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Daniel Gromer
Name

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Title The Relationship Between the Immunogenicity and Reactogenicity of a Seasonal Influenza Vaccine Delivered by Microneedle Patch or Hypodermic Needle

Author Daniel Gromer

Degree Master of Science

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Approved by the Committee

DocuSigned by:
Jordan Kempker
39DC6753655C4B8...

Jordan Kempker
Advisor

DocuSigned by:
Amita Manatunga
AB1827790028472...

Amita Manatunga
Advisor

DocuSigned by:
DBB19F748947457...

Nadine Rouphael
Committee Member

DocuSigned by:
Daniel Graciaa
4F20987BEE764A2...

Daniel Graciaa
Committee Member

Committee Member

Committee Member

Accepted by the Laney Graduate School:

_____

Kimberly Jacob Arriola, Ph.D, MPH
Dean, James T. Laney Graduate School

_____

Date

The Relationship Between the Immunogenicity and Reactogenicity of a Seasonal Influenza Vaccine Delivered by Microneedle Patch or Hypodermic Needle

By

Daniel Gromer

B.A., University of Pennsylvania, 2012

M.D., University of Pennsylvania, 2018

Advisor: Nadine Rouphael, M.D., M.Sc.

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Abstract

The Relationship Between the Immunogenicity and Reactogenicity of a Seasonal Influenza Vaccine Delivered by Microneedle Patch or Hypodermic Needle

By Daniel Gromer

Background: Vaccine immunogenicity and reactogenicity each depend on recipient and vaccine characteristics. An association between these two vaccine outcomes would have major implications for clinical care, public health, and vaccine development. We hypothesized that healthy adults who reported higher reactogenicity from seasonal inactivated influenza vaccine (IIV) developed higher antibody titers compared with those who reported lower reactogenicity.

Methods: We performed a secondary analysis of the TIV-MNP 2015 study, a randomized phase 1 clinical trial comparing the immunogenicity and reactogenicity of a trivalent IIV delivered by microneedle patch (MNP) or intramuscular injection (IM). We created composite scores of solicited adverse events (Global, Systemic, and Local) as the exposure and hemagglutination inhibition (HAI) antibody titers against the H1N1, H3N2, and B antigens in the vaccine as the outcome. To account for longitudinal outcome data, we used mixed model analysis of variance to estimate geometric mean titers (GMTs), GMT ratios (GMRs), and titer fold change ratios (FCRs) and modified Poisson generalized estimating equations to estimate risk ratios (RRs) of HAI seroprotection and seroconversion. We adjusted for several confounders and generated estimates separately by vaccine delivery method.

Results: The IM (n=25) and MNP (n=50) groups were balanced in baseline characteristics. Longitudinal estimates of H3N2 HAI GMTs were associated with the Systemic and Local scores among the IM group. Within the IM group, those with high reaction scores had lower baseline H3N2 HAI GMTs (Global GMR 0.5, p=0.06; Systemic GMR 0.4, p=0.01; Local GMR 0.3, p=0.01) and twice the titer fold change by day 28 (Global FCR 2.0, p=0.04; Systemic FCR 1.9, p=0.07; Local FCR 2.0, p=0.15) compared with those with low reaction scores. Those with high Local scores had a higher risk of HAI seroconversion (RR 1.4, p=0.03).

Conclusion: These results suggest that heightened reactogenicity to intramuscular inactivated influenza vaccine is related to low baseline humoral immunity to an included antigen. Participants with greater reactogenicity developed greater antibody titer fold change after 4 weeks, though the overall magnitude of response was similar or lower compared with low-reactogenicity participants.

The Relationship Between the Immunogenicity and Reactogenicity of a Seasonal Influenza Vaccine Delivered by Microneedle Patch or Hypodermic Needle

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Table of Contents

Introduction

Vaccines are an effective public health measure for preventing and mitigating infectious diseases. A vaccine's immunity-boosting capacity, termed immunogenicity, and near-term adverse event profile, termed reactogenicity, depend on recipient characteristics, such as age,[1–4] assigned sex,[1,5,6] and potentially body mass index (BMI),[1,7–12] as well as prior immunization[13,14] and the platform, route, and dose of vaccine.[2,4,15–18] Limited information exists regarding whether vaccine reactogenicity and immunogenicity are related to each other.

There are several reasons to pursue this question. One reason is to determine whether post-vaccine symptoms could serve as a clinical indicator of population immunity in the early stages of a pandemic emergency. Knowing that a specific reaction or cadre of reactions implies that a vaccine has been effective in generating adequate immunity could allow public health officials to allocate a scarce vaccine better among a large group of people. More likely, learning about the balance between immunogenicity and reactogenicity will positively impact patient-provider communication by helping clinicians communicate with their patients. Over recent decades, numerous studies have linked low vaccine confidence to patient and parent fears about vaccine adverse events and higher vaccine confidence to the presence of a strong patient-provider relationship and trust in the medical and public health establishments.[19–26] Defining the association between immunogenicity and reactogenicity may help clinicians to counsel patients about the meaning behind post-vaccine adverse events and build the trust needed to inspire vaccine confidence.

Beyond affecting how clinicians communicate about what adverse events mean, investigating the immunogenicity-reactogenicity relationship can also provide them and their patients with guidance on how to manage adverse events. When discussing post-vaccine symptoms, a frequent and logical question is whether taking antipyretic analgesics to suppress vaccine reactions affects vaccine-derived immunity. Because fever after vaccination may herald increased immunity,[27] the concern is that taking antipyretics around the time of vaccination may blunt a necessary inflammatory response and dampen immunogenicity. Some evidence in children suggests that antipyretics taken at the time of vaccination may negatively impact immunogenicity,[28] but minimal investigation has been done in adults, who can provide more detailed reactogenicity data. Determining whether immunogenicity and reactogenicity are linked in analyses of adult participant data can serve as an appropriate starting point for future prospective studies of vaccines and medications such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs). Ultimately, this line of study can enable medical providers and public health institutions to accurately coach people about the risks and benefits of taking medications to make tolerating vaccines easier.

From a biomedical research perspective, understanding the immunogenicity-reactogenicity relationship also has important implications for the future of vaccine design. Because vaccine and recipient characteristics impact vaccine responses, increasing the granularity of our knowledge about how variables such as sex, hormonal milieu, vaccine dose, and inclusion of adjuvants alter these responses may bring a new age of personalized vaccinology.

Though questions about the association between immunogenicity and reactogenicity had been asked infrequently prior to 2020, the pace of the implementation of SARS-CoV-2 vaccines to address the COVID-19 pandemic reinvigorated inquiry about the immunogenicity-reactogenicity relationship in the scientific literature and the lay press.[29–31] Several publications featuring large samples, advanced biostatistical methods, and different SARS-CoV-2 vaccines subsequently found positive associations between reactogenicity and immunogenicity, particularly after multiple doses of mRNA vaccines or after participants with prior infection were exposed to vaccine antigen.[32–39] Specific post-vaccination reactions (e.g., fever) and composite scores of reactions were both associated with increased immunity.[34–37,40–48] While the estimation of this relationship for SARS-CoV-2 vaccines represents an exciting step, it is unclear if vaccines against other pathogens, such as influenza, carry the same immunogenicity-reactogenicity association.

Strains of influenza virus mutate perpetually, contributing to their ongoing evasion of human immunity through antigenic drift.[49–51] As a result, the seasonal inactivated influenza vaccine (IIV) is manufactured yearly, anticipating antigenic drift, and is universally recommended in the United States (US) for individuals aged 6 months and older.[4,52–54] To address the variety of distinct influenza viruses that circulate in each season, the seasonal IIV delivers multiple antigens designed to stimulate short-lived immunity against predicted strains. During the 2010s, seasonal IIVs approved in the US were either trivalent or quadrivalent, containing 1 influenza A/H1N1 antigen, 1 influenza A/H3N2 antigen, and 1 or 2 influenza B antigens.

The antigens contained within seasonal IIVs and the strains they are intended to generate immunity against differ regarding their effects on and importance to human health. An A/H1N1 influenza strain caused the first influenza pandemic of the 21st century in 2009.[55–57] Between 2010 and 2015, the seasonal IIV included a stable H1N1 antigen similar to the 2009 pandemic strain.[58,59] As of the 2014–2015 influenza season, A/H1N1 influenza made up a minority of circulating virus and contributed to a minority of severe cases of disease.[58,59]

In contrast, influenza A/H3N2 is usually dominant or co-dominant as the circulating virus and leads to most adult hospitalizations and deaths.[59–61] The H3N2 antigen included in the seasonal IIV changes on a yearly basis and mismatches can occur between the circulating virus and the predicted antigen contained within the vaccine. This was the case in the 2014–2015 influenza season, when the vaccine's H3N2 antigen did not closely match the dominant circulating H3N2 virus.[59,60]

Influenza B presents a different prediction problem than influenza A/H3N2. Since the 1980s, there have been 2 distinct co-circulating lineages of influenza B, which undergoes antigenic drift more slowly than influenza A.[62,63] Efforts to predict which of the 2 lineages will dominate in a specific influenza season have largely failed, with trivalent IIVs in the US containing the dominant antigen less than 50% of the time between 2000 and early 2011.[63] Though influenza B causes fewer hospitalizations and deaths in adults, it is particularly notable for causing disease in children.[64] To mitigate the negative impact of frequent vaccine-strain mismatches, many trivalent influenza vaccines (TIVs) were transitioned to quadrivalent influenza vaccines (QIVs) by including 2 influenza B antigens during the 2010s and early 2020s.[52,53,63,65,66]

One major challenge in estimating the immunogenicity-reactogenicity relationship of influenza vaccines comes in the form of confounding by human variation. Even in a clinical trial without variation in the platform, route, and dose of the vaccine of interest, heterogeneity in recipient characteristics may impact the estimation of the effect of reactogenicity on immunogenicity. Among multiple SARS-CoV-2 vaccines, younger age, female sex, and prior COVID-19 have all been associated with increases in both immunogenicity and reactogenicity, while the effect of BMI has yet to be shown consistently.[33–35,37–39,67,68] Age[1–4] and assigned sex[1,5,6] affect seasonal IIV responses similarly, though prior antigen exposure via vaccination may be associated with decreased immunogenicity due to imprinting.[13,14] The available data are inconsistent on the topic of the effect of BMI on influenza vaccine responses, perhaps in part due to differences in how BMI is categorized and expressed in various modeling approaches.[1,7–12] Though differential vaccine effects are widely recognized and have been for decades, the implications of this heterogeneity in outcomes remains understudied and the approach to vaccination of large populations often follows a one-size-fits-all model. Seasonal influenza is currently the only pathogen for which we have dedicated vaccines for older (≥ 65) and younger (< 65) adult populations,[53,69–71] and there is currently no tailored vaccine approach to address differences in sex, BMI, vaccination history, or any other variable. Thus, models investigating the association between reactogenicity and immunogenicity must adjust for multiple confounding variables to achieve accurate estimation.

Heterogeneity in the platform, route, and dose of influenza vaccines represents a second major challenge in estimating the immunogenicity-reactogenicity relationship. The number and dose of

antigen, as well as the presence of an adjuvant, vary among licensed seasonal IIVs, precluding simple comparisons between trial participants who have received vaccines from different manufacturers. One way to circumvent this latter issue may be to analyze trials of the same vaccine delivered across distinct routes.

The seasonal IIV is generally offered, as with most vaccines worldwide, as an intramuscular (IM) injection via hypodermic needle.[16,72] While manufacturing, regulatory, and distribution systems have matured around the constraints of this convention, global dominance of this method of vaccine delivery presents several challenges. IM vaccines require an intact cold chain, large amounts of storage space, and trained healthcare workers to administer them. Needle use results in frequent adverse safety events and needle phobia.[73] Non-single-use vials of vaccine result in wasted doses. Besides these significant logistical and acceptability issues, IM vaccines also suffer from immunologic drawbacks.[15,74,75] The intramuscular compartment contains low numbers of antigen-presenting cells (APCs) as compared to mucosal sites and the skin. Exposing these alternative sites to vaccine antigens has repeatedly resulted in improved immunity against numerous infections.[16,74,76]

As a result, there is growing enthusiasm for novel methods of achieving mucosal or intradermal vaccination. One such method is the microneedle patch (MNP), an array of sub-millimeter needles attached to a patch backing that delivers antigens to the intradermal space.[15,16] Along with the logistical benefits that MNPs promise, such as low-cost manufacturing, thermostability, easy transportation, lack of waste, increased safety, and possibly the convenience of self-administration,[54,77–80] several preclinical and clinical studies of MNP vaccines have shown equal

or enhanced immunogenicity over traditional IM vaccination.[74,81] MNPs have also demonstrated a distinct adverse event profile from their IM counterparts, causing a higher frequency of redness, swelling, and itching where the patches were applied.[54,82–84]

Therefore, to assess the relationship between reactogenicity and immunogenicity with a seasonal influenza vaccine, we performed a secondary analysis of TIV-MNP 2015, the completed first-in-human phase 1 clinical trial of a seasonal IIV delivered by MNP, which was compared with IM injection of the same IIV.[54] Our aim was to estimate the effect of reactogenicity on immunogenicity for each delivery method, adjusting for measured confounding variables. We hypothesized that healthy, non-pregnant adults who reported higher reactogenicity from IIV developed markers of increased immunity compared with those who reported lower reactogenicity.

Methods

Parent Study Design

We used primary data obtained in the TIV-MNP 2015 trial,[54] a partly blinded, randomized, placebo-controlled, phase 1 study, described in brief below.

Setting and Participants

The TIV-MNP 2015 study included 100 healthy, non-pregnant, immunocompetent adults aged 18–49 years, who were recruited by the Hope Clinic of the Emory Vaccine Center in Atlanta, Georgia in the summer of 2015. Key exclusion criteria included influenza infection or vaccination during the 2014–2015 season, BMI > 35 kg/m^2, recent blood donation, vaccination, or experimental product receipt, various acute and chronic medical or psychiatric conditions, and receipt of specified immunosuppressive or immunomodulatory medications. Please see clinicaltrials.gov, NCT02438423, for further details.

Randomization and Procedures

Participants were randomized in a 1:1:1:1 fashion to receive either IIV via microneedle patch ($MNP_{IIV\text{-}HCW}$), IIV via intramuscular injection (IM_{IIV}), or placebo via microneedle patch ($MNP_{placebo}$), all administered by a healthcare worker, or IIV via microneedle patch self-administered by the study participant ($MNP_{IIV\text{-}self}$) under healthcare worker supervision. The IIV was composed of 15μg of each of the following influenza vaccine strains: A/Christchurch/16/2010, NIB-74 (H1N1), A/Texas/50/2012, NYMC X-223 (H3N2), B/Massachusetts/2/2012, NYMC BX-51 (B). Participants were followed for 180 days. Solicited

local and systemic adverse events were assessed daily for 8 days after study product administration, by questionnaire initially and clinic visit if necessary. Adverse events were graded by severity (0 representing no event and 4 representing life-threatening event) based on the Food and Drug Administration toxicity grading schema.[85] Blood samples were drawn for immunogenicity testing on days 0, 28, and 180. We excluded all participants in the $MNP_{placebo}$ group, as the association of interest was specific to those receiving IIV. Two additional participants did not provide blood samples, and therefore did not have any immunogenicity measurements available. We treated these values as missing at random and excluded the outcome data from statistical comparisons. There was no loss to follow up.

Variables

Reactogenicity

We chose measurements of reactogenicity derived from solicited adverse events as the exposure variables. Reactogenicity was divided into local and systemic adverse events. Local adverse events included swelling (induration), pain, redness (erythema), itching (pruritus), and tenderness. Systemic adverse events included fatigue, joint pain (arthralgia), body ache (myalgia), fever, shivering or shaking body movements, malaise, nausea, sweating, and headache.

Primary Measures of Reactogenicity

The number of unique solicited adverse events recorded by each participant at any point during the 8-day reporting period was summed to make continuous variables. We generated separate sums for local events, systemic events, and all events, and termed these Local, Systemic, and

Global reaction scores. We examined the distributions of these scores using descriptive statistics and univariable and bivariable plots, visually determined cut points to separate high and low levels of each score, and generated dichotomous categorical variables as our primary reactogenicity measures.

Secondary Measures of Reactogenicity

We grouped participants by the severity of adverse events. Any participant recording an event of grade 2 or greater at any point during the study was included in the high severity group, and all others were included in the low severity group.

We also grouped participants by the duration of adverse events. Any participant with an event beginning on day 0 or day 1 and lasting greater than 2 continuous days (i.e., still present on day 2 or day 3, respectively), was included in the prolonged duration group, and all others were included in the short duration group.

Immunogenicity

We chose measurements of immunogenicity as the outcome variables. Immunogenicity was measured by hemagglutination inhibition (HAI) antibody titer. HAI measurement was performed by blinded Hope Clinic Laboratory staff using previously described methods.[86]

Primary Measure of Immunogenicity

We chose HAI antibody titers as the primary measure of immunogenicity. HAI titers were inverted and represented as continuous numerical data. They were log-transformed for regression

analyses and then back-transformed to generate geometric mean titers (GMTs) and the ratios between GMTs (geometric mean titer ratios, or GMRs).

Secondary Measures of Immunogenicity

We modeled HAI titer fold change as a measure of immunogenicity. We calculated HAI titer fold change by subtracting baseline log-transformed HAI from post-vaccine (day 28 or day 180) log-transformed HAI for each participant.

We also chose HAI seroprotection and HAI seroconversion as dichotomous categorical measures of immunogenicity. Seroprotection is defined as an HAI titer ≥ 1:40. Seroconversion is defined as a post-vaccination measurement with a minimum 4-fold increase in HAI titer (if the baseline titer is ≥ 1:10) or an HAI titer ≥ 1:40 (if the baseline titer is < 1:10).

Covariates

Other measured covariates, recorded at enrollment, included BMI, sex, race, ethnicity, and IIV receipt in the prior two influenza seasons. All covariates were recorded by participant questionnaire except for BMI, which was measured with standard equipment in the clinic. All were represented as categorical variables. BMI was categorized as ≤ 25, 25 to < 30, and ≥ 30.

Sensitivity Analysis

To assess the robustness of findings generated with our primary measures of reactogenicity, we repeated our analyses using multiple different cut points for dichotomizing reaction scores. Additionally, we subsetted the participants using the 15 highest and 15 lowest values of each

reaction score and performed comparisons between these groups of participants with extreme values of each reaction score. For example, we compared those with the 15 highest Global scores to those with the 15 lowest Global scores.

Statistical Analysis

We constructed a directed acyclic graph (DAG, Figure 1) to express our conceptual model of the effect of reactogenicity on immunogenicity and determine which variables to adjust for in multivariable modeling. To further characterize potential confounders and effect modifiers, we performed bivariable comparisons between selected variables and both the exposure and outcome measures. Covariates with statistically significant associations with both the exposure and outcome at the 95% confidence level were marked as potential confounders for adjustment in the multivariable analyses. Additionally, we used multiplicative terms to assess for interactions to include in multivariable models.

For the primary analyses, we used mixed model analysis of variance (ANOVA) to estimate GMTs and GMRs, using the exposure variable as the between-participant factor, time as the within-participant factor, and a compound symmetric covariance structure. We included vaccine delivery method (IM or MNP) in interaction terms with these factors, including a three-way interaction term, to generate separate estimates for participants receiving IM or MNP IIV on each study day. We used analogous methods for models estimating HAI titer fold change, which were expressed as ratios between groups (fold change ratios, or FCRs). For analyses with HAI seroprotection and seroconversion outcomes, we used modified Poisson regression[87,88] and generalized estimating equations (GEEs)[89,90] to generate risk ratios (RRs).

All statistical testing was performed in SAS version 9.4 (Cary, NC, USA). We used the MIXED procedure to generate estimates with mixed model ANOVAs and the GENMOD procedure to generate estimates with modified Poisson regression and GEEs to account for repeated outcome measures. Data visualization was performed with R v4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The initial enrolled sample included 100 participants. The original trial flow diagram is displayed in Figure 2. After the 25 participants in the $MNP_{placebo}$ group were excluded, we excluded the outcome data of 2 additional participants who did not submit blood samples for analysis. We combined the $MNP_{IIV\text{-}HCW}$ and $MNP_{IIV\text{-}self}$ groups into a single MNP group to compare with the IM group. The two groups were balanced in baseline characteristics (Table 1). The median H1N1 HAI antibody titer was 80 and the median H3N2 and B HAI titers were 40.

Figure 3 displays the fraction of participants who received either IM or MNP IIV and had each type of reaction. The IM group reported more systemic events of every type except fever, which was only reported by a single participant, who was in the MNP group. The MNP group reported more local pruritus, redness, and swelling events, and the IM group reported more local pain events. Redness and swelling peaked within 24 hours of vaccination, resolving within 48 hours for the IM group and over 1–2 weeks for the MNP group (Figure 4).

Figure 5 displays the correlation matrix between all types of adverse events. Most Pearson correlation coefficients were close to 0 and there was no discernible hierarchical clustering pattern between adverse events.

Based on the distributions of the Global, Systemic, and Local reaction scores generated for all participants, we visually dichotomized the scores into high and low levels (Figures 6 and 7).

Those with a Global reaction score ≥ 4, a Systemic reaction score ≥ 3, or a Local reaction score ≥ 3 were assigned the high level for the corresponding score.

To screen for significant associations between reactogenicity and immunogenicity, we generated unadjusted and adjusted longitudinal GMR estimates and hypothesis tests for each vaccine antigen comparing participants in the IM or MNP groups with high and low Global, Systemic, and Local reaction scores (Table 2). We found no significant associations using the Global score but noted a consistent association among the IM group between antibody titers against the H3N2 antigen and both the Systemic and Local scores. We also noted associations between the Local score and the H1N1 and H3N2 HAI titers in the MNP group. These associations were not robust to changes in the dichotomization cut points of the reaction scores (Table 3A-C). In this sensitivity analysis, we also noted associations among the IM group between the B antigen and both the Global and Systemic scores with the lowest cut points, but these were not statistically significant after adjustment (Table 3A-B).

When we used interaction terms to generate time-specific estimates (Table 4A-C), we found that the previously noted associations among the MNP group were not driven by any significant difference on any specific day. In contrast, the IM group associations were driven by baseline differences in H3N2 HAI titer (reproduced from Table 4B in Table 5). Among participants in the IM group, adjusting for BMI, sex, race, ethnicity, and prior IIV, those with a high Systemic reaction score had 0.4 (95% CI 0.2, 0.8) times the day 0 H3N2 HAI GMT compared with those who had a low Systemic score. Similarly, those with a high Local score had 0.3 (95% CI 0.1, 0.8) times the day 0 H3N2 HAI GMT compared with those who had a low Local score. Those in

the IM group with a high Systemic score developed similar H3N2 GMTs on day 28 compared with those who had a low Systemic score. In contrast, those with a high Local score appeared to develop lower H3N2 GMTs on day 28 compared with those who had a low Local score, though this did not reach statistical significance. Finally, those with high Systemic and Local scores appeared to demonstrate lower H3N2 GMTs on day 180 compared with their low-score counterparts, though these findings also did not reach statistical significance (Table 5, Figure 8).

We generated analogous unadjusted and adjusted estimates for the secondary outcome of HAI titer fold change (Table 6A-C). Among the IM group, prior to adjustment for confounding variables, those with any type of high reaction score had more than 2 times the day 28 H3N2 HAI titer fold change compared with their low-score counterparts. This finding remained similar after adjustment and was statistically significant when using the Global reaction score (Table 6B). Though it was not indicated by the longitudinal GMR estimates, we found a similar pattern among the MNP group using the Global reaction score and the B antigen, where a high score was associated with lower day 0 HAI titer (Table 4C) and greater day 28 HAI titer fold change (Table 6C).

As a sensitivity analysis, we selected participants with the 15 highest and 15 lowest values of each reaction score and performed unadjusted comparisons between these smaller groups of participants with extreme values. Among the IM group, those with the highest Local scores had lower H3N2 HAI GMTs compared to those with the lowest Local scores (Table 7), and those with the highest Systemic scores had a higher H3N2 HAI titer fold change on day 28 compared to those with the lowest Systemic scores (Table 8). Additionally, those in the IM group with the

highest Local reaction scores had higher H1N1 GMTs and a lower H1N1 HAI titer fold change on day 28 compared to those with the lowest Local scores, but this association was not found in the primary analyses.

We then grouped participants by either reaction severity or reaction duration, agnostic of reaction type, and estimated the relationship between these secondary reactogenicity measures and either HAI GMT or HAI titer fold change. We found no association between moderate or greater reaction severity and either longitudinal HAI GMT (Table 9) or HAI titer fold change (Table 10). We also found no association between a "prolonged" reaction duration greater than 48 continuous hours and either longitudinal HAI GMT (Table 11) or HAI titer fold change (Table 12).

Using modified Poisson GEEs, we then generated unadjusted and adjusted longitudinal estimates for the secondary outcome of HAI seroprotection (Table 13). There were no significant associations between any reaction score and HAI seroprotection, and models generating time-specific estimates frequently failed to converge (data not shown) due to the high proportion of participants with HAI seroprotection, including at baseline (Table 14). There were also no significant associations between any reaction score and HAI seroprotection after changing the dichotomization cut points of the scores (Table 15A-C).

Similarly, we generated unadjusted and adjusted longitudinal estimates for the secondary outcome of HAI seroconversion (Table 16), noting only an association between the Systemic reaction score and H3N2 HAI seroconversion in the MNP group after adjustment for

confounding variables. When we generated adjusted time-specific estimates (Table 17A-C), we found that those with a high Local score had 1.4 (95% CI 1.0, 1.9) times the risk of day 28 H3N2 HAI seroconversion compared to those with a low Local score among the IM group (Table 17B). Those with a high Systemic score had 2.9 (95% CI 1.4, 6.0) times the risk of day 180 H3N2 HAI seroconversion compared to those with a low Systemic score. These findings were not robust to changes in the dichotomization cut points of the reaction scores (Table 18A-C, Table 19A-C).

Discussion

In this secondary analysis of a phase 1 clinical trial comparing the same seasonal IIV when delivered by IM injection or MNP, we found evidence of a relationship between reactogenicity and immunogenicity in the IM group. Our results suggest that heightened reactogenicity to IM IIV is related to low baseline HAI antibody titers to included antigens, in this case, H3N2. Participants with greater reactogenicity developed greater H3N2 HAI titer fold change after 4 weeks, though the overall magnitude of HAI response was similar or lower compared with low-reactogenicity participants. For those with relatively greater local reactogenicity, who appear to have had the lowest baseline H3N2 HAI titers, the greater titer fold change was associated with a greater probability of seroconversion.

These findings appear to conflict with the SARS-CoV-2 vaccine literature, in which participants without prior infection reported greater reactogenicity and developed most of their immunity from the second (boost) dose of a prime-boost vaccine series. As a result, we had hypothesized that those with greater reactogenicity would have greater resulting immunity. Our results instead show that those with greater reactogenicity had lower baseline markers of humoral immunity. The antibody response of these participants in the short term was higher in the relative sense and similar or lower in the absolute sense than their low-reactogenicity counterparts. The reasons behind these differences between our expectations and our findings are not clear, though there are multiple plausible explanations.

Regarding why those with lower baseline HAI titers might have greater reactogenicity, one possibility is that those with greater preexisting immunity do not undergo the same inflammatory cascade as those with less preexisting immunity, perhaps due to antibody-mediated precipitation and destruction of injected antigen without resulting presentation by APCs in the draining lymph node. This could also explain why the difference was only noted in our study in the IM group, though the MNP group was larger and had more statistical power – the muscle has a sparse distribution of APCs and lymphatic vessels compared to the dermis. Instead of rapid delivery from the skin to the draining lymph node, either by an APC or free within lymphatic vessels, antigen may move more slowly to the draining lymph node from the muscle, increasing the chances that it is bound along the way by IgG, the primary immunoglobulin isotype found in the extracellular space within body tissues.[91–93]

A complicating factor in this analysis that might explain why participants had differing levels of baseline H3N2 HAI titers is that we are unable to accurately discern each participant's personal history of antigen exposure. We do not have granular data about trial participants' seasonal IIV and influenza infection histories, including what antigens and strains they have been exposed to, how many times, in what order, and how recently. All these pieces of information may affect a person's baseline HAI titer to specific antigens, such as the A/Texas/50/2012, NYMC X-223 used as the H3N2 component in the TIV-MNP 2015 study. We adjusted for receipt of influenza vaccine within the prior 2 years, as recent vaccination may affect responses to a new influenza vaccine via imprinting, but we were unable to adjust for most influenza antigen exposure in the participants' lifetimes. The mismatch of H3N2 vaccine antigen and influenza strain from the preceding influenza season may also have been important here, as having had undiagnosed

influenza A/H3N2 during the prior winter would not have primed participants perfectly for the H3N2 antigen they were exposed to in the trial.

This may also help to explain why these models only generated consistent and significant findings with the H3N2 HAI titer outcome. The H1N1 antigen used in influenza vaccines over the previous 5 years, including in a standalone pandemic influenza vaccine, had not changed. Individuals exposed to pandemic A/H1N1 influenza in 2009, vaccinated against this strain specifically during the pandemic emergency, or vaccinated against seasonal influenza between 2010 and 2014 were effectively primed with the same H1N1 antigen used in the TIV-MNP 2015 trial. This is supported by our findings that median baseline H1N1 HAI titers were higher than H3N2 or B HAI titers and that over 75% of participants had baseline seroprotection against A/H1N1 influenza.

Why we did not find a consistent association between B HAI titer and reactogenicity is harder to rationalize. The median baseline HAI titer was the same against H3N2 and B antigens, and the percentage of participants with baseline seroprotection was lower for the B antigen than for the H3N2 antigen. However, the IM group had higher baseline seroprotection against influenza B than against influenza A/H3N2, and the MNP group had lower baseline seroprotection against influenza B. Consistent with this, among the MNP group, a high Global reaction score was associated with lower baseline B HAI titer and a higher day 28 B HAI titer fold change, mirroring the more durable findings we focused on throughout the study. Despite this signal, we must exercise caution when interpreting influenza B HAI results from a study of a trivalent vaccine for the same reasons discussed above. Many participants most likely had unique personal

histories of influenza B antigen and virus exposure, leading to heterogeneity in their baseline HAI titers and their anamnestic responses to vaccination with an influenza B antigen from one of two co-circulating lineages.

Taken together, it is challenging to compare IIV data to SARS-CoV-2 vaccine data. The COVID-19 pandemic resulted in a novel pathogen exposure and novel vaccines. SARS-CoV-2 vaccine study participants did not have long histories of intermittent exposure to several sequences of antigen at varying ages and periods of immune system development. The observation that people have greater reactogenicity and greater immunity after an initial pair of sequential exposures to a similar antigen is not specific to SARS-CoV-2, but whether this holds true for influenza remains to be seen.

Moreover, our assessment of the relationship between HAI and reactogenicity does not account for other measures of humoral immunity, such as neuraminidase inhibition (NAI) antibody titers, and components of the cellular immune compartment, such as T cells. This is a key limitation of our study (and many others in this space). Several studies suggest that, although it is not the gold standard measurement, NAI functions as an independent correlate of protection against influenza.[94–98] Similarly, a vast literature has shown that while most approved influenza vaccines do not elicit effective cross-reactive T cell immunity, intranasal live-attenuated influenza vaccine and influenza infection generate such cellular immune responses to a broad range of influenza antigens.[99–101] Thus, we cannot assume that participants with unknown exposure histories and lower baseline HAI titers have lower baseline NAI titers or lower numbers of preexisting central memory T cells, for example. Further, since symptoms like fever and headache are well accepted

to be driven by cytokines, which are predominantly secreted by the cellular immune compartment, attempts to correlate HAI titers with reactogenicity, particularly systemic reactogenicity, may be utilizing a measure of humoral immunity as a surrogate for measures of cellular immunity. Though HAI titer remains a standard correlate of protection for FDA approval of vaccines against influenza, in part due to the simplicity and reproducibility of testing peripheral blood, this metric cannot entirely explain vaccine immunogenicity or reactogenicity. Whenever possible, future investigations should incorporate measurements of NAI and various cell populations, particularly from tissues such as draining lymph nodes, to evaluate additional associations with reactogenicity. Ultimately, one goal of this line of investigation is to find reactogenicity correlates of vaccine efficacy, a relationship that we expect may be mediated by multiple variables that collectively describe immunogenicity.

Our study has some additional limitations. We took an a priori approach to defining reactogenicity variables as composite scores. These unweighted scores assume that each type of adverse event has the same impact on immunogenicity (e.g., fatigue and myalgia carry the same amount of meaning). However, we also recognize that adverse event grades are highly subjective. Summing grades of events instead of numbers of events could have introduced additional subjectivity to our models. Moreover, using sums of grades injects another assumption: that individual event grades (e.g., headache grades 1, 2, and 3) are equal distances apart. Ultimately, we chose our primary measure of reactogenicity because it is generalizable to future research, interpretable, and can be implemented clinically. Creating a bespoke, weighted composite reactogenicity score in future studies, for example by using LASSO regression techniques or unsupervised learning methods, to find a smaller number of more important

adverse event types would be instructive in determining if certain events matter more than others in the generation of immunity. However, given the small sample size in this study, such an approach would likely forfeit generalizability to larger databases if it were feasible at all. Our approach is supported by multiple publications in the SARS-CoV-2 literature, in which numbers of adverse events were added together to define groups of participants or variables for modeling. Though there is no metric to confirm the construct validity of our score definition or our dichotomization cut points, the fact that the findings were consistent and robust to at least some sensitivity analyses lends credence to our chosen approach.

The small sample size, large number of variables, and multitude of model outputs collectively represent another limitation of the study. Incorporating longitudinal data into mixed models ANOVA and GEEs mitigated the statistical power limitations (and prevented additional statistical comparisons) to some extent, but it remained challenging to prioritize and interpret model outputs. In some cases, particularly those involving the relative variables of titer fold change and seroconversion, using longitudinal models may have reduced the clarity of the findings. As an example, day 180 seroconversion carries less meaning than day 28 seroconversion, and its inclusion in GEEs obscured the association between local reactogenicity and seroconversion in the longitudinal estimates. At the same time, this study accomplished the function of a screening experiment successfully. We used advanced biostatistical methods on a small sample to identify signals for future investigation and generate hypotheses for future testing.

One more limitation is the low generalizability of our sample, which only included young and middle-aged adults without a wide range of self-reported racial and ethnic diversity. Though race and ethnicity should not have biologic significance regarding vaccine responses, we still adjusted for these variables due to associations we found in our preliminary bivariable comparisons and because they may act as proxies for unmeasured confounding variables that do affect vaccine biology. Future analyses of the influenza immunogenicity-reactogenicity relationship will need to include a more generalizable sample of healthy participants.

To address the intriguing findings and limitations of this pilot study, our group has acquired data from 12 completed phase 1 and 2 multicenter clinical trials of vaccines designed against pandemic strains of influenza. These vaccines delivered novel antigens to previously unexposed participants via intramuscular injection on a prime-boost schedule. With a larger database, broader source population, and sequential vaccine schedule, free of variation in participant exposure history, we will be able to meta-analyze these studies with better control of confounding to bridge the knowledge gap between our understanding of the immunogenicity-reactogenicity relationships of SARS-CoV-2 vaccines and influenza vaccines. Beyond this, we also plan to investigate the molecular underpinnings of reactogenicity, a pursuit that may help us to ultimately answer several critical outstanding questions, including questions about significant vaccine-associated adverse events such as Guillain-Barré syndrome and myocarditis.

In conclusion, greater reactogenicity of intramuscular seasonal inactivated influenza vaccine was associated with lower baseline H3N2 immunity and greater antibody titer fold change after 28 days, though the overall magnitude of the response was similar or lower compared with low-

reactogenicity participants. Future analyses of influenza vaccine trials will further elucidate their immunogenicity-reactogenicity relationship, which has wide-ranging implications for patient care, public health, and vaccine development.

Tables

Table 1. Baseline Participant Characteristics

Characteristic*	IM (n = 25)	MNP (n = 50)	All (n = 75)
Age, year	28.0 (7.0)	26.0 (10.0)	27.0 (9.0)
BMI, kg/m^2	24.8 (6.9)	24.8 (5.3)	24.8 (6.0)
≤ 25	14 (56.0%)	26 (52.0%)	40 (53.3%)
25 – 30, not inclusive	9 (36.0%)	17 (34.0%)	26 (34.7%)
≥ 30	2 (8.0%)	7 (14.0%)	9 (12.0%)
Sex			
Female	11 (44.0%)	24 (48.0%)	35 (46.7%)
Male	14 (56.0%)	26 (52.0%)	40 (53.3%)
Race			
White	12 (48.0%)	25 (50.0%)	37 (49.3%)
Black	8 (32.0%)	16 (32.0%)	24 (32.0%)
Other	5 (20.0%)	9 (18.0%)	14 (18.7%)
Ethnicity			
Not LatinX	22 (88.0%)	48 (96.0%)	70 (93.3%)
LatinX	3 (12.0%)	2 (4.0%)	5 (6.7%)
IIV in prior 2 seasons			
No	17 (68.0%)	34 (68.0%)	51 (68.0%)
Yes	8 (32.0%)	16 (32.0%)	24 (32.0%)
Baseline HAI titer^			
H1N1	80.0 (80.0)	80.0 (140.0)	80.0 (120.0)
H3N2	40.0 (60.0)	40.0 (65.0)	40.0 (60.0)
B	40.0 (40.0)	20.0 (30.0)	40.0 (70.0)
Missing	0	2 (4%)	2 (2.7%)

BMI: body mass index; IIV: inactivated influenza vaccine; HAI: hemagglutination inhibition
*continuous variables: median (IQR); categorical variables: N (%)
^represented as inverse titers

Table 2. Longitudinal Associations Between Reaction Score and HAI GMT

Reaction Type	Antigen	Study Group	Unadjusted GMR* (95% CI)	p-value	Adjusted GMR*^ (95% CI)	p-value
Global	H1N1	IM	1.07 (0.53, 2.16)	0.85	0.98 (0.58, 1.66)	0.95
		MNP	0.87 (0.50, 1.52)	0.62	0.85 (0.58, 1.24)	0.38
	H3N2	IM	0.83 (0.47, 1.49)	0.54	0.74 (0.45, 1.22)	0.23
		MNP	0.81 (0.50, 1.29)	0.37	0.78 (0.54, 1.12)	0.17
	B	IM	1.21 (0.80, 1.83)	0.36	0.95 (0.63, 1.42)	0.79
		MNP	0.80 (0.53, 1.22)	0.30	0.81 (0.58, 1.14)	0.22
Systemic	H1N1	IM	0.73 (0.35, 1.52)	0.40	0.65 (0.38, 1.12)	0.12
		MNP	0.91 (0.36, 2.27)	0.83	0.85 (0.45, 1.60)	0.61
	H3N2	IM	0.64 (0.35, 1.18)	0.15	**0.54 (0.33, 0.89)**	**0.02**
		MNP	1.43 (0.66, 3.10)	0.36	1.42 (0.77, 2.60)	0.26
	B	IM	1.07 (0.69, 1.65)	0.77	0.81 (0.53, 1.24)	0.33
		MNP	0.84 (0.42, 1.66)	0.61	0.78 (0.45, 1.36)	0.38
Local	H1N1	IM	0.59 (0.22, 1.54)	0.27	0.6 (0.30, 1.21)	0.15
		MNP	1.53 (0.90, 2.61)	0.12	**1.54 (1.05, 2.25)**	**0.03**
	H3N2	IM	0.46 (0.21, 1.01)	0.05	**0.37 (0.19, 0.72)**	**<0.01**
		MNP	0.72 (0.46, 1.13)	0.15	**0.63 (0.44, 0.90)**	**0.01**
	B	IM	1.06 (0.60, 1.87)	0.85	0.89 (0.51, 1.55)	0.68
		MNP	0.97 (0.65, 1.45)	0.89	1.00 (0.71, 1.41)	0.99

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 3A. Longitudinal Associations Between Alternative Cut Point Global Reaction Score and HAI GMT

Reaction Type	Antigen	Study Group	Unadjusted GMR* (95% CI)	p-value	Adjusted GMR*^ (95% CI)	p-value
Global (≥ 3 vs ≤ 2)	H1N1	IM	0.96 (0.51, 1.81)	0.90	0.93 (0.58, 1.51)	0.77
		MNP	0.94 (0.56, 1.58)	0.81	0.91 (0.64, 1.29)	0.59
	H3N2	IM	0.97 (0.57, 1.64)	0.90	0.84 (0.53, 1.33)	0.45
		MNP	0.81 (0.53, 1.26)	0.35	0.78 (0.56, 1.10)	0.15
	B	IM	**1.47 (1.02, 2.12)**	**0.04**	1.16 (0.80, 1.69)	0.42
		MNP	1.04 (0.71, 1.53)	0.82	1.05 (0.76, 1.44)	0.77
Global (≥ 5 vs ≤ 4)	H1N1	IM	0.85 (0.39, 1.87)	0.68	0.81 (0.45, 1.45)	0.47
		MNP	1.03 (0.57, 1.86)	0.91	0.95 (0.64, 1.43)	0.82
	H3N2	IM	0.82 (0.43, 1.58)	0.55	0.73 (0.42, 1.26)	0.25
		MNP	1.00 (0.61, 1.64)	1.0	0.94 (0.64, 1.39)	0.76
	B	IM	0.91 (0.57, 1.45)	0.69	0.69 (0.44, 1.08)	0.10
		MNP	0.91 (0.59, 1.42)	0.68	0.90 (0.63, 1.28)	0.54

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 3B. Longitudinal Associations Between Alternative Cut Point Systemic Reaction Score and HAI GMT

Reaction Type	Antigen	Study Group	Unadjusted GMR* (95% CI)	p-value	Adjusted GMR*^ (95% CI)	p-value
Systemic (≥ 1 vs 0)	H1N1	IM	1.15 (0.60, 2.18)	0.67	1.12 (0.70, 1.81)	0.63
		MNP	0.81 (0.49, 1.35)	0.42	0.8 (0.57, 1.13)	0.21
	H3N2	IM	0.95 (0.56, 1.63)	0.86	0.84 (0.53, 1.32)	0.44
		MNP	1.02 (0.66, 1.56)	0.94	1.03 (0.74, 1.44)	0.87
	B	IM	**1.54 (1.07, 2.22)**	**0.02**	1.22 (0.84, 1.76)	0.29
		MNP	0.87 (0.60, 1.28)	0.48	0.89 (0.66, 1.21)	0.47
Systemic (≥ 2 vs ≤ 1)	H1N1	IM	1.03 (0.55, 1.93)	0.94	1.0 (0.63, 1.59)	0.99
		MNP	1.04 (0.59, 1.84)	0.89	1.12 (0.76, 1.65)	0.56
	H3N2	IM	0.79 (0.47, 1.33)	0.37	0.72 (0.46, 1.11)	0.13
		MNP	0.99 (0.61, 1.60)	0.95	0.99 (0.68, 1.44)	0.94
	B	IM	1.42 (0.99, 2.05)	0.06	1.17 (0.82, 1.67)	0.39
		MNP	0.86 (0.56, 1.33)	0.50	0.87 (0.62, 1.23)	0.43

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 3C. Longitudinal Associations Between Alternative Cut Point Local Reaction Score and HAI GMT

Reaction Type	Antigen	Study Group	Unadjusted GMR* (95% CI)	p-value	Adjusted GMR*^ (95% CI)	p-value
Local (≥ 1 vs 0)	H1N1	IM	1.40 (0.71, 2.73)	0.32	1.34 (0.81, 2.21)	0.25
		MNP	1.18 (0.51, 2.71)	0.69	1.00 (0.57, 1.77)	1.0
	H3N2	IM	1.01 (0.58, 1.77)	0.97	0.92 (0.57, 1.49)	0.73
		MNP	1.00 (0.49, 2.01)	0.99	0.94 (0.54, 1.63)	0.81
	B	IM	1.18 (0.80, 1.76)	0.40	0.95 (0.64, 1.40)	0.79
		MNP	1.19 (0.64, 2.21)	0.59	1.13 (0.68, 1.87)	0.64
Local (≥ 2 vs ≤ 1)	H1N1	IM	1.10 (0.58, 2.09)	0.78	1.02 (0.63, 1.66)	0.92
		MNP	1.19 (0.69, 2.05)	0.53	1.05 (0.72, 1.55)	0.79
	H3N2	IM	1.22 (0.72, 2.08)	0.46	1.05 (0.66, 1.67)	0.83
		MNP	0.79 (0.50, 1.26)	0.32	0.73 (0.50, 1.05)	0.09
	B	IM	1.20 (0.83, 1.75)	0.33	0.92 (0.63, 1.34)	0.66
		MNP	1.30 (0.87, 1.96)	0.20	1.25 (0.89, 1.76)	0.19

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 4A. Reaction Score and H1N1 HAI GMT by Study Day

Reaction Type	Study Group	Day 0 GMR* (95% CI)	p-value	Day 28 GMR* (95% CI)	p-value	Day 180 GMR* (95% CI)	p-value
Unadjusted							
Global	IM	1.0 (0.4, 2.4)	0.99	1.1 (0.5, 2.5)	0.87	1.1 (0.5, 2.7)	0.77
	MNP	1.2 (0.6, 2.3)	0.59	0.7 (0.4, 1.4)	0.36	0.7 (0.4, 1.4)	0.35
Systemic	IM	0.6 (0.2, 1.4)	0.21	0.9 (0.4, 2.1)	0.78	0.8 (0.3, 1.9)	0.58
	MNP	1.5 (0.5, 4.2)	0.48	0.7 (0.2, 2.0)	0.50	0.7 (0.3, 2.1)	0.56
Local	IM	1.0 (0.3, 3.3)	0.94	0.5 (0.1, 1.5)	0.19	0.4 (0.1, 1.3)	0.14
	MNP	1.6 (0.9, 2.9)	0.14	1.5 (0.8, 2.8)	0.18	1.5 (0.8, 2.8)	0.18
Adjusted^							
Global	IM	0.9 (0.4, 2.2)	0.86	1.0 (0.4, 2.3)	0.98	1.0 (0.4, 2.4)	0.92
	MNP	1.2 (0.6, 2.2)	0.64	0.7 (0.4, 1.4)	0.32	0.7 (0.4, 1.4)	0.31
Systemic	IM	0.5 (0.2, 1.2)	0.13	0.8 (0.3, 1.9)	0.59	0.7 (0.3, 1.7)	0.41
	MNP	1.4 (0.5, 3.9)	0.55	0.7 (0.2, 1.9)	0.42	0.7 (0.2, 2.0)	0.48
Local	IM	1.1 (0.3, 3.3)	0.91	0.5 (0.2, 1.5)	0.20	0.4 (0.1, 1.3)	0.14
	MNP	1.6 (0.9, 2.9)	0.14	1.5 (0.8, 2.8)	0.19	1.5 (0.8, 2.8)	0.19

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 4B. Reaction Score and H3N2 HAI GMT by Study Day

Reaction Type	Study Group	Day 0 GMR* (95% CI)	p-value	Day 28 GMR* (95% CI)	p-value	Day 180 GMR* (95% CI)	p-value
Unadjusted							
Global	IM	0.5 (0.2, 1.1)	0.09	1.1 (0.5, 2.4)	0.82	1.0 (0.5, 2.2)	0.93
	MNP	0.8 (0.4, 1.5)	0.53	0.7 (0.4, 1.4)	0.32	0.9 (0.5, 1.6)	0.69
Systemic	IM	**0.4 (0.2, 0.9)**	**0.03**	1.0 (0.5, 2.3)	0.96	0.6 (0.3, 1.4)	0.25
	MNP	1.3 (0.5, 3.6)	0.63	1.5 (0.5, 4.1)	0.47	1.6 (0.6, 4.4)	0.40
Local	IM	**0.3 (0.1, 0.9)**	**0.03**	0.7 (0.2, 1.9)	0.48	0.5 (0.2, 1.3)	0.13
	MNP	0.6 (0.4, 1.2)	0.16	0.7 (0.4, 1.3)	0.29	0.8 (0.4, 1.4)	0.43
Adjusted^							
Global	IM	0.5 (0.2, 1.0)	0.06	1.0 (0.4, 2.2)	0.95	0.9 (0.4, 2.1)	0.85
	MNP	0.8 (0.4, 1.5)	0.45	0.7 (0.4, 1.3)	0.26	0.8 (0.5, 1.6)	0.60
Systemic	IM	**0.4 (0.2, 0.8)**	**0.01**	0.9 (0.4, 1.9)	0.7	0.5 (0.2, 1.2)	0.12
	MNP	1.3 (0.5, 3.5)	0.63	1.4 (0.5, 4.0)	0.47	1.5 (0.6, 4.3)	0.40
Local	IM	**0.3 (0.1, 0.8)**	**0.01**	0.6 (0.2, 1.6)	0.28	0.4 (0.1, 1.1)	0.07
	MNP	0.6 (0.3, 1.0)	0.06	0.6 (0.4, 1.1)	0.13	0.7 (0.4, 1.2)	0.22

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 4C. Reaction Score and B HAI GMT by Study Day

Reaction Type	Study Group	Day 0 GMR* (95% CI)	p-value	Day 28 GMR* (95% CI)	p-value	Day 180 GMR* (95% CI)	p-value
Unadjusted							
Global	IM	1.2 (0.6, 2.3)	0.54	1.2 (0.6, 2.3)	0.58	1.2 (0.6, 2.3)	0.55
	MNP	0.6 (0.3, 1.0)	0.05	1.1 (0.6, 1.9)	0.84	0.9 (0.5, 1.6)	0.66
Systemic	IM	0.9 (0.5, 1.8)	0.83	1.1 (0.6, 2.1)	0.80	1.2 (0.6, 2.4)	0.60
	MNP	1.0 (0.4, 2.5)	0.92	0.9 (0.4, 2.5)	0.90	0.7 (0.2, 1.7)	0.39
Local	IM	1.2 (0.5, 3.0)	0.66	0.8 (0.3, 2.0)	0.68	1.2 (0.5, 2.9)	0.73
	MNP	0.7 (0.4, 1.3)	0.26	1.1 (0.6, 2.0)	0.71	1.1 (0.6, 2.0)	0.65
Adjusted^							
Global	IM	1.0 (0.5, 1.8)	0.89	0.9 (0.5, 1.8)	0.85	0.9 (0.5, 1.8)	0.87
	MNP	**0.6 (0.3, 1.0)**	**0.04**	1.1 (0.6, 1.9)	0.80	0.9 (0.5, 1.5)	0.67
Systemic	IM	0.7 (0.4, 1.4)	0.32	0.8 (0.4, 1.6)	0.59	0.9 (0.5, 1.8)	0.80
	MNP	0.9 (0.4, 2.2)	0.81	0.9 (0.3, 2.2)	0.78	0.6 (0.2, 1.5)	0.29
Local	IM	1.0 (0.4, 2.5)	0.95	0.7 (0.3, 1.7)	0.42	1.0 (0.4, 2.4)	0.98
	MNP	0.7 (0.4, 1.3)	0.29	1.1 (0.7, 2.0)	0.62	1.2 (0.7, 2.0)	0.57

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 5. IM Study Group Reaction Score and H3N2 HAI GMT by Study Day

Reaction Type	Day 0 Adjusted GMR*^ (95% CI)	p-value	Day 28 Adjusted GMR*^ (95% CI)	p-value	Day 180 Adjusted GMR*^ (95% CI)	p-value
Global	0.5 (0.2, 1.0)	0.06	1.0 (0.4, 2.2)	0.95	0.9 (0.4, 2.1)	0.85
Systemic	**0.4 (0.2, 0.8)**	**0.01**	0.9 (0.4, 1.9)	0.7	0.5 (0.2, 1.2)	0.12
Local	**0.3 (0.1, 0.8)**	**0.01**	0.6 (0.2, 1.6)	0.28	0.4 (0.1, 1.1)	0.07

IM: intramuscular; HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 6A. Reaction Score and H1N1 HAI Titer Fold Change by Study Day

Reaction Type	Study Group	Day 28 FCR* (95% CI)	p-value	Day 180 FCR* (95% CI)	p-value
Unadjusted					
Global	IM	1.1 (0.3, 3.7)	0.92	1.1 (0.3, 3.9)	0.85
	MNP	0.6 (0.3, 1.4)	0.26	0.6 (0.3, 1.4)	0.25
Systemic	IM	1.5 (0.4, 5.6)	0.51	1.4 (0.4, 5.0)	0.63
	MNP	0.5 (0.1, 1.8)	0.28	0.5 (0.1, 1.9)	0.31
Local	IM	0.4 (0.1, 2.4)	0.34	0.4 (0.1, 2.2)	0.28
	MNP	1.0 (0.4, 2.1)	0.91	1.0 (0.4, 2.1)	0.91
Adjusted^					
Global	IM	1.0 (0.3, 3.1)	0.94	1.0 (0.3, 3.3)	0.99
	MNP	0.7 (0.3, 1.4)	0.30	0.7 (0.3, 1.4)	0.29
Systemic	IM	1.1 (0.3, 4.0)	0.84	1.0 (0.3, 3.5)	1.0
	MNP	0.6 (0.2, 2.0)	0.41	0.6 (0.2, 2.1)	0.45
Local	IM	0.4 (0.1, 2.2)	0.32	0.4 (0.1, 2.0)	0.27
	MNP	1.0 (0.5, 2.2)	0.90	1.0 (0.5, 2.2)	0.90

HAI: hemagglutination inhibition; FCR: fold change ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated fold change ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 6B. Reaction Score and H3N2 HAI Titer Fold Change by Study Day

Reaction Type	Study Group	Day 28 FCR* (95% CI)	p-value	Day 180 FCR* (95% CI)	p-value
Unadjusted					
Global	IM	**2.1 (1.1, 4.1)**	**0.03**	**2 (1.0, 3.9)**	**0.04**
	MNP	0.9 (0.5, 1.6)	0.68	1.1 (0.6, 1.9)	0.81
Systemic	IM	**2.4 (1.2, 5.0)**	**0.02**	1.5 (0.7, 3.1)	0.27
	MNP	1.1 (0.4, 2.9)	0.79	1.2 (0.5, 3.1)	0.69
Local	IM	2.2 (0.8, 5.7)	0.12	1.4 (0.5, 3.8)	0.48
	MNP	1.1 (0.6, 1.9)	0.69	1.2 (0.7, 2.1)	0.48
Adjusted^					
Global	IM	**2 (1.0, 3.7)**	**0.04**	1.9 (1.0, 3.5)	0.05
	MNP	0.9 (0.6, 1.6)	0.83	1.1 (0.7, 1.9)	0.61
Systemic	IM	1.9 (1.0, 3.8)	0.07	1.2 (0.6, 2.4)	0.64
	MNP	1.3 (0.6, 3.1)	0.49	1.4 (0.6, 3.3)	0.40
Local	IM	2 (0.8, 4.9)	0.15	1.3 (0.5, 3.2)	0.58
	MNP	1.1 (0.7, 1.9)	0.66	1.2 (0.7, 2.0)	0.44

HAI: hemagglutination inhibition; FCR: fold change ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated fold change ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 6C. Reaction Score and B HAI Titer Fold Change by Study Day

Reaction Type	Study Group	Day 28 FCR* (95% CI)	p-value	Day 180 FCR* (95% CI)	p-value
Unadjusted					
Global	IM	1.0 (0.4, 2.3)	0.97	1.0 (0.4, 2.4)	0.99
	MNP	1.9 (1.0, 3.6)	0.05	1.6 (0.8, 3.0)	0.16
Systemic	IM	1.2 (0.5, 2.9)	0.73	1.3 (0.5, 3.2)	0.58
	MNP	1.0 (0.3, 2.9)	0.98	0.7 (0.2, 2.0)	0.49
Local	IM	0.7 (0.2, 2.2)	0.52	1.0 (0.3, 3.1)	0.94
	MNP	1.5 (0.8, 2.9)	0.17	1.6 (0.8, 2.9)	0.15
Adjusted^					
Global	IM	0.8 (0.4, 1.6)	0.46	0.8 (0.4, 1.6)	0.48
	MNP	**1.8 (1.1, 3.2)**	**0.03**	1.5 (0.9, 2.6)	0.14
Systemic	IM	0.9 (0.4, 1.9)	0.70	0.9 (0.4, 2.1)	0.88
	MNP	1.1 (0.4, 2.9)	0.79	0.8 (0.3, 2.0)	0.61
Local	IM	0.5 (0.2, 1.5)	0.22	0.7 (0.3, 2.1)	0.58
	MNP	1.3 (0.7, 2.2)	0.39	1.3 (0.7, 2.3)	0.35

HAI: hemagglutination inhibition; FCR: fold change ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated fold change ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 7. Extreme Reaction Scores and HAI GMT by Study Day

Reaction Type	Antigen	Study Group	Day 0 GMR* (95% CI)	p-value	Day 28 GMR* (95% CI)	p-value	Day 180 GMR* (95% CI)	p-value
Global	H1N1	IM	0.9 (0.3, 2.8)	0.87	0.8 (0.3, 2.5)	0.74	0.9 (0.3, 2.8)	0.87
		MNP	1.7 (0.5, 5.3)	0.35	1.6 (0.5, 4.8)	0.44	1.4 (0.4, 4.2)	0.60
	H3N2	IM	0.6 (0.2, 1.6)	0.28	1.2 (0.4, 3.4)	0.73	1.1 (0.4, 3.2)	0.84
		MNP	0.5 (0.2, 1.5)	0.24	0.7 (0.3, 1.9)	0.47	0.9 (0.3, 2.4)	0.75
	B	IM	0.9 (0.4, 1.8)	0.68	1.2 (0.5, 2.5)	0.68	1.5 (0.7, 3.2)	0.29
		MNP	0.8 (0.4, 1.8)	0.60	1.0 (0.5, 2.3)	0.95	1.1 (0.5, 2.4)	0.86
Systemic	H1N1	IM	0.5 (0.2, 1.7)	0.30	0.7 (0.2, 2.3)	0.55	0.5 (0.2, 1.6)	0.24
		MNP	1.5 (0.5, 4.5)	0.44	0.8 (0.3, 2.3)	0.63	0.6 (0.2, 1.8)	0.35
	H3N2	IM	0.4 (0.1, 1.3)	0.14	1.2 (0.4, 3.8)	0.76	0.8 (0.2, 2.4)	0.65
		MNP	0.5 (0.2, 1.1)	0.08	0.6 (0.3, 1.4)	0.23	0.7 (0.3, 1.5)	0.38
	B	IM	1.0 (0.4, 2.6)	1.0	1.5 (0.6, 4.0)	0.36	1.5 (0.6, 4.0)	0.36
		MNP	1.2 (0.6, 2.4)	0.69	0.7 (0.4, 1.5)	0.41	0.6 (0.3, 1.3)	0.20
Local	H1N1	IM	**4.4 (1.1, 17.7)**	**0.04**	0.3 (0.1, 1.1)	0.07	0.6 (0.2, 2.6)	0.54
		MNP	1.2 (0.5, 2.9)	0.61	1.2 (0.5, 2.7)	0.73	1.4 (0.6, 3.1)	0.47
	H3N2	IM	**0.2 (0.1, 0.8)**	**0.02**	0.3 (0.1, 1.1)	0.06	**0.2 (0.1, 0.8)**	**0.02**
		MNP	0.5 (0.2, 1.4)	0.17	0.6 (0.2, 1.8)	0.40	0.7 (0.2, 1.8)	0.42
	B	IM	2.0 (0.9, 4.3)	0.08	0.9 (0.4, 2.0)	0.82	1.4 (0.7, 3.1)	0.37
		MNP	0.8 (0.4, 1.9)	0.63	0.9 (0.4, 2.1)	0.87	1.3 (0.5, 2.9)	0.58

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher score category compared with those in the lower score category

Table 8. Extreme Reaction Scores and HAI Titer Fold Change by Study Day

Reaction Type	Antigen	Study Group	Day 0 FCR* (95% CI)	p-value	Day 28 FCR * (95% CI)	p-value
Global	H1N1	IM	0.9 (0.2, 5.3)	0.92	1.0 (0.2, 5.9)	1.0
		MNP	0.9 (0.3, 2.8)	0.87	0.8 (0.3, 2.5)	0.68
	H3N2	IM	2.1 (1.0, 4.7)	0.06	2 (0.9, 4.4)	0.09
		MNP	1.3 (0.5, 3.1)	0.61	1.6 (0.6, 3.8)	0.33
	B	IM	1.4 (0.8, 2.4)	0.29	1.7 (1.0, 3.1)	0.06
		MNP	1.3 (0.5, 3.0)	0.59	1.3 (0.5, 3.2)	0.52
Systemic	H1N1	IM	1.3 (0.2, 8.4)	0.78	0.9 (0.1, 6.0)	0.92
		MNP	0.5 (0.2, 1.4)	0.18	0.4 (0.1, 1.1)	0.07
	H3N2	IM	**2.8 (1.2, 6.5)**	**0.02**	1.8 (0.8, 4.2)	0.15
		MNP	1.3 (0.6, 2.6)	0.54	1.4 (0.7, 3.0)	0.34
	B	IM	1.5 (0.7, 3.3)	0.25	1.5 (0.7, 3.3)	0.25
		MNP	0.6 (0.3, 1.5)	0.31	0.5 (0.2, 1.3)	0.17
Local	H1N1	IM	**0.1 (0.0, 0.5)**	**0.01**	0.1 (0.0, 1.2)	0.07
		MNP	0.9 (0.3, 3.1)	0.91	1.1 (0.3, 3.7)	0.88
	H3N2	IM	1.4 (0.5, 4.2)	0.52	1.1 (0.4, 3.2)	0.87
		MNP	1.3 (0.6, 3.2)	0.52	1.4 (0.6, 3.3)	0.48
	B	IM	0.5 (0.2, 1.2)	0.11	0.7 (0.3, 1.9)	0.47
		MNP	1.1 (0.4, 3.0)	0.79	1.5 (0.6, 4.1)	0.38

HAI: hemagglutination inhibition; FCR: fold change ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated fold change ratio for those in the higher score category compared with those in the lower score category

Table 9. Reaction Severity and Longitudinal HAI GMT

Reaction	Antigen	Study Group	Unadjusted GMR* (95% CI)	p-value	Adjusted GMR*^ (95% CI)	p-value
Severe (Yes vs. No)	H1N1	IM	1.1 (0.6, 2.2)	0.69	1.1 (0.6, 2.2)	0.75
		MNP	1.3 (0.7, 2.3)	0.42	1.4 (0.8, 2.5)	0.30
	H3N2	IM	0.9 (0.5, 1.6)	0.80	0.8 (0.5, 1.5)	0.52
		MNP	0.8 (0.5, 1.3)	0.41	0.8 (0.5, 1.3)	0.38
	B	IM	1.4 (0.9, 2.0)	0.11	1.1 (0.8, 1.7)	0.58
		MNP	1.1 (0.7, 1.7)	0.77	1.2 (0.8, 1.8)	0.47

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the higher severity category compared with those in the lower severity category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 10. Reaction Severity and HAI Titer Fold Change by Study Day

Reaction	Antigen	Study Group	Day 28 FCR* (95% CI)	p-value	Day 180 FCR* (95% CI)	p-value
Unadjusted						
Severe (Yes vs. No)	H1N1	IM	0.7 (0.2, 2.0)	0.46	0.7 (0.2, 2.3)	0.60
		MNP	0.5 (0.2, 1.2)	0.12	0.6 (0.3, 1.5)	0.28
	H3N2	IM	1.7 (0.9, 3.2)	0.12	1.5 (0.8, 2.8)	0.23
		MNP	0.9 (0.5, 1.7)	0.80	1.5 (0.8, 2.7)	0.20
	B	IM	1.0 (0.5, 2.3)	0.91	1.1 (0.5, 2.4)	0.82
		MNP	1.0 (0.5, 2.0)	0.96	1.2 (0.6, 2.3)	0.66
Adjusted^						
Severe (Yes vs. No)	H1N1	IM	0.6 (0.2, 1.9)	0.40	0.7 (0.2, 2.1)	0.53
		MNP	0.6 (0.3, 1.3)	0.17	0.7 (0.3, 1.6)	0.41
	H3N2	IM	1.5 (0.8, 2.8)	0.17	1.4 (0.7, 2.5)	0.32
		MNP	1.0 (0.6, 1.7)	0.99	1.6 (0.9, 2.7)	0.09
	B	IM	1.0 (0.5, 2.1)	0.91	1.1 (0.5, 2.2)	0.80
		MNP	1.1 (0.6, 1.9)	0.87	1.2 (0.7, 2.3)	0.46

HAI: hemagglutination inhibition; FCR: fold change ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated fold change ratio for those in the higher severity category compared with those in the lower severity category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 11. Reaction Duration and Longitudinal HAI GMT

Reaction	Antigen	Study Group	Unadjusted GMR* (95% CI)	p-value	Adjusted GMR*^ (95% CI)	p-value
Prolonged (Yes vs. No)	H1N1	IM	1.0 (0.6, 2.0)	0.90	1.0 (0.5, 2.0)	0.94
		MNP	1.4 (0.8, 2.3)	0.26	1.3 (0.8, 2.3)	0.34
	H3N2	IM	1.2 (0.7, 2.0)	0.58	1.1 (0.6, 1.9)	0.85
		MNP	1.0 (0.6, 1.5)	0.89	1.0 (0.6, 1.6)	1.0
	B	IM	1.3 (0.9, 1.9)	0.18	1.0 (0.7, 1.5)	0.99
		MNP	0.9 (0.6, 1.4)	0.77	1.0 (0.7, 1.5)	0.94

HAI: hemagglutination inhibition; GMT: geometric mean titer; GMR: geometric mean titer ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated geometric mean titer ratio for those in the prolonged category compared with those not in the prolonged category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 12. Reaction Duration and HAI Titer Fold Change by Study Day

Reaction	Antigen	Study Group	Day 28 FCR* (95% CI)	p-value	Day 180 FCR* (95% CI)	p-value
Unadjusted						
Prolonged (Yes vs. No)	H1N1	IM	0.7 (0.2, 2.2)	0.55	0.8 (0.3, 2.5)	0.72
		MNP	0.9 (0.4, 2.0)	0.83	0.8 (0.4, 1.7)	0.58
	H3N2	IM	1.1 (0.6, 2.1)	0.72	1.5 (0.8, 2.9)	0.19
		MNP	1.1 (0.6, 1.8)	0.82	1.4 (0.8, 2.3)	0.25
	B	IM	1.1 (0.5, 2.5)	0.72	1.3 (0.6, 2.8)	0.55
		MNP	0.9 (0.5, 1.7)	0.82	1.0 (0.5, 1.8)	0.92
Adjusted^						
Prolonged (Yes vs. No)	H1N1	IM	0.9 (0.3, 2.8)	0.87	1.0 (0.3, 3.2)	0.93
		MNP	0.7 (0.4, 1.4)	0.33	0.6 (0.3, 1.2)	0.18
	H3N2	IM	1.2 (0.6, 2.2)	0.62	1.6 (0.9, 3.0)	0.14
		MNP	0.9 (0.5, 1.5)	0.66	1.1 (0.7, 1.9)	0.58
	B	IM	1.3 (0.6, 2.6)	0.48	1.4 (0.7, 2.9)	0.34
		MNP	0.6 (0.4, 1.1)	0.08	0.6 (0.4, 1.1)	0.10

HAI: hemagglutination inhibition; FCR: fold change ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated fold change ratio for those in the prolonged category compared with those not in the prolonged category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 13. Longitudinal Associations Between Reaction Score and HAI Seroprotection

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Global	H1N1	IM	1.01 (0.90, 1.12)	0.88	0.99 (0.89, 1.12)	0.92
		MNP	1.03 (0.94, 1.13)	0.55	1.02 (0.93, 1.12)	0.66
	H3N2	IM	0.88 (0.64, 1.20)	0.41	0.87 (0.65, 1.15)	0.33
		MNP	0.94 (0.74, 1.18)	0.58	0.93 (0.75, 1.14)	0.47
	B	IM	1.02 (0.87, 1.19)	0.83	0.96 (0.81, 1.15)	0.67
		MNP	0.98 (0.80, 1.20)	0.83	1.00 (0.82, 1.22)	0.99
Systemic	H1N1	IM	0.92 (0.79, 1.07)	0.28	0.92 (0.80, 1.07)	0.28
		MNP	1.01 (0.86, 1.19)	0.92	0.99 (0.84, 1.15)	0.86
	H3N2	IM	0.75 (0.49, 1.13)	0.16	0.74 (0.51, 1.07)	0.11
		MNP	1.16 (0.96, 1.40)	0.11	1.13 (0.97, 1.33)	0.12
	B	IM	0.99 (0.84, 1.18)	0.94	0.92 (0.78, 1.10)	0.37
		MNP	1.00 (0.69, 1.46)	1.0	0.98 (0.65, 1.48)	0.92
Local	H1N1	IM	0.93 (0.76, 1.14)	0.50	0.91 (0.76, 1.09)	0.32
		MNP	1.08 (1.00, 1.18)	0.06	1.08 (0.98, 1.19)	0.10
	H3N2	IM	0.63 (0.33, 1.21)	0.17	0.61 (0.35, 1.09)	0.10
		MNP	0.84 (0.66, 1.06)	0.15	0.81 (0.65, 1.02)	0.07
	B	IM	0.99 (0.80, 1.24)	0.96	0.95 (0.73, 1.23)	0.70
		MNP	1.00 (0.82, 1.22)	1.0	1.02 (0.84, 1.25)	0.81

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated risk ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 14. HAI Seroprotection* by Vaccine Route and Day

Antigen	Study Day	IM (n = 25)	MNP^ (n = 48)	All (n = 73)
H1N1	0	21 (84.0%)	35 (72.9%)	56 (76.7%)
	28	25 (100.0%)	48 (100.0%)	73 (100.0%)
	180	25 (100.0%)	48 (100.0%)	73 (100.0%)
H3N2	0	18 (72.0%)	29 (60.4%)	47 (64.4%)
	28	25 (100.0%)	47 (97.9%)	72 (98.6%)
	180	20 (80.0%)	39 (81.25%)	59 (80.8%)
B	0	19 (76.0%)	23 (47.9%)	42 (57.5%)
	28	25 (100.0%)	47 (97.9%)	72 (98.6%)
	180	23 (92.0%)	38 (79.2%)	61 (83.6%)

*Number (%) of participants with hemagglutination inhibition antibody titer ≥ 1:40
^2 participants had missing outcome data

Table 15A. Longitudinal Associations Between Alternative Cut Point Global Reaction Score and HAI Seroprotection

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Global (≥ 3 vs ≤ 2)	H1N1	IM	1.01 (0.91, 1.12)	0.79	0.99 (0.88, 1.10)	0.79
		MNP	1.05 (0.96, 1.16)	0.31	1.04 (0.94, 1.15)	0.47
	H3N2	IM	0.98 (0.76, 1.26)	0.89	0.94 (0.73, 1.20)	0.60
		MNP	0.96 (0.79, 1.17)	0.70	0.95 (0.79, 1.14)	0.57
	B	IM	1.09 (0.92, 1.30)	0.30	0.99 (0.83, 1.17)	0.87
		MNP	1.12 (0.90, 1.39)	0.30	1.15 (0.94, 1.41)	0.18
Global (≥ 5 vs ≤ 4)	H1N1	IM	0.98 (0.86, 1.13)	0.80	0.98 (0.85, 1.13)	0.77
		MNP	1.01 (0.91, 1.12)	0.85	1.01 (0.91, 1.12)	0.89
	H3N2	IM	0.85 (0.56, 1.27)	0.42	0.83 (0.58, 1.21)	0.33
		MNP	0.97 (0.76, 1.22)	0.77	0.95 (0.77, 1.18)	0.66
	B	IM	0.96 (0.80, 1.16)	0.69	0.90 (0.75, 1.09)	0.28
		MNP	1.00 (0.82, 1.22)	1.0	1.02 (0.82, 1.26)	0.86

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 15B. Longitudinal Associations Between Alternative Cut Point Systemic Reaction Score and HAI Seroprotection

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Systemic (≥ 1 vs 0)	H1N1	IM	1.02 (0.92, 1.14)	0.67	1.01 (0.90, 1.13)	0.91
		MNP	1.00 (0.91, 1.10)	0.98	1.00 (0.91, 1.10)	0.99
	H3N2	IM	1.01 (0.78, 1.31)	0.92	0.97 (0.76, 1.24)	0.81
		MNP	1.03 (0.84, 1.25)	0.81	1.03 (0.86, 1.23)	0.78
	B	IM	1.12 (0.93, 1.34)	0.22	1.02 (0.85, 1.21)	0.86
		MNP	1.02 (0.83, 1.25)	0.86	1.05 (0.87, 1.28)	0.59
Systemic (≥ 2 vs ≤ 1)	H1N1	IM	1.00 (0.90, 1.10)	0.93	0.98 (0.88, 1.08)	0.68
		MNP	1.06 (0.97, 1.16)	0.20	1.04 (0.95, 1.13)	0.37
	H3N2	IM	0.92 (0.72, 1.19)	0.54	0.90 (0.71, 1.14)	0.37
		MNP	0.99 (0.80, 1.24)	0.95	0.97 (0.79, 1.19)	0.77
	B	IM	1.12 (0.96, 1.30)	0.16	1.04 (0.89, 1.21)	0.64
		MNP	1.08 (0.88, 1.34)	0.45	1.08 (0.88, 1.31)	0.47

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 15C. Longitudinal Associations Between Alternative Cut Point Local Reaction Score and HAI Seroprotection

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Local (≥ 1 vs 0)	H1N1	IM	1.05 (0.93, 1.18)	0.45	1.03 (0.91, 1.17)	0.63
		MNP	1.06 (0.89, 1.25)	0.54	1.08 (0.92, 1.27)	0.32
	H3N2	IM	0.94 (0.74, 1.20)	0.63	0.91 (0.71, 1.17)	0.47
		MNP	1.00 (0.73, 1.36)	0.99	1.00 (0.77, 1.29)	0.98
	B	IM	0.96 (0.83, 1.12)	0.62	0.86 (0.73, 1.01)	0.06
		MNP	1.14 (0.76, 1.71)	0.53	1.16 (0.83, 1.62)	0.39
Local (≥ 2 vs ≤ 1)	H1N1	IM	0.98 (0.88, 1.09)	0.67	0.97 (0.87, 1.08)	0.57
		MNP	1.03 (0.93, 1.15)	0.53	1.05 (0.94, 1.17)	0.42
	H3N2	IM	0.99 (0.76, 1.28)	0.92	0.96 (0.75, 1.23)	0.74
		MNP	0.89 (0.74, 1.06)	0.18	0.89 (0.74, 1.07)	0.21
	B	IM	1.01 (0.87, 1.18)	0.87	0.92 (0.78, 1.09)	0.35
		MNP	1.13 (0.87, 1.47)	0.37	1.15 (0.88, 1.51)	0.31

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 16. Longitudinal Associations Between Reaction Score and HAI Seroconversion

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Global	H1N1	IM	1.05 (0.59, 1.87)	0.86	1.05 (0.62, 1.78)	0.85
		MNP	0.96 (0.75, 1.24)	0.77	0.98 (0.78, 1.24)	0.86
	H3N2	IM	1.65 (0.99, 2.76)	0.05	1.59 (0.96, 2.63)	0.07
		MNP	0.97 (0.65, 1.45)	0.89	0.97 (0.71, 1.33)	0.85
	B	IM	1.29 (0.37, 4.41)	0.69	1.20 (0.43, 3.31)	0.73
		MNP	1.53 (0.96, 2.43)	0.07	1.38 (0.89, 2.14)	0.15
Systemic	H1N1	IM	1.10 (0.59, 2.05)	0.76	1.06 (0.59, 1.91)	0.84
		MNP	0.97 (0.69, 1.37)	0.87	1.04 (0.71, 1.52)	0.83
	H3N2	IM	**1.69 (1.08, 2.65)**	**0.02**	1.53 (0.91, 2.58)	0.11
		MNP	1.25 (0.64, 2.45)	0.52	**1.50 (1.21, 1.85)**	**<0.01**
	B	IM	2.26 (0.69, 7.39)	0.18	1.83 (0.70, 4.79)	0.22
		MNP	1.10 (0.53, 2.30)	0.80	1.26 (0.72, 2.20)	0.41
Local	H1N1	IM	0.51 (0.10, 2.54)	0.41	0.52 (0.11, 2.53)	0.41
		MNP	1.02 (0.81, 1.29)	0.87	1.00 (0.79, 1.26)	0.99
	H3N2	IM	1.54 (0.94, 2.55)	0.09	1.57 (0.88, 2.80)	0.13
		MNP	1.16 (0.82, 1.64)	0.40	1.06 (0.80, 1.40)	0.70
	B	IM	1.47 (0.26, 8.29)	0.66	1.35 (0.28, 6.55)	0.71
		MNP	1.52 (0.95, 2.43)	0.08	1.14 (0.69, 1.90)	0.61

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 17A. Reaction Score and H1N1 HAI Seroconversion by Study Day

Reaction Type	Study Group	Day 28 RR* (95% CI)	p-value	Day 180 RR* (95% CI)	p-value
Unadjusted					
Global	IM	0.9 (0.5, 1.4)	0.56	1.5 (0.6, 3.5)	0.38
	MNP	1 (0.8, 1.2)	0.87	0.9 (0.5, 1.6)	0.77
Systemic	IM	0.8 (0.4, 1.4)	0.44	1.8 (0.8, 4.1)	0.15
	MNP	1.1 (1.0, 1.2)	0.08	0.8 (0.3, 2.2)	0.69
Local	IM	0.4 (0.1, 1.9)	0.25	0.7 (0.1, 3.9)	0.71
	MNP	1.0 (0.9, 1.2)	1.0	1.1 (0.7, 1.7)	0.83
Adjusted^					
Global	IM	0.9 (0.5, 1.4)	0.59	1.5 (0.7, 3.3)	0.33
	MNP	1.0 (0.8, 1.2)	0.98	0.9 (0.6, 1.5)	0.81
Systemic	IM	0.8 (0.4, 1.4)	0.41	1.8 (0.8, 3.9)	0.14
	MNP	1.1 (0.9, 1.4)	0.18	0.9 (0.3, 2.4)	0.78
Local	IM	0.4 (0.1, 2.0)	0.26	0.8 (0.2, 3.9)	0.75
	MNP	1.0 (0.8, 1.2)	0.84	1.0 (0.7, 1.6)	0.89

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated risk ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 17B. Reaction Score and H3N2 HAI Seroconversion by Study Day

Reaction Type	Study Group	Day 28 RR* (95% CI)	p-value	Day 180 RR* (95% CI)	p-value
Unadjusted					
Global	IM	1.2 (0.8, 1.8)	0.42	7.7 (1.0, 62.2)	0.06
	MNP	0.9 (0.6, 1.3)	0.45	1.4 (0.5, 4.0)	0.54
Systemic	IM	**1.5 (1.1, 2.0)**	**0.01**	3.2 (0.6, 17.9)	0.19
	MNP	0.9 (0.5, 1.7)	0.84	2.4 (0.8, 7.6)	0.12
Local	IM	**1.4 (1.1, 1.8)**	**0.01**	2.4 (0.4, 16.6)	0.36
	MNP	1 (0.8, 1.4)	0.8	1.7 (0.6, 4.6)	0.33
Adjusted^					
Global	IM	1.1 (0.8, 1.7)	0.56	7.3 (0.9, 56.5)	0.06
	MNP	0.9 (0.6, 1.2)	0.37	1.4 (0.5, 3.6)	0.52
Systemic	IM	1.3 (0.9, 1.8)	0.11	2.8 (0.5, 16.5)	0.24
	MNP	1.1 (0.8, 1.6)	0.57	**2.9 (1.4, 6.0)**	**<0.01**
Local	IM	**1.4 (1.0, 1.9)**	**0.03**	2.5 (0.3, 17.8)	0.37
	MNP	0.9 (0.7, 1.3)	0.71	1.5 (0.6, 3.8)	0.38

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated risk ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 17C. Reaction Score and B HAI Seroconversion by Study Day

Reaction Type	Study Group	Day 28 RR* (95% CI)	p-value	Day 180 RR* (95% CI)	p-value
Unadjusted					
Global	IM	1.5 (0.5, 4.8)	0.45	0.9 (0.1, 6.9)	0.88
	MNP	1.3 (0.9, 2.0)	0.15	2.1 (0.9, 5.1)	0.11
Systemic	IM	1.9 (0.6, 5.7)	0.25	3.2 (0.6, 17.9)	0.19
	MNP	1.2 (0.6, 2.2)	0.60	0.9 (0.2, 5.4)	0.92
Local	IM	1.0 (0.2, 5.8)	0.96	2.4 (0.4, 16.6)	0.36
	MNP	1.3 (0.8, 1.9)	0.26	2.3 (0.9, 5.8)	0.07
Adjusted^					
Global	IM	1.3 (0.5, 3.3)	0.52	0.7 (0.1, 6.3)	0.79
	MNP	1.3 (0.8, 2.0)	0.32	2.0 (0.9, 4.4)	0.11
Systemic	IM	1.6 (0.7, 3.9)	0.26	2.7 (0.5, 15.1)	0.25
	MNP	1.3 (0.7, 2.7)	0.43	1.0 (0.3, 3.6)	0.96
Local	IM	1.1 (0.2, 5.1)	0.92	2.5 (0.4, 15.6)	0.32
	MNP	1.0 (0.6, 1.7)	0.96	1.9 (0.7, 4.7)	0.18

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;
*Estimated risk ratio for those in the higher score category compared with those in the lower score category
^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 18A. Longitudinal Associations Between Alternative Cut Point Global Reaction Score and HAI Seroconversion

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Global (≥ 3 vs ≤ 2)	H1N1	IM	1.09 (0.67, 1.76)	0.73	1.21 (0.76, 1.93)	0.43
		MNP	0.84 (0.68, 1.04)	0.11	0.86 (0.69, 1.06)	0.16
	H3N2	IM	1.02 (0.61, 1.72)	0.94	0.98 (0.59, 1.64)	0.95
		MNP	0.88 (0.62, 1.24)	0.46	0.87 (0.64, 1.18)	0.36
	B	IM	1.57 (0.43, 5.69)	0.49	1.61 (0.53, 4.88)	0.40
		MNP	0.94 (0.58, 1.52)	0.80	0.84 (0.53, 1.32)	0.45
Global (≥ 5 vs ≤ 4)	H1N1	IM	0.96 (0.45, 2.05)	0.92	0.97 (0.47, 1.98)	0.93
		MNP	1.04 (0.80, 1.35)	0.79	1.02 (0.79, 1.31)	0.87
	H3N2	IM	**1.75 (1.12, 2.74)**	**0.01**	1.63 (0.93, 2.86)	0.09
		MNP	1.08 (0.73, 1.60)	0.69	1.04 (0.77, 1.41)	0.78
	B	IM	1.33 (0.34, 5.26)	0.68	1.21 (0.38, 3.81)	0.74
		MNP	1.71 (1.10, 2.68)	0.02	1.42 (0.91, 2.22)	0.13

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 18B. Longitudinal Associations Between Alternative Cut Point Systemic Reaction Score and HAI Seroconversion

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Systemic (≥ 1 vs 0)	H1N1	IM	1.06 (0.64, 1.73)	0.83	1.15 (0.71, 1.87)	0.57
		MNP	0.94 (0.75, 1.18)	0.60	0.95 (0.77, 1.16)	0.60
	H3N2	IM	1.04 (0.61, 1.75)	0.89	1.02 (0.61, 1.71)	0.93
		MNP	1.04 (0.73, 1.47)	0.83	1.03 (0.77, 1.37)	0.84
	B	IM	2.00 (0.45, 8.83)	0.36	2.16 (0.58, 8.05)	0.25
		MNP	0.93 (0.57, 1.50)	0.76	0.86 (0.58, 1.29)	0.47
Systemic (≥ 2 vs ≤ 1)	H1N1	IM	0.89 (0.55, 1.46)	0.65	0.95 (0.60, 1.50)	0.81
		MNP	0.93 (0.71, 1.22)	0.60	1.04 (0.80, 1.35)	0.79
	H3N2	IM	0.99 (0.58, 1.71)	0.98	1.06 (0.65, 1.74)	0.81
		MNP	0.87 (0.58, 1.32)	0.52	0.98 (0.71, 1.35)	0.90
	B	IM	1.52 (0.42, 5.44)	0.52	1.8 (0.62, 5.23)	0.28
		MNP	1.01 (0.60, 1.70)	0.97	1.38 (0.87, 2.20)	0.17

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 18C. Longitudinal Associations Between Alternative Cut Point Local Reaction Score and HAI Seroconversion

Reaction Type	Antigen	Study Group	Unadjusted RR* (95% CI)	p-value	Adjusted RR*^ (95% CI)	p-value
Local (≥ 1 vs 0)	H1N1	IM	0.75 (0.49, 1.14)	0.17	0.74 (0.50, 1.11)	0.15
		MNP	0.96 (0.61, 1.51)	0.86	0.87 (0.56, 1.34)	0.52
	H3N2	IM	0.88 (0.53, 1.46)	0.63	1.02 (0.65, 1.60)	0.95
		MNP	1.02 (0.57, 1.83)	0.94	0.93 (0.61, 1.44)	0.76
	B	IM	0.94 (0.27, 3.30)	0.92	1.01 (0.37, 2.77)	0.98
		MNP	0.74 (0.49, 1.10)	0.14	**0.42 (0.24, 0.72)**	**<0.01**
Local (≥ 2 vs ≤ 1)	H1N1	IM	1.24 (0.77, 1.99)	0.39	1.28 (0.81, 2.03)	0.30
		MNP	1.07 (0.82, 1.41)	0.61	0.98 (0.77, 1.25)	0.88
	H3N2	IM	1.64 (0.99, 2.71)	0.05	1.54 (0.97, 2.46)	0.07
		MNP	0.94 (0.66, 1.33)	0.72	0.87 (0.64, 1.17)	0.35
	B	IM	2.10 (0.63, 7.01)	0.23	1.75 (0.65, 4.69)	0.27
		MNP	1.36 (0.83, 2.23)	0.22	0.81 (0.47, 1.40)	0.44

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 19A. Alternative Cut Point Global Reaction Score and HAI Seroconversion by Study Day

Reaction Type	Antigen	Study Group	Day 28 RR* (95% CI)	p-value	Day 180 RR* (95% CI)	p-value
Unadjusted						
Global (≥ 3 vs ≤ 2)	H1N1	IM	1.0 (0.6, 1.4)	0.84	1.4 (0.5, 3.5)	0.51
		MNP	1.0 (0.8, 1.1)	0.76	0.7 (0.4, 1.0)	0.08
	H3N2	IM	0.9 (0.6, 1.3)	0.54	2.4 (0.3, 19.7)	0.43
		MNP	0.9 (0.7, 1.2)	0.38	0.9 (0.3, 2.4)	0.77
	B	IM	1.3 (0.4, 4.3)	0.66	2.4 (0.3, 19.7)	0.43
		MNP	0.9 (0.6, 1.3)	0.50	1.1 (0.4, 3.0)	0.78
Adjusted^						
Global (≥ 3 vs ≤ 2)	H1N1	IM	1.1 (0.7, 1.7)	0.74	1.5 (0.6, 3.7)	0.34
		MNP	1.0 (0.8, 1.2)	0.87	0.7 (0.4, 1.0)	0.07
	H3N2	IM	0.8 (0.5, 1.3)	0.43	2.3 (0.3, 18.5)	0.44
		MNP	0.9 (0.6, 1.2)	0.37	0.8 (0.3, 2.2)	0.74
	B	IM	1.4 (0.5, 4.1)	0.49	2.6 (0.3, 21.5)	0.38
		MNP	0.8 (0.5, 1.3)	0.33	1.0 (0.4, 2.5)	0.92
Unadjusted						
Global (≥ 5 vs ≤ 4)	H1N1	IM	0.7 (0.3, 1.5)	0.36	1.5 (0.6, 3.7)	0.37
		MNP	1 (0.8, 1.2)	0.76	1.1 (0.7, 1.9)	0.59
	H3N2	IM	**1.4 (1.1, 1.9)**	**0.01**	4.0 (0.7, 21.8)	0.11
		MNP	0.9 (0.6, 1.3)	0.70	1.7 (0.6, 4.9)	0.31
	B	IM	1.3 (0.4, 4.7)	0.66	1.3 (0.2, 10.3)	0.78
		MNP	1.4 (1.0, 2.1)	0.06	**2.6 (1.1, 6.2)**	**0.03**
Adjusted^						
Global (≥ 5 vs ≤ 4)	H1N1	IM	0.7 (0.4, 1.5)	0.38	1.5 (0.7, 3.6)	0.31
		MNP	1.0 (0.8, 1.2)	0.72	1.1 (0.7, 1.8)	0.60

	H3N2	IM	1.3 (0.9, 1.8)	0.11	3.7 (0.6, 21.2)	0.14
		MNP	0.9 (0.6, 1.2)	0.49	1.6 (0.6, 4.2)	0.31
	B	IM	1.2 (0.4, 3.3)	0.72	1.2 (0.2, 9.3)	0.86
		MNP	1.2 (0.8, 2.0)	0.34	2.2 (1.0, 5.1)	0.05

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 19B. Alternative Cut Point Systemic Reaction Score and HAI Seroconversion by Study Day

Reaction Type	Antigen	Study Group	Day 28 RR* (95% CI)	p-value	Day 180 RR* (95% CI)	p-value
Unadjusted						
Systemic (≥ 1 vs 0)	H1N1	IM	1.0 (0.7, 1.5)	1.0	1.2 (0.5, 3.0)	0.75
		MNP	1.0 (0.8, 1.1)	0.65	0.9 (0.6, 1.4)	0.67
	H3N2	IM	0.9 (0.6, 1.4)	0.69	2 (0.2, 16.6)	0.52
		MNP	0.9 (0.7, 1.3)	0.67	1.5 (0.5, 4.4)	0.48
	B	IM	2.0 (0.5, 8.0)	0.33	2.0 (0.2, 16.6)	0.52
		MNP	0.9 (0.6, 1.4)	0.63	1.0 (0.4, 2.5)	0.98
Adjusted^						
Systemic (≥ 1 vs 0)	H1N1	IM	1.1 (0.7, 1.7)	0.69	1.3 (0.5, 3.1)	0.59
		MNP	1.0 (0.8, 1.1)	0.71	0.9 (0.6, 1.4)	0.66
	H3N2	IM	0.9 (0.6, 1.4)	0.66	2.0 (0.2, 16.0)	0.52
		MNP	0.9 (0.7, 1.2)	0.63	1.5 (0.5, 3.9)	0.45
	B	IM	2.2 (0.6, 7.6)	0.23	2.2 (0.3, 17.7)	0.48
		MNP	0.9 (0.6, 1.3)	0.44	0.9 (0.4, 2.1)	0.86
Unadjusted						
Systemic (≥ 2 vs ≤ 1)	H1N1	IM	0.9 (0.6, 1.3)	0.56	0.9 (0.4, 2.2)	0.82
		MNP	1.0 (0.8, 1.2)	0.81	0.9 (0.5, 1.5)	0.59
	H3N2	IM	0.8 (0.5, 1.3)	0.31	3.2 (0.4, 27.2)	0.28
		MNP	1.0 (0.7, 1.4)	0.82	0.6 (0.1, 2.4)	0.47
	B	IM	1.8 (0.5, 6.0)	0.33	1.1 (0.2, 6.5)	0.93
		MNP	1.1 (0.7, 1.7)	0.67	0.8 (0.3, 2.5)	0.71
Adjusted^						
Systemic (≥ 2 vs ≤ 1)	H1N1	IM	0.9 (0.6, 1.4)	0.78	1.0 (0.4, 2.2)	0.92
		MNP	1.1 (0.9, 1.3)	0.42	0.9 (0.5, 1.7)	0.86

	H3N2	IM	0.8 (0.5, 1.3)	0.42	3.5 (0.4, 27.5)	0.24
		MNP	1.1 (0.8, 1.5)	0.63	0.7 (0.2, 2.4)	0.54
	B	IM	2.0 (0.7, 5.5)	0.18	1.2 (0.2, 6.9)	0.84
		MNP	1.5 (0.9, 2.3)	0.12	1.1 (0.4, 3.0)	0.89

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Table 19C. Alternative Cut Point Local Reaction Score and HAI Seroconversion by Study Day

Reaction Type	Antigen	Study Group	Day 28 RR* (95% CI)	p-value	Day 180 RR* (95% CI)	p-value
Unadjusted						
Local (≥ 1 vs 0)	H1N1	IM	**0.7 (0.5, 1.0)**	**0.03**	0.8 (0.3, 2.0)	0.67
		MNP	1.2 (0.8, 1.9)	0.44	0.7 (0.4, 1.2)	0.22
	H3N2	IM	0.8 (0.5, 1.2)	0.30	1.4 (0.2, 11.5)	0.75
		MNP	1.0 (0.6, 1.6)	0.96	1.2 (0.2, 7.3)	0.87
	B	IM	0.8 (0.2, 2.5)	0.68	1.4 (0.2, 11.5)	0.75
		MNP	**0.6 (0.5, 0.8)**	**<0.01**	1.4 (0.2, 8.6)	0.72
Adjusted^						
Local (≥ 1 vs 0)	H1N1	IM	0.7 (0.5, 1.0)	0.06	0.8 (0.4, 1.9)	0.66
		MNP	1.1 (0.7, 1.6)	0.78	0.6 (0.4, 1.1)	0.08
	H3N2	IM	0.9 (0.6, 1.4)	0.70	1.6 (0.2, 12.8)	0.64
		MNP	0.9 (0.6, 1.4)	0.66	1.1 (0.2, 5.4)	0.94
	B	IM	0.9 (0.4, 2.3)	0.86	1.7 (0.2, 13.9)	0.64
		MNP	**0.4 (0.2, 0.6)**	**<0.01**	0.8 (0.1, 5.8)	0.86
Unadjusted						
Local (≥ 2 vs ≤ 1)	H1N1	IM	1.0 (0.7, 1.5)	1.0	1.8 (0.7, 4.3)	0.19
		MNP	1.1 (0.9, 1.4)	0.29	1.0 (0.6, 1.7)	0.97
	H3N2	IM	1.3 (0.9, 2.0)	0.15	4.5 (0.5, 37.4)	0.16
		MNP	0.9 (0.7, 1.2)	0.34	1.2 (0.4, 3.9)	0.75
	B	IM	1.5 (0.5, 4.7)	0.48	4.5 (0.5, 37.4)	0.16
		MNP	1.0 (0.6, 1.5)	0.84	5.5 (0.8, 38.2)	0.09
Adjusted^						
Local (≥ 2 vs ≤ 1)	H1N1	IM	1.0 (0.7, 1.6)	0.83	1.9 (0.8, 4.3)	0.13
		MNP	1.0 (0.8, 1.2)	0.81	0.9 (0.6, 1.5)	0.75

	H3N2	IM	1.3 (0.9, 1.9)	0.24	4.2 (0.5, 33.3)	0.17
		MNP	0.8 (0.6, 1.1)	0.11	1.1 (0.4, 3.4)	0.84
	B	IM	1.4 (0.6, 3.4)	0.45	4.2 (0.5, 35.8)	0.19
		MNP	0.7 (0.4, 1.1)	0.13	3.8 (0.5, 28.1)	0.20

HAI: hemagglutination inhibition; RR: risk ratio; CI: confidence interval; IM: intramuscular; MNP: microneedle patch;

*Estimated risk ratio for those in the higher score category compared with those in the lower score category

^Adjusted for body mass index category, sex, prior inactivated influenza vaccination, race, ethnicity, and study day

Figures

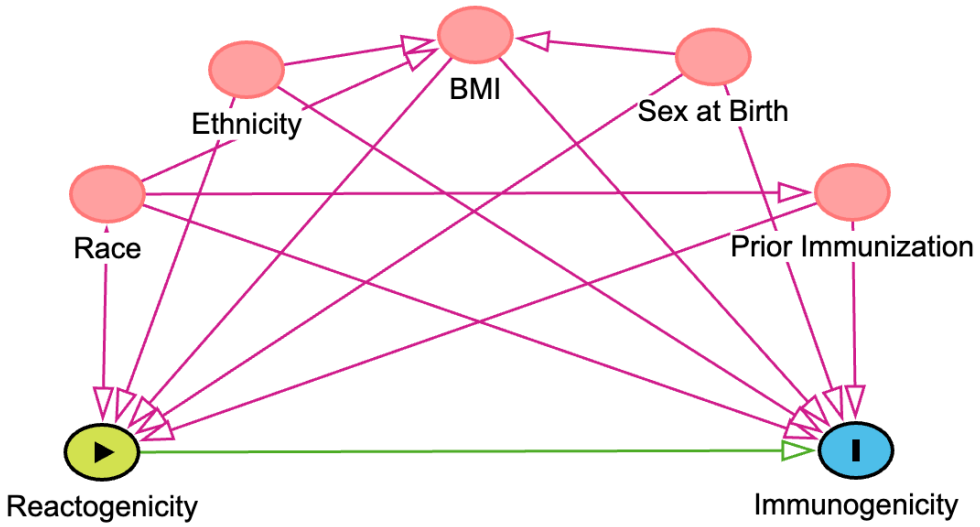

Figure 1. Conceptual Model. Directed acyclic graph (DAG) of the relationship between reactogenicity (green, exposure) and immunogenicity (blue, outcome) with confounding variables (salmon).

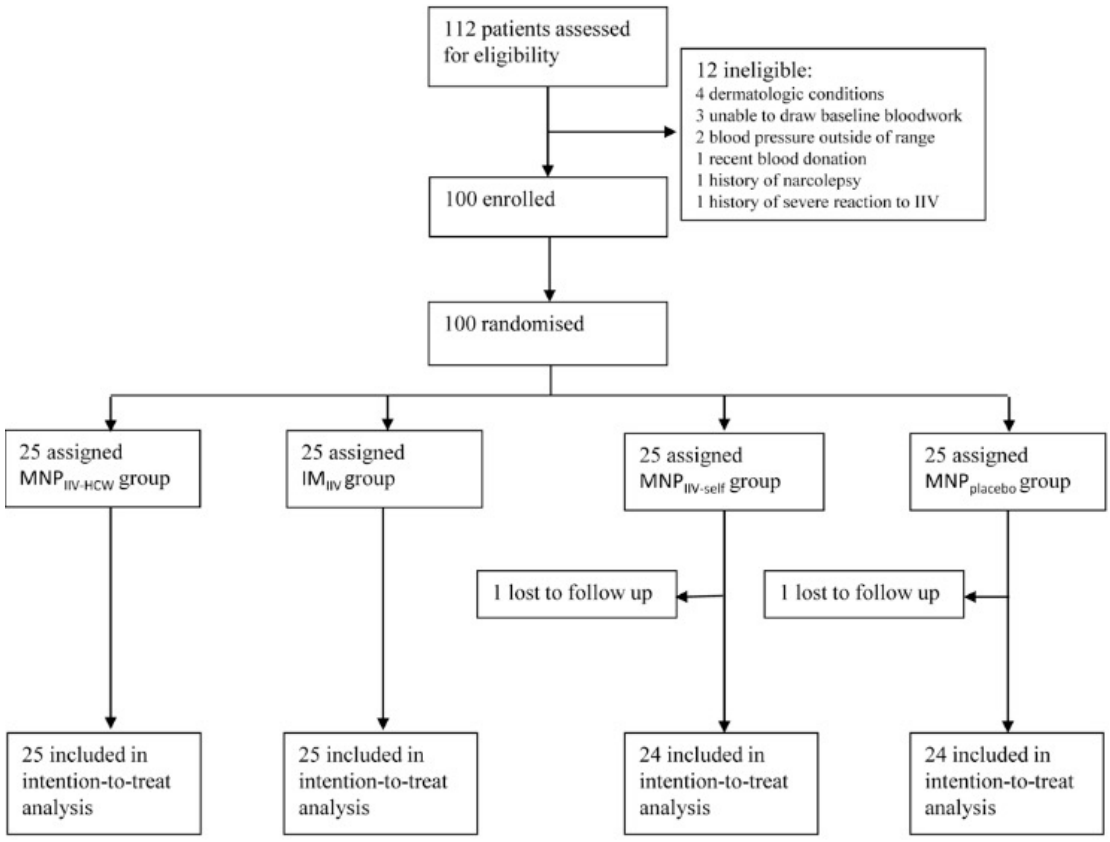

Figure 2. Parent Study CONSORT Diagram. CONSORT (flow) diagram from TIV-MNP 2015[54].

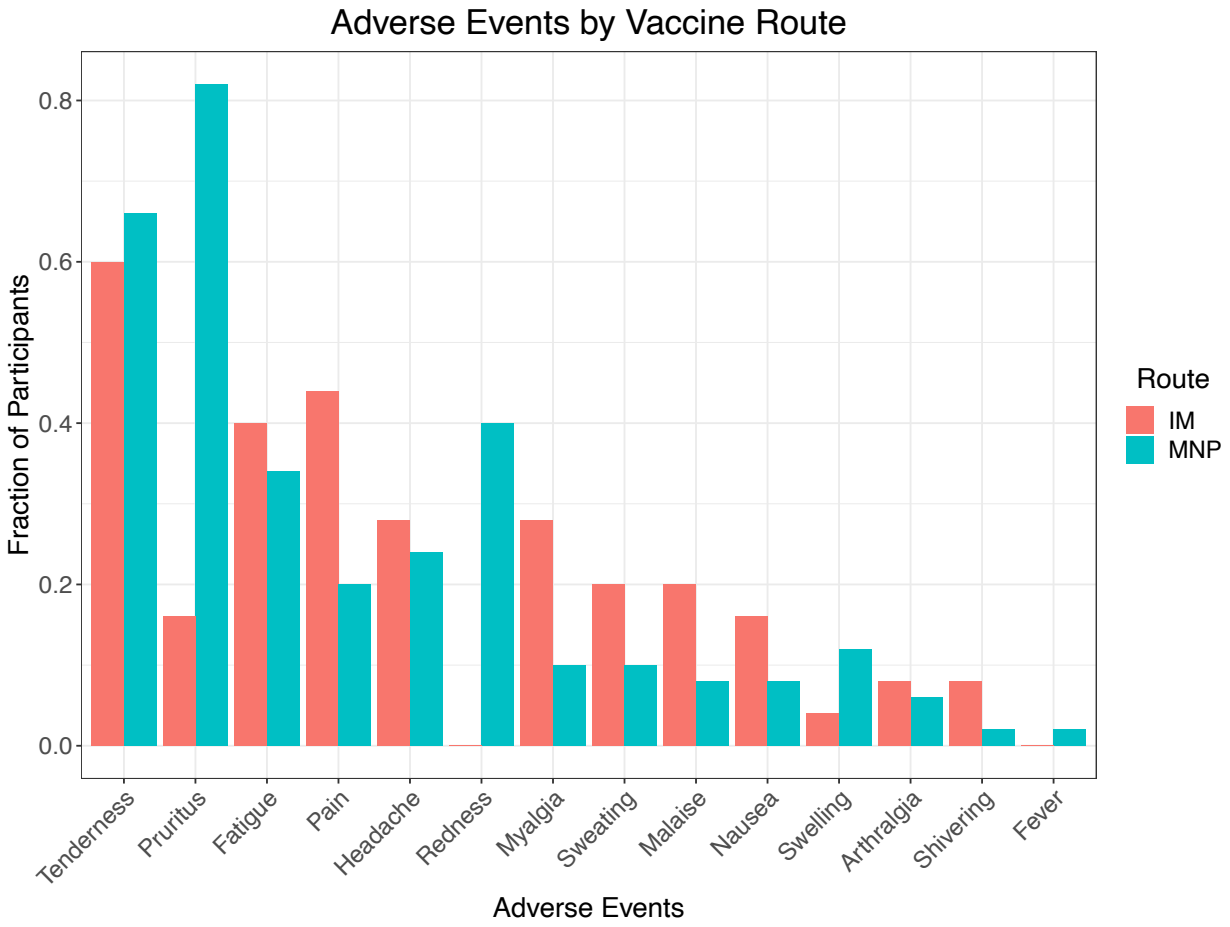

Figure 3. Adverse Events by Vaccine Route. Fraction of participants in the intramuscular (IM, red) and microneedle patch (MNP, teal) group who developed each type of adverse event.

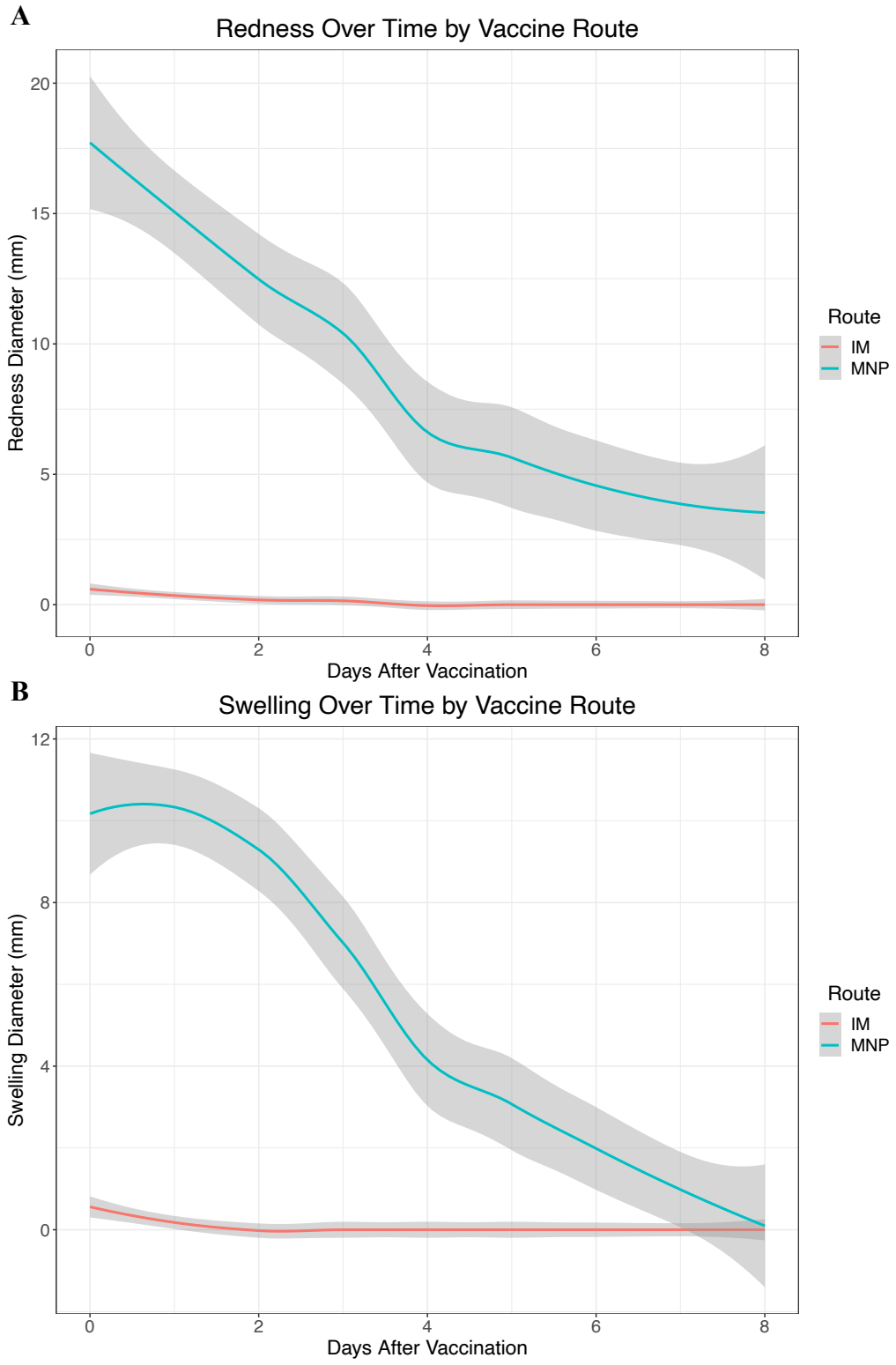

Figure 4. Select Local Adverse Events by Time and Vaccine Route. Redness (A) and swelling (B) diameter in millimeters over time after vaccination for those in the intramuscular (IM, red) and microneedle patch (MNP, teal) groups.

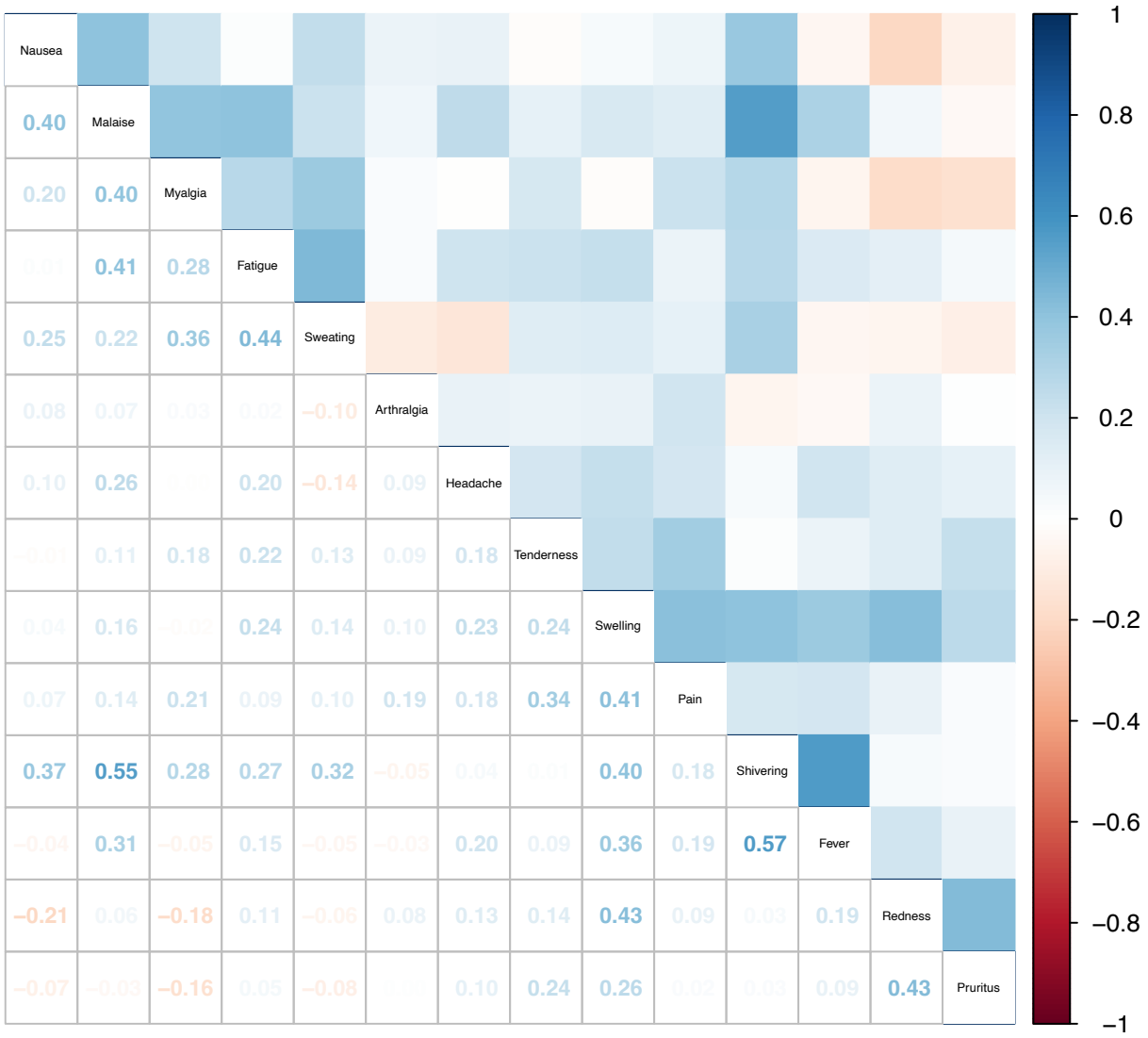

Figure 5. Correlation Between Adverse Events. Correlation plot of all solicited adverse event types.

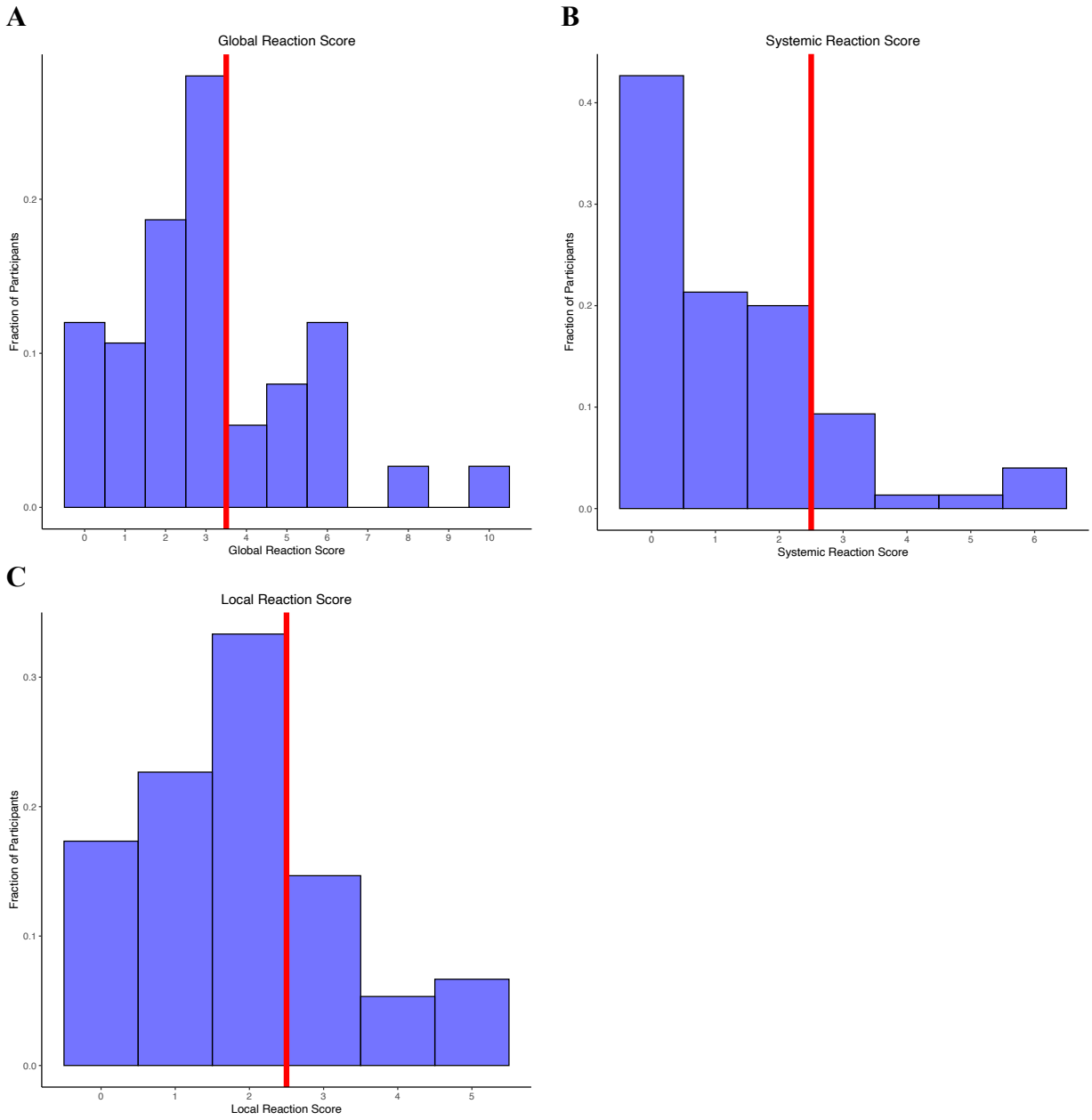

Figure 6. Reaction Score Univariable Distributions. Histograms of the Global (A), Systemic (B), and Local (C) reaction scores. Vertical red lines depict the dichotomization cut points chosen for the primary analyses.

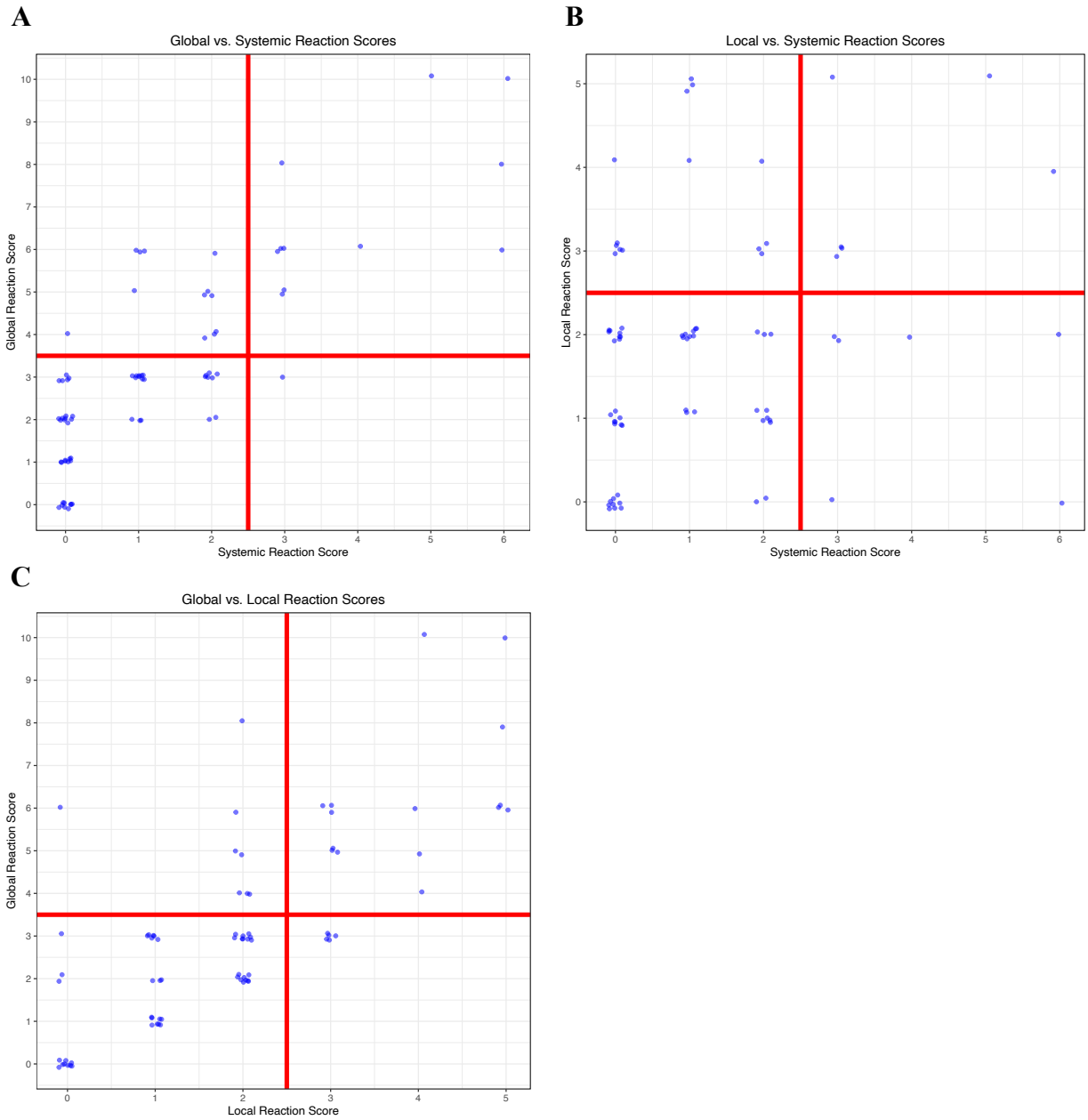

Figure 7. Reaction Score Bivariable Distributions. Scatterplots of the Global and Systemic (A), Local and Systemic (B), and Global and Local (C) reaction scores. Red lines depict the dichotomization cut points chosen for the primary analyses.

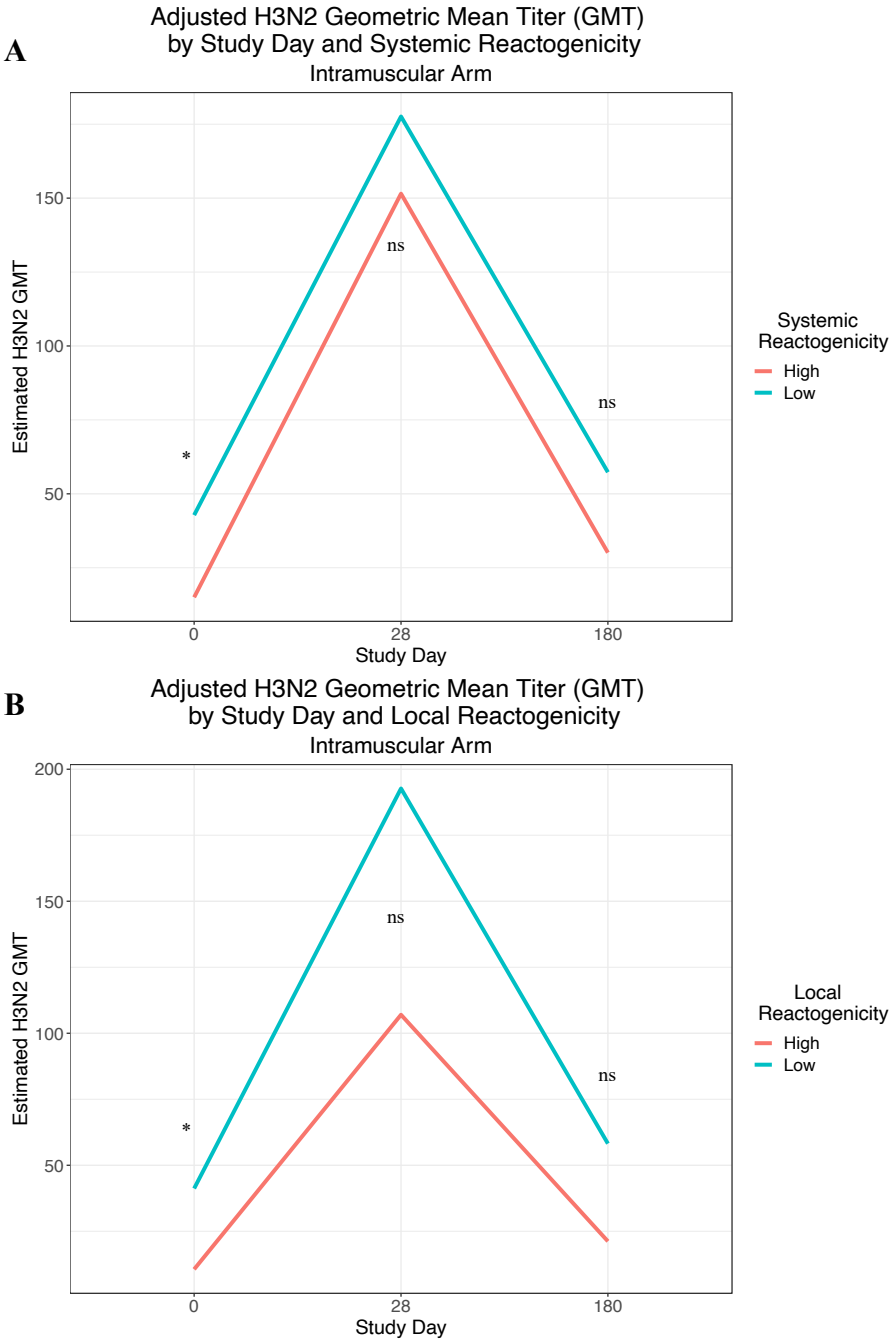

Figure 8. H3N2 HAI Titer by Time and Reaction Score. Estimated adjusted geometric mean titer against the H3N2 antigen of intramuscular seasonal inactivated influenza vaccine by study day, comparing low and high score levels for systemic reactogenicity (A) and local reactogenicity (B). * p = 0.01.

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