fold rise in titer of antibody to NA was detected in only 37% of IIV recipients, compared with 77% as detected by the HAI assay. Because of the different nature of the NAI assay, some investigators have reported a ≥2-fold rise in titer as the standard for response [20]. However, when a ≥2-fold rise in

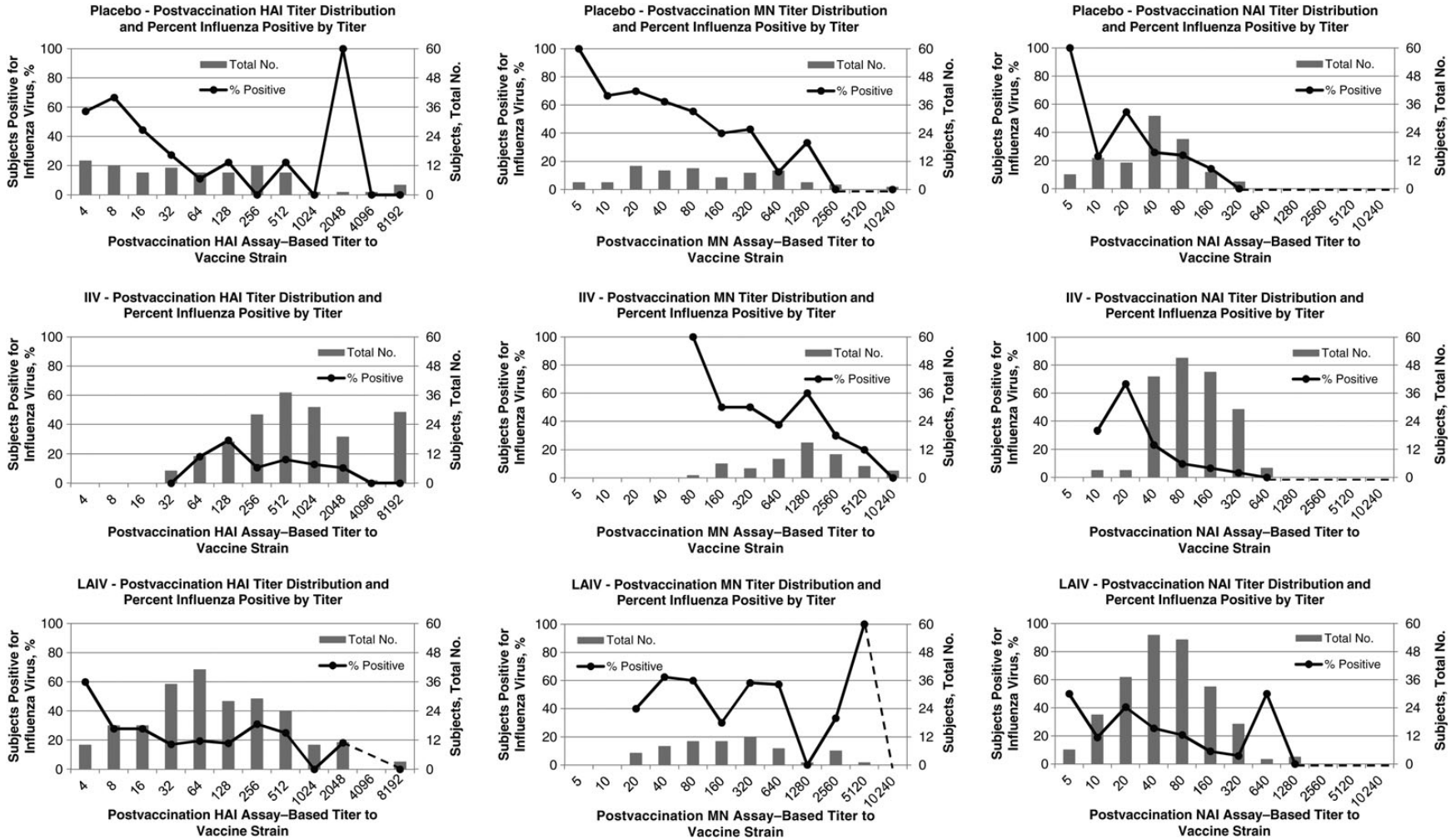

Figure 2. Postvaccination titers detected by hemagglutination inhibition (HAI), microneutralization (MN), and neuraminidase inhibition (NAI) assays and percentage of influenza A(H3N2) positivity, by titer, for subjects receiving placebo, inactivated influenza vaccine (IIV), or live-attenuated influenza vaccine (LAIV).

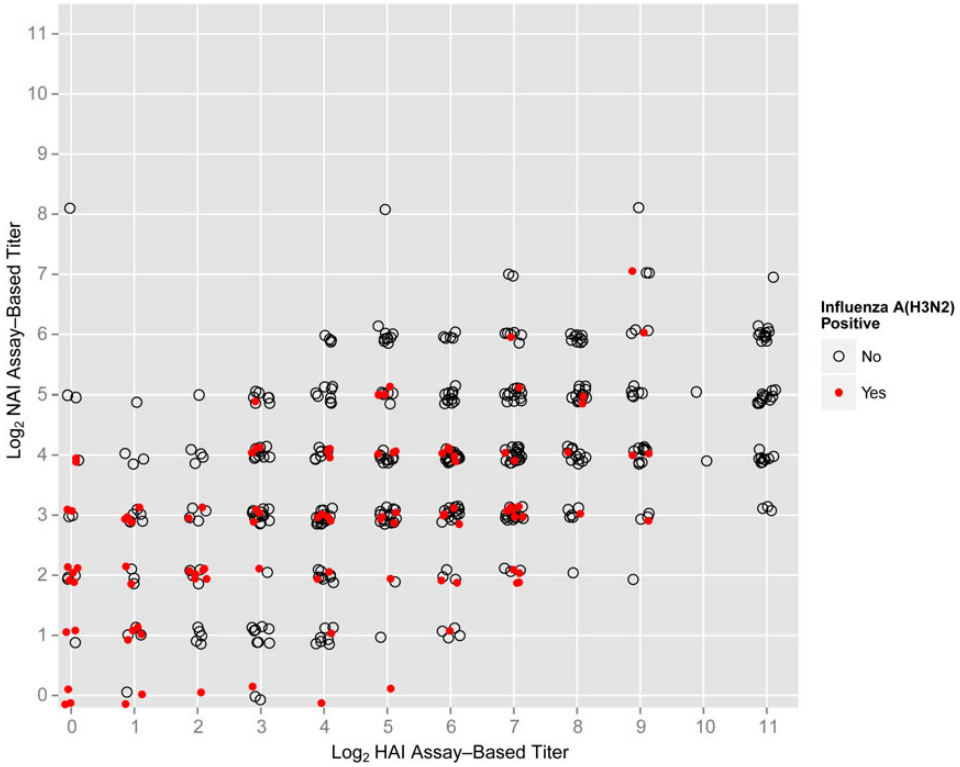

Figure 3. Scatterplot of postvaccination $\log_2$ titers detected by hemagglutination inhibition (HAI) and neuraminidase inhibition (NAI) assays, by influenza A(H3N2) infection status.

NAI titer was considered here, the proportion of placebo recipients exhibiting a vaccine immune response increased from 5% (a ≥4-fold rise) to 26%, indicating an unacceptable increase in detection of false responses. Somewhat surprising was the poor antibody response as measured by NAI in the LAIV recipients; even among those in whom a response was detected by the HAI assay, confirming that they were infected by the vaccine virus, only 15% had a rise in titer detected by the NAI assay.

The proportion of influenza A(H3N2) cases among IIV recipients declined not only with increasing HAI assay titer, but also with increasing titers measured by the MN and NAI assays. That would be expected with titers based on the MN assay because this assay is measuring antibody to viral HA. However, the relationship between high NAI assay titers and protection appeared stronger than with high HAI assay titers, with fewer failures at higher titers; this may be related to titers measured

	Before Vaccination			After Vaccination			After Influenza Season		
Placebo									
	HAI	MN	NAI	HAI	MN	NAI	HAI	MN	NAI
HAI	1.00	0.87	0.47	1.00	0.87	0.55	1.00	0.84	0.53
MN		1.00	0.41		1.00	0.51		1.00	0.56
NAI			1.00			1.00			1.00
Inactivated Influenza Vaccine									
	HAI	MN	NAI	HAI	MN	NAI	HAI	MN	NAI
HAI	1.00	0.87	0.40	1.00	0.78	0.37	1.00	0.78	0.31
MN		1.00	0.46		1.00	0.38		1.00	0.40
NAI			1.00			1.00			1.00
Live Attenuated Influenza Vaccine									
	HAI	MN	NAI	HAI	MN	NAI	HAI	MN	NAI
HAI	1.00	0.81	0.42	1.00	0.88	0.37	1.00	0.87	0.45
MN		1.00	0.61		1.00	0.52		1.00	0.41
NAI			1.00			1.00			1.00

Correlation
>0.75
0.50–0.75
<0.50

Figure 4. Spearman rank correlation coefficients for $\log_2$ antibody titers, by intervention group, measured by hemagglutination inhibition (HAI), microneutralization (MN), and neuraminidase inhibition (NAI) assays at prevaccination, postvaccination, and postseason time points.

Table 2. Independent Effectiveness of Antibody Titers, as Measured in Hemagglutinin Inhibition (HAI) and Neuraminidase Inhibition (NAI) Assays, in Preventing Influenza A(H3N2) Infection

Assay	Placebo Group[a] Effectiveness, % (95% CI)	IIV Group[a] Effectiveness, % (95% CI)	LAIV Group[a] Effectiveness, % (95% CI)	Overall[b] Effectiveness, % (95% CI)
HAI	26 (8–41)	11 (−14 to 31)	6 (−8 to 19)	14 (5–22)
NAI	24 (−12 to 48)	48 (19–67)	24 (5–39)	29 (16–41)

Effectiveness was calculated as [(1 − odds ratio)×100] and denotes the percentage reduction in the odds of influenza virus infection associated with a 1-$\log_2$ (2-fold) increase in titer measured by the HAI (or NAI) assay.

Abbreviations: CI, confidence interval; IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine.

[a] The logistic regression model included the following covariates: $\log_2$ HAI titer (continuous), $\log_2$ NAI titer (continuous), IIV receipt (0, 1), LAIV receipt (0, 1), interaction between $\log_2$ HAI titer and IIV, interaction between $\log_2$ HAI titer and LAIV receipt, interaction between $\log_2$ NAI titer and IIV receipt, and interaction between $\log_2$ NAI titer and LAIV. All interaction terms were nonsignificant ($P > .05$), indicating that the effect of a 1-$\log_2$ (2-fold) increase in titer measured by the HAI or NAI assay did not significantly differ by intervention.

[b] The logistic regression model included the covariates $\log_2$ HAI titer (continuous) and $\log_2$ NAI titer (continuous).

by the HAI assay being more widely distributed than those obtained by the NAI assay. Those who were ultimately infected (cases) had lower prevaccination titers measured by all 3 assays than those who did not become infected (noncases), indicating the critical role of prior exposures, probably to natural infection, in protection.

A major question has been the contribution of antibodies to viral NA to actual protection against influenza in humans. Studies, particularly those in animals, have suggested that antibodies to NA do not protect against asymptomatic infection but lower the risk of clinical disease [21]. Since only symptomatic illnesses were identified in this study, such a distinction, if present, could not be made. However, several lines of evidence presented here suggest a contribution to protection that is independent of antibody to viral HA. One is the correlation coefficients, which were high for titers measured in the HAI and MN assays but lower for titers measured by the NAI assay. Since this analysis involved sera collected at 3 time points, the correlation coefficients, while suggesting independence, cannot be taken as related directly to protection. The same can be said for the finding that, among cases who had received IIV, a rise in titer indicating an infection-based immune response was more likely to be detected by the NAI assay than by the HAI assay. The strongest evidence of an independent contribution, in terms of protection, comes from the regression analysis. Here it appeared that a 1-unit (2-fold) increase in NAI assay titer was associated with a greater decrease in the odds of infection than a similar increase in HAI assay titer. However, the difference in magnitude might be related to the more constrained scale of NAI assay titers, compared with HAI assay titers. It was of interest that this observation was consistent in recipients of both vaccines, as well as in placebo recipients, indicating a similar role of both vaccine-induced antibodies and those coming from past infections. Protection by infection-induced antibodies to NA has recently been demonstrated in a study of influenza A(H1N1)pdm09 outcomes [22].

The question, then, is whether more attention should be paid to producing a response to NA by vaccination. Given the increasing evidence that antibodies to NA contribute to protection, should consideration be given to developing influenza vaccines that regularly produce such antibodies? [20]. Not only might protection be increased in years when the HA of the vaccine strains are well matched to those of the circulating viruses, but there would be the possibility of some protection in years with HA drift but little NA drift. Attention has begun to be directed toward how this can be done, in terms of both the amount and stability of the NA component in the vaccine [23]. As the move toward improved influenza vaccines continues, it is now clear that it would be a mistake to continue to forget NA.

Notes

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