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Effect of Medication Usage on Immune Response to Inactivated Influenza Vaccine among Residents of a Virginia Life Care Facility, 1995-96

By

Patrick Gallagher
MPH

Global Epidemiology

_____
Kevin Sullivan, PhD, MPH, MHA
Committee Chair

_____
Lynnette Brammer, MPH
Committee Member

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By

Patrick Gallagher

BBA
University of Notre Dame
2005

Thesis Committee Chair:
Kevin Sullivan, PhD, MPH, MHA

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Abstract

Effect of Medication Usage on Immune Response to Inactivated Influenza Vaccine among Residents of a Virginia Life Care Facility, 1995-96

By Patrick Gallagher

Background: It is widely recognized that the immune response to influenza vaccination in the elderly may be influenced by medication usage. Previous studies have pointed to immunosuppresants, including steroids, as medications which hinder immune response to influenza vaccination.

Objective: To determine if medication use is associated with the height and persistence of immune response to influenza vaccination in the elderly.

Methods: In 1995, in a life care facility for the elderly in Virginia, 64 subjects received an inactivated, trivalent influenza vaccine. In 1996, 68 subjects received a similar vaccine at the same facility. The majority of subjects were self-sufficient and living in independent apartments. Both vaccines contained A(H1N1), A(H3N2), and B components. Blood sera samples were collected at the time of vaccination, and 1, 2, 3, 4, 12, 20, and 28 weeks post vaccination. Hemagglutination inhibition antibody titers were determined from the sera. A prospective cohort study design was employed. All of the subjects' medications were categorized according to their medicinal purpose into four categories: 1) cardiac anti-hypertensives, 2) anti-inflammatory analgesics, 3) steroids, and 4) bronchodilators. The outcomes of interest were seroprotection and seroconversion. Kruskal-Wallis tests were used to examine HI antibody titers in users of specific medication categories compared to non-users. Chi-square tests were used to examine seroprotection and seroconversion in users compared to non-users. Logistic regression was used to determine which predictors best explained seroprotection and seroconversion.

Results: In both vaccine years, subjects were mostly female, over 80 years old, possessed a normal BMI, and had received influenza vaccine each of the previous five years. In 1995, at at least one point during the four weeks after vaccination, 67.2% were seroprotected against A(H1N1), 43.8% against A(H3N2), and 68.8% against influenza B. In 1996, at at least one point during the four weeks after vaccination, 72.1% were seroprotected against A(H1N1), 44.1% against A(H3N2), and 77.9% against B. No medications demonstrated a conclusive effect on seroprotection or seroconversion.

Conclusions: There was no clear evidence that any of the medications examined led to a consistent, discernible effect on immune response to the influenza vaccine.

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Table of Contents

BACKGROUND

Influenza is a contagious respiratory disease of moderate severity in immunocompetent persons, but potentially severe in the immunocompromised, including the elderly. Recent years have reported as many as 55,000 influenza-related deaths, with the majority occurring in persons over age 65 years (1).

While public health officials currently recommend near universal annual influenza vaccination, they emphasize vaccination among the elderly because of their poorer immune responses and more severe health outcomes (1, 2). The importance of influenza immunization in older individuals is substantial, as research suggests that influenza vaccination is associated with a 10% to 50% reduction in mortality among community-dwelling elderly persons (3, 4).

However, influenza vaccination does not always lead to protection in the elderly. It is widely recognized that the immune response in the elderly may be influenced by other factors, including medication usage, underlying health conditions, and influenza vaccination history (1).

In 1995, in a life care facility for the elderly in Virginia, 64 subjects received an inactivated, trivalent influenza vaccine. In 1996, 68 subjects received a similar vaccine at the same facility. Information, including medication usage, was gathered from the subjects. Determining if medication use is associated with the height and persistence of immune response to influenza vaccination in the elderly is the primary goal of this study.

Literature Review

Published, peer-reviewed papers which evaluated the immune response to the influenza vaccine *in the elderly* (defined as a mean age over 60 years), as influenced by their

medications, were identified through PubMed. Only studies published in English were considered.

Most studies compared blood sera drawn on the day of vaccination and 3-6 weeks after vaccination to evaluate immune response. All studies used the same criteria for evaluating immune response: seroprotection, defined as hemagglutination inhibition (HI) antibody titers ≥1:40 after vaccination; and seroconversion, defined as a 4-fold increase in HI titers after vaccination.

One study examined 104 subjects receiving warfarin, an anticoagulant, and their immune response to the 2004-05 trivalent influenza vaccine. The mean age of the subjects was 71.3 years. Sera were evaluated for HI antibodies before and 28 days after vaccination. Seroprotection 28 days after vaccination ranged from 92.0% of subjects against the A(H1N1) component to 100.0% against the A(H3N2) component. Seroconversion ranged from 33.0% against A(H1N1) to 82.0% against A(H3N2). Precise seroprotection and seroconversion figures for influenza B were not supplied. There was no comparison group (i.e. no individuals not receiving warfarin) (5).

Another study from the same author examined the 1995-96 trivalent influenza vaccination in 131 elderly Italian females receiving "mostly" antihypertensive/inotropic drugs and benzodiazepines. The mean age of the subjects was 77.3 years. Sera were drawn before and 30 days after vaccination. Seroprotection 30 days after vaccination ranged from 44.3% of subjects against the A(H1N1) component to 61.8% against the influenza B component. Seroconversion data were not published. The authors noted significantly better immune responses in women who resided in a nursing home versus those who did not, but no non-medicated comparison groups were included (6).

A study of 146 subjects compared the response of cancer patients, some of whom were taking myelosuppressive chemotherapy, to the monovalent pandemic A(H1N1) 2009 vaccine. The mean age of the subjects on therapy was 62.4 years. Sera from before and 2-6 weeks after vaccination were compared. Seroprotection 2-6 weeks after vaccination for those on myelosuppressive chemotherapy was 79.0% compared to 90.5% not on therapy, but the difference was not statistically significant. Similarly, seroconversion for those on myelosuppressive chemotherapy was 72.2% compared to 87.0% not on therapy, but the difference was not significant (7).

A study of 162 German subjects examined the immune response to the 2001-02 trivalent influenza vaccine. All subjects had chronic obstructive pulmonary disease, and 74% were taking prednisolone or inhaled corticosteroids. The mean age of the subjects was 71.3 years. Sera were evaluated before vaccination and 4 and 24 weeks after vaccination. Assessing seroprotection and seroconversion against all three antigens 4 weeks and 24 weeks after vaccination, no significant differences in immune response were found between subjects taking prednisolone, inhaled steroids, or no medication (8).

One hundred Canadian patients were randomized to receive acetaminophen prophylaxis or a placebo after 1990-91 trivalent vaccine administration. The mean age was 73 years. Each treatment group experienced statistically similar immune responses (seroprotection and seroconversion) across antigens when blood sera were analyzed 4-6 weeks after vaccination (9).

The effect of aspirin (acetylsalicylic acid) has also been studied. A randomized controlled trial with 281 patients (mean age 76 years) examined aspirin as an adjuvant to trivalent influenza vaccination. Subjects receiving aspirin experienced seroconversion against the

A(H3N2) antigen more often than subjects not receiving aspirin ($p<0.05$). The difference was even greater in subjects over 75 years ($P<0.01$) (10).

A broader literature review, examining papers which evaluated the antibody response to influenza vaccine in medicated populations of *any age* - not only the elderly - was also conducted.

Immunosuppressants usually hinder immune responsiveness to an influenza vaccine, at least to the pandemic A(H1N1) 2009 vaccine. Patients with rheumatoid arthritis and similar autoimmune diseases receiving immunosuppressant therapy showed significantly less seroprotection and seroconversion than those not on immunosuppressant medications (11). Subjects with systemic lupus erythematosus receiving immunosuppressant therapy demonstrated a similar impaired immune response (12, 13). Children with inflammatory bowel disease demonstrated a similar impairment in response to the 2005-06 trivalent vaccine (14). Recent kidney transplant recipients who received a trivalent influenza vaccine demonstrated a similar response, especially with the use of mycophenolate mofetil (MMF) (15).

A systematic review of the effect of various immunosuppressants (methotrexate, anti-TNF agents, adalimumab, disease modifying anti-rheumatic drugs, rituximab, mycophenalate mofetil, prednisone, and azathioprine) on the immune response to trivalent influenza vaccines reached the conclusion that immunosuppressive medications may partially dampen responses, especially when multiple medications are used concurrently (16). Nevertheless, at least one study not in the systematic review (due to its monovalent vaccine focus) found no association between immunosuppressant use (specifically, the glucocorticoid prednisone) and the immune response to the pandemic A(H1N1) 2009 vaccine (17).

Rheumatoid arthritis patients on anti-rheumatic medication showed significantly less seroprotection and seroconversion compared to those not on the medication. Abatacept, rituximab, and methotrexate were particular hindrances to immune response (11, 18).

Glucocorticoids have also been shown to hinder pandemic A(H1N1) 2009 vaccine immune response in the non-elderly. Studies examining patients with autoimmune diseases and systemic lupus erythematosus both showed significantly less seroprotection and seroconversion in glucocorticoid users compared to those not on glucocorticoid medications (11, 12, 17, 18). It is not clear whether glucocorticoids are a greater hindrance than other immunosuppressants, however (12).

Several drugs have not shown a significant effect on immune response in the non-elderly: the antimalarial chloroquine had no significant effect on pandemic A(H1N1) 2009 vaccine immune response in older children (12). Similarly, in younger children, the antipyretics ibuprofen and paracetamol showed no effect on the response to the pandemic A(H1N1) 2009 vaccine (19). Combinations of cytostatic chemotherapy drugs in a range of Polish adults have also been shown to have no significant effect on the 1995-96 trivalent vaccine response (20). A randomized control trial showed supplemental vitamin D had no effect on response to the 2008-09 trivalent vaccine in HIV-infected adults (21).

METHODS

In 1995, in a life care facility for the elderly in Virginia, 64 subjects received an inactivated, trivalent influenza vaccine. In 1996, 68 subjects received a similar vaccine at the same facility. Information, including medication usage, was gathered from the subjects. Determining if medication use is associated with the height and persistence of immune response to influenza vaccination in the elderly is the primary goal of this study.

Vaccine Administration

The three components of the 1995-96 vaccine (administered to participants in mid-November 1995) were A/Texas/36/91 (H1N1), A/Johannesburg/33/94 (H3N2), and B/Harbin/07/94. The three components of the 1996-97 vaccine (administered to participants in mid-October 1996) were A/Texas/36/91 (H1N1), A/Nanchang/933/95 (H3N2), and B/Harbin/07/94. Subjects received the vaccine in a dosage of 45 mcg (15 mcg per antigen), administered by the study nurse employed by the life care facility. The manufacturer of the vaccines was not recorded.

Data and Data Collection

Subjects were recruited from all residents of a life care facility in Virginia. The life care facility included independent apartments, an assisted living facility, and a skilled 24-hour nursing care facility. The majority of residents were self-sufficient and living in independent apartments. Data collectors initially sought 100 people to join the study each year. Recruiting was conducted through short presentations in common areas at the facility, with a large majority of residents present.

Data collection took place on or near the day of vaccination as noted above. Subjects were voluntarily enrolled by signing a three-page consent agreement (Appendix III), without any incentives.

Blood sera samples were drawn by a study nurse employed at the life care facility. Sera samples were collected at the time of vaccination, and 1, 2, 3, 4, 12, 20, and 28 weeks post vaccination. HI titers against the three antigens in each year's influenza vaccine were determined at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA. The HI assay was conducted by preparing two-fold serial dilutions of a virus, mixed with red blood cells, and added to the wells of a plastic tray. The red blood cells that are not bound by influenza virus sink to the bottom of a well and form a button. The red blood cells that are attached to influenza virus particles form a lattice that coats the well. The assay can be performed within 30 minutes, and is therefore a quick indicator of the relative quantities of virus particles (22).

The study nurse also administered questionnaires. Subjects' medication usage at the time of vaccination was queried and visually confirmed. No data were collected on medication dosage or frequency or length of use.

Data on each subject's sex, race, age (in years), height (in inches), weight (in pounds), year of entrance into the life care facility, and residence location (independent apartment, assisted living, or 24-hour nursing care unit) were collected.

Additionally, data about each subject's health status was obtained on a yes/no basis on the following: arteriosclerosis, angina, myocardial infarction, congestive heart failure, hypertension, peripheral vascular disease, other heart disease, chronic lung disease, diabetes, insulin use, hypothyroidism, kidney disease, current dialysis use, cancer, diseases of immune system, mental depression, arthritis, osteoporosis, degenerative join

disease, other bone or join disease, stroke history, Parkinson's disease, multiple sclerosis, other neurologic/neuromuscular disorder, anemia, and other blood disorders.

Influenza vaccination history over the previous five years was collected, and classified as never, 1-2 times, 3-4 times, or 5 times. History of any pneumococcal vaccination was also collected.

In 1996 only, data on subjects' current smoking status were collected. They were not included in any analysis, as only one year of data was obtained.

Lastly, data on subjects' activity level were collected, and classified as ambulatory and active, ambulatory and sedentary, wheelchair and active, wheelchair and inactive, not ambulatory or mobile, or bedridden.

Several (n=37) subjects were a part of the study during both the 1995 and 1996 vaccine years. All other subjects (n=58) participated in only one of the vaccine years.

CDC's Institutional Review Board (IRB) gave approval to conduct the study in the early 1990s. Emory University's IRB gave an exemption for analysis of a de-identified dataset.

Statistical Analysis

A prospective cohort design was used in this study. In 1995, data were collected on 70 subjects. Five subjects were excluded from analysis because sera were not taken at vaccination. One more subject was excluded because there was serologic evidence that she acquired influenza infection during follow-up. Analyses were conducted on the remaining 64 subjects.

In 1996, data were collected on 74 subjects. Six subjects were excluded because there was serologic evidence that they acquired influenza infection during follow-up. Analyses were conducted on the remaining 68 subjects.

Descriptive statistics were performed on HI titers, medication usage, and several other characteristics of the subjects.

All of the subjects' medications were categorized according to their medicinal purpose into four main categories: 1) cardiac anti-hypertensives, 2) anti-inflammatory analgesics, 3) steroids, and 4) bronchodilators.

All subjects were considered either 1) users of an aforementioned medication category, or 2) non-users of that category.

Hemagglutination inhibition (HI) IgG antibody titer is the most established correlate of vaccine protection (1). This study used HI titers as the outcome measure of immune response. Three standard measures were examined:

1. Seroprotection: HI antibody titers $\geq$ 1:40 post-vaccination;

2. Seroconversion: a 4-fold increase in antibody titers;

3. Geometric mean titer (GMT, the mean factor increase in antibody titer) of HI antibody achieved post-vaccination. GMT is the standard method of reporting mean titers for groups. It is calculated by taking the group mean of the log of each individual's titer, then the antilog of that group mean.

Seroprotection and seroconversion are the main measures of immune response outcome used in this study. Seroconversion is a less consistent measure because it does not take

into account subjects with high titers at baseline, for whom it will be very difficult to have a 4-fold increase in antibody titers.

Most analysis was limited to four time points: the time of vaccination, and two, three, and twelve weeks after vaccination. The study team concluded these time-points were an adequate representation of the baseline, height, and persistence of HI titers.

Kruskal-Wallis tests were conducted comparing the mean titer ranking scores of specific medication category users to non-users. Titers were examined by each vaccine year's antigens. The Kruskal-Wallis test was used because the outcome, HI titer, is an ordinal variable with values of 5, 10, 20, 40, 80, 160, 320, etc.

Chi-square tests, using seroprotection as the dichotomous outcome, and medication category as the dichotomous exposure, were conducted. Odds ratios, 95% confidence intervals, and two-sided Mantel-Haenszel or Fisher's Exact p-values were calculated. Odds ratios were calculated as the odds of seroprotection among subjects using a certain medication category divided by the odds of seroprotection among subjects not using a certain medication category. Tests were conducted for each antigen in each year. The same tests were done with seroconversion as the outcome.

All associations yielding statistically significant results were assessed for interaction and confounding by age, sex, baseline HI titers, body mass index, physical activity level, and recent influenza vaccination history. Interaction was assessed using p-values from the Breslow-Day test for differing odds ratios across strata. Confounding was assessed using the standard data-based "10% rule": if there was a ≥10% difference between the crude and adjusted odds ratios, confounding was deemed to exist and the adjusted odds ratio was reported. Otherwise, the crude odds ratio was reported.

Logistic regression was performed. One set of models examined the dichotomous outcome of seroprotection, while a second set examined dichotomized seroconversion. For both sets of models, the predictor of interest was medication use category.

Additional exposures evaluated as potential confounders included age, baseline HI titers, body mass index, physical activity level, and recent influenza vaccination history. Due to concerns about the small sample size and therefore the stability of estimates, only relevant confounders were included. Based on the approach described below, these were deemed to be baseline HI titers, body mass index, and recent influenza vaccination history. Age was not considered a potential confounder due to high statistical non-significance when evaluating models and relative biological irrelevance in this particular study. (See Appendix IV for further details.)

Interaction terms were created with each of these variables and the exposure of interest, medication category.

The hierarchical backward elimination approach was used to reduce the initial model containing all possible confounders and interaction terms to a final model containing only statistically significant interaction terms, their lower order components, relevant confounders, and the predictor of interest. After logistic regression was performed, only the association between the exposure of interest (medication category) and the outcome (seroprotection or serconversion) was reported.

Data Manipulation

For 1995 and 1996, age was dichotomized as >82 years or ≤82 years, which was the approximate mean age of subjects.

Individual baseline HI titers were dichotomized at that antigen's sample median (HI titers are ordinal variables with values of 5, 10, 20, 40, 80, 160, 320, etc., so dichotomizing at the mean was not appropriate). In 1995, for the A(H1N1) and B antigens, they were split as >20 or ≤20. For A(H3N2), they were split as >5 or ≤5. In 1996, for the A(H1N1) and B antigens, they were split as >40 or ≤40. For A(H3N2), they were split as >10 or ≤10.

Body mass index (BMI) was dichotomized as ≥25.0 or <25.0, the standard cutoff between normal and overweight BMI. BMI is calculated as (weight in kilograms / height in meters^2).

Subjects' influenza vaccination history in the last five years was dichotomized as "vaccinated all five years" or "not vaccinated all five years".

Physical activity level of subjects was dichotomized as "ambulatory and active" or "not ambulatory and/or not active".

All tests for significance were conducted at a 5% alpha level.

All statistical analyses were conducted using SAS version 9.3, SAS Institute Inc., Cary, NC.

RESULTS

Descriptive statistics were performed on HI titers, medication usage, and several other characteristics of the subjects (Table 1). In both vaccine years, subjects were mostly female, over 80 years old, possessed a normal BMI, and had received influenza vaccine each of the previous five years.

During the 1995-96 influenza season, 335 sera were collected from 64 participants (5.2 sera per person). During the 1996-97 influenza season, 434 sera were collected from 68 participants (6.4 sera per person). In 1995, geometric mean titers against the A(H3N2) and B antigens peaked two weeks after vaccination, while A(H1N1) titers peaked in the third week (Figure A). In 1996, geometric mean titers against all three antigens peaked three weeks after vaccination (Figure B).

In 1995, the most common HI titer level at vaccination for A(H1N1) and B was 20 (29.7% and 28.1% of subjects, respectively). For A(H3N2), 56.3% of subjects had an HI titer of 5 on the day of vaccination (Figure C).

In 1996, the most common HI titer levels at vaccination for A(H1N1) were 20 and 40 (both at 23.5% of subjects). For A(H3N2), 44.1% of subjects had an HI titer of 5 on the day of vaccination. For influenza B, 23.5% of subjects had an HI titer of 80 on the day of vaccination, followed by 20.6% of subjects at an HI titer level of 20 (Figure D).

In 1995, seroprotection against influenza B was most common, followed by A(H1N1). Of subjects for which sera were obtained, 79.5% demonstrated protective titers against influenza B two weeks after vaccination, with 56.1% maintaining titers ≥ 40 after 12 weeks (Figure E). Seroconversion against A(H3N2) two and three weeks after vaccination occurred for 48.7% and 46.3% of subjects, respectively (i.e. their A(H3N2)

titers increased at least 4-fold over baseline two and three weeks after vaccination). Serum antibody levels of all subjects returned to less than 4-fold of baseline values by 20 weeks post-vaccination for the H1N1 antigen (Figure F).

In 1996, the trends were similar, with seroprotection against influenza B most common, followed by A(H1N1). Of subjects for which sera were obtained, 82.7% were seroprotected against influenza B three weeks after vaccination, with 75.5% seroprotected after 12 weeks (Figure G). Influenza A(H3N2) experienced the highest level of seroconversion, followed by B (Figure H).

Highlights described above are also available in table format (Table 2). In 1995, at at least one point during the four weeks after vaccination, 67.2% were seroprotected against A(H1N1), 43.8% against A(H3N2), and 68.8% against influenza B. In 1996, at at least one point during the four weeks after vaccination, 72.1% were seroprotected against A(H1N1), 44.1% against A(H3N2), and 77.9% against B.

In 1995, at at least one point during the four weeks after vaccination, 20.3% seroconverted against A(H1N1), 39.1% against A(H3N2), and 31.3% against B. In 1996, at at least one point during the four weeks after vaccination, 19.1% seroconverted against A(H1N1), 41.2% against A(H3N2), and 26.5% against B (Table 2).

In 1995, 64 subjects reported use of a total of 336 medications, for a mean of 5.3 medications per person. In 1996, 68 subjects reported use of a total of 376 medications, for a mean of 5.5 medications per person. The four main categories of medications used were cardiac anti-hypertensives, anti-inflammatory analgesics, steroids, and bronchodilators. Cardiac anti-hypertensives and anti-inflammatory analgesics were the most common medications in both vaccine years. In 1995, 57.8% of subjects used cardiac anti-hypertensive medication, while 64.1% used anti-inflammatory analgesic

medication. In 1996, 61.8% of subjects used cardiac anti-hypertensive medication, while 60.3% used anti-inflammatory analgesic medication (Table 3).

In 1995, 94% of subjects reported at least one of the chronic health conditions included on the questionnaire. In 1996, 92% of subjects reported at least one condition. During both years, the most common conditions were arthritis and hypertension (Table 4).

Kruskal-Wallis tests were conducted comparing the mean titer ranking scores of specific medication category users to non-users. These tests yielded the following statistically significant results: In 1995, on the day of vaccination, non-users of steroids had higher A(H3N2) titers than subjects using steroids (p=0.0108). Three weeks after vaccination, subjects using anti-inflammatory analgesic medication had higher influenza B titers than non-users (p=0.0474). Twelve weeks after vaccination, non-users of steroids had higher A(H3N2) and B titers than subjects using steroids (p=0.0362 and p=0.0267 respectively). (See Tables 5-8 for full 1995 Kruskal-Wallis results.)

In 1996, on the day of vaccination and three and twelve weeks afterward, subjects not using cardiac anti-hypertensives had higher B titers than subjects using cardiac anti-hypertensives (p=0.0392, p=0.0128, and p=0.0239, respectively). (See Tables 9-12 for full 1996 Kruskal-Wallis results.)

The association between medication use and immune response was examined in several ways. Chi-square tests of association yielded the following statistically significant results: In 1995, subjects taking steroidal medication were more likely be seroprotected against influenza A(H3N2) at vaccination than those not taking steroidal medication (OR=6.53 (95% CI: 1.37, 31.23), p=0.0272). Also, subjects taking steroidal medication were more likely to seroconvert against A(H1N1) two and three weeks after vaccination than those not taking steroidal medication (two weeks OR=10.83 (95% CI: 1.68, 69.93),

p=0.0122; three weeks OR=16.80 (95% CI: 2.61, 108.12), p=0.0022). (See Tables 13-16 for 1995 chi-square test results.)

In 1996, subjects taking cardiac anti-hypertensive medication were more likely to have protective levels of antibody against influenza B at vaccination, and three and twelve weeks afterward, than those not taking that medication (at vaccination OR=2.88 (95% CI: 1.05, 7.92), p=0.0392; three weeks OR=7.25 (95% CI: 1.33, 39.53), p=0.0220; twelve weeks OR=4.73 (95% CI: 1.18, 19.02), p=0.0392) (See Tables 17-20 for 1996 chi-square test results.)

All significant chi-square test results were assessed for interaction and confounding by age, sex, baseline HI titers, body mass index, physical activity level, and recent influenza vaccination history. No interaction was present in any significant association. In 1996, the association between subjects taking cardiac anti-hypertensive medication and their seroprotection against influenza B at the time of vaccination (crude OR=2.88 (95% CI: 1.05, 7.92)) was confounded by physical activity level (adjusted OR=3.57 (95% CI: 1.21, 10.50)). No other confounded associations were discovered.

Using logistic regression to evaluate the association between medication use and seroprotection and seroconversion outcomes, controlling for relevant confounders, led to the following statistically significant results: In 1995, subjects taking cardiac anti-hypertensive medication were more likely to seroconvert against influenza B at two weeks after vaccination than those not taking the medication (OR=7.89 (95% CI: 1.07, 58.30), p=0.0431). Those taking steroidal medication were more likely have protective levels of antibodies against influenza A(H3N2) at vaccination than those not taking steroids (OR=7.62 (95% CI: 1.49, 38.94), p=0.0148). (See Tables 13-16 for 1995 logistic

regression results). There were no significant associations in 1996. No interaction was found in logistic regression analyses.

During the 1995-96 season there was no virologic evidence of influenza activity among study participants. During the 1996-97 season, two influenza A(H3N2) isolates were obtained from study participants, one of whom died within a month of illness onset.

DISCUSSSION

Overall, subjects from both vaccine years experienced a mild immune response, with the highest antibody titers peaking three weeks after the 1996-97 vaccine administration with a GMT of 71.9 to influenza B. Of 48 calculated GMTs across two years, three antigens, and eight blood draws, only twice did GMTs exceed 60.0 (Figures A and B). This mild immune response is expected, as previous studies have shown that those with low titers before vaccination – such as these two groups of elderly subjects – do not often acquire sufficient HI antibody titers (23).

Subjects from 1996 showed higher GMT and seroprotection than 1995 subjects. However, 1995 subjects performed better when using the seroconversion measure (Figures A-H). Possibly, differing A(H3N2) components in the two vaccines were the cause. Results from 1996 are likely more reliable, as that year had a greater number of subjects (68 versus 64) giving more blood sera on average (6.4 versus 5.2) than 1995.

Subjects from both years experienced higher levels of seroprotection than seroconversion. This is consistent with results from other studies in elderly populations (5, 7). Though elderly persons may reach a protective level of antibodies, achieving a 4-fold increase is a unlikely in the elderly, particularly if they have detectable antibodies at the time of vaccination.

After GMT for all antigens peaked two weeks after vaccination in 1995, and three weeks after vaccination in 1996, declines were observed. Persistence of GMT was consistent from 12 weeks to 28 weeks after vaccination in both years, though the levels differed little from GMT at vaccination (Figures A and B). Subjects' antibody titers peaked quickly, within 4 weeks, then receded to baseline level within 12 weeks.

In 1995, less than 60% of subjects had protective levels of antibody, and seroconverted less than 25% of the time (Figures E and F). In 1996, less than 85% of subjects had protective levels of antibody, and seroconverted less than 30% of the time (Figures G and H). As mentioned earlier, this was not unexpected (23).

A small number of meaningful patterns were detected when examining the relationship between medication use and immune response. In 1996, Kruskal-Wallis tests show significantly lower antibody titers for subjects using cardiac anti-hypertensives at vaccination, and the association persisted at three and twelve weeks (Tables 9-12). This suggests that cardiac anti-hypertensive mediations moderately suppress immune response to the influenza vaccine. Alternatively, it may suggests that those with cardiac health conditions have a poorer immune response to influenza vaccination.

Using other methods, however, the relationship does not hold. Bivariate chi-square tests examining seroprotection to influenza B in 1995 yielded statistically significant results at the same time points, but those became non-significant after controlling for confounding variables (Table 17).

Using chi-square and logistic regression methods, no statistically significant associations were found concerning use of anti-inflammatory analgesic medications and immune response (Tables 14 and 18). We therefore deem the effect of anti-inflammatory analgesic medications to be inconclusive.

Due to their immunosuppressive nature, the study team hypothesized that steroidal medication usage would hinder immune response. This was not the case, as a majority of associations showed odds ratios greater than 1, some significantly so, even when controlling for confounders (Tables 15 and 19). Kruskal-Wallis tests in 1995 showed higher titers against A(H3N2) in non-users compared to users at vaccination and 12

weeks, and higher titers to influenza B in non-users compared to users after 12 weeks (Tables 5 and 8). The overall picture is inconclusive, which is consistent with at least one other study (8).

No statistically significant associations were found concerning use of bronchodilators and immune response (Tables 5-12, 16, and 20). Due to sparse data, several point estimates and confidence intervals could not be precisely quantified when using logistic regression. The overall picture is inconclusive.

A visual examination of statistically significant results by antigen suggests that medications were not associated with the immune response to one antigen over another. Likewise, a visual examination of time-points suggests that medications did not begin (or cease) to be associated with the immune response at a certain point in time.

Overall, this study yielded few statistically significant findings. As a large number of analyses were conducted, and statistically significant associations did not occur in any discernible pattern, they are very possibly due to random error.

Future Directions

There was no clear evidence that any of the medications examined led to a consistent, discernible decrease in immune response to the influenza vaccine. Nevertheless, simply conducting this study begs the question: if a patient's medication is known to hinder the immune response to influenza vaccination, should the patient cease its use for a certain amount of time prior to vaccination? The answer is complicated, as it depends on at least these factors: the patient's age and susceptibility to severe influenza infection; the severity of the patient's other health conditions compared to influenza; the necessity of medication to ameliorate the patient's other health conditions; the type and virulence of

circulating influenza strains, and their novelty to the patient's immune system; and the availability of an influenza booster vaccine.

However, while vaccine administration prior to onset of shorter term treatment might be beneficial, evidence from this study does not suggest modifying medication use prior to vaccination.

Alternatively, the patient could receive the influenza vaccine at a much higher dose to "overcome" any hindering effect of medication usage that might exist (as found in other studies). New influenza vaccines for the elderly do, in fact, contain a higher dose. Fluzone High-Dose (manufactured by Sanofi Pasteur) contains 60 mcg of hemagglutination per vaccine strain (rather than 15 mcg per strain as in the standard vaccine), and is available as an alternative trivalent inactivated vaccine for persons aged ≥65 years (2).

As demonstrated in the Background section, most studies of immune response to influenza vaccination have evaluated titers 2-6 weeks after vaccination, which usually corresponds with peak titer levels. While evaluation of peak titers is useful, it ignores the evaluation of titer persistence, when they are needed most due to peak influenza activity.

As such, the study team proposes a slight modification in methodology in studies of influenza vaccine immune response. In the United States, vaccination is recommended in October or November of each year, in order to protect against the earliest circulating strains (2). However, peak influenza activity usually occurs in January or February, several weeks after titers have peaked (24).

Evaluation of titers at two times - 2-6 weeks and 8-16 weeks after vaccination - would give a more complete picture of both the height and persistence of immune responses.

At the least, it is time for a discussion of the public health implications of research that has mostly focused on evaluation of titers 2-6 weeks after evaluation, instead of during peak influenza circulation. A substantial knowledge gap exists regarding optimal timing of influenza vaccination, particularly among immunocompromised elderly people.

In an effort to optimally time influenza vaccination so that peak HI antibody titers more closely coincide with peak influenza activity (or at least a level above the epidemic threshold), healthcare providers should pay careful attention to local influenza surveillance data. Admittedly, this is difficult, as influenza is notoriously unpredictable, but any increase in timing awareness would lead to improved patient outcomes.

For their part, health departments at all levels should continue to strive to publish accurate surveillance data as quickly as possible – and continue to inch toward real-time surveillance.

Regarding this study, it should be noted that an unusually early influenza season saw peak circulation in week 51 of 1995 and week 52 of 1996, as measured by the percentage of positive isolate samples received by CDC laboratories (25) . In 1995, this corresponded to approximately five weeks after subjects' vaccinations on November 14; in 1996, it corresponded to approximately 10 weeks after subjects' vaccinations on October 21.

Limitations

This study has limitations. Methodologically, these include its small sample size of 64 subjects in 1995 and 68 subjects in 1996. In nearly all analyses, fewer subjects are considered because of incomplete serologic data. In some analyses, particularly logistic regression, this led to point estimates with very large confidence intervals. The reader

should be aware that the odds ratios likely estimate the risk ratios in this study, due to low percentages of seroprotection and seroconversion among subjects. A literature review suggests a greater sample size is needed for this type of study, in order to increase statistical power.

Selection bias may exist in some associations. While blood sera from all subjects were drawn at the time of vaccination, it's unclear why sera were not drawn from all subjects at subsequent time points.

This study considers medication *use* only, not dosage or duration. At least one study has shown steroid dosage to be an important factor in predicting immune responses to influenza vaccination (12). Small or infrequent dosages might explain the unanticipated positive association between steroidal medication usage and immune response. The study also did not consider drug interactions between concurrently used medications. Additionally, despite having comparison groups which didn't use the medications of interest, the study lacks a comparison group with subjects who did not use any medications whatsoever.

The study did not consider underlying health conditions as part of the analysis, largely due to concerns of multicollinearity with medication use. As data was collected from 1995-97, the study was unable to evaluate the effects of recently released medications. Some medications considered are no longer marketed. As such, healthcare providers should closely examine Appendix I before acting on the results of the study.

The study population is relatively homogenous in sex and race, limiting the study's generalizability across populations. All participants were non-Hispanic whites, and around 80% were women.

This study assumes, as several others do, that HI titers are the best method to assess immune response in the elderly (1). Other measures may have equal or greater validity, however. Similarly, though seroprotection and seroconversion are the most established means of quantifying immune response, future innovation in the field may prove them obsolete.

Lastly, study subjects consisted of elderly persons residing in a life care facility. While the public health implications of influenza vaccination may be grave in this particular subgroup, the results of the study may not be generalized to elderly persons in the general population due to possible differences in health and immune status. On the other hand, the subjects in this study reside at a life care facility. As such, they are likely healthier than nursing home residents, to whom this study may not be generalizable either.

REFERENCES

1. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review. *Vaccine* 2006;24(8):1159-69.
2. ACIP. Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. *Morbidity and Mortality Weekly Report (MMWR)* 2011;60(33):1128-32.
3. Voordouw AC, Sturkenboom MC, Dieleman JP, et al. Annual revaccination against influenza and mortality risk in community-dwelling elderly persons. *JAMA* 2004;292(17):2089-95.
4. Vu T, Farish S, Jenkins M, et al. A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community. *Vaccine* 2002;20(13-14):1831-6.
5. Iorio A, Basileo M, Marcucci M, et al. Influenza vaccination and vitamin K antagonist treatment: a placebo-controlled, randomized, double-blind crossover study. *Arch Intern Med* 2010;170(7):609-16.
6. Iorio A, Alatri A, Camilloni B, et al. Antibody response to 1995-1996 influenza vaccine in institutionalized and non-institutionalized elderly women. *Gerontology* 1999;45(1):31-8.
7. Xu Y. Immunogenicity of an Inactivated Monovalent 2009 Influenza A (H1N1) Vaccine in Patients Who Have Cancer. *Oncologist* 2012;Epub ahead of print.
8. deRoux A, Marx A, Burkhardt O, et al. Impact of corticosteroids on the immune response to a MF59-adjuvanted influenza vaccine in elderly COPD-patients. *Vaccine* 2006;24(10):1537-42.

9. Chernesky M, O'Neill D, Pickard L, et al. Immunogenicity and adverse reactions of influenza vaccination in elderly patients given acetaminophen or placebo. *Clin Diagn Virol* 1993;1(2):129-36.

10. Hsia J, Tang T, Parrott M, et al. Augmentation of the immune response to influenza vaccine by acetylsalicylic acid: a clinical trial in a geriatric population. *Methods Find Exp Clin Pharmacol* 1994;16(9):677-83.

11. Adler S, Krivine A, Weix J, et al. Protective effect of A/H1N1 vaccination in immune-mediated disease--a prospectively controlled vaccination study. *Rheumatology (Oxford)* 2011;[Epub ahead of print].

12. Borba E, Saad C, Pasoto S, et al. Influenza A/H1N1 vaccination of patients with SLE: can antimalarial drugs restore diminished response under immunosuppressive therapy? *Rheumatology (Oxford)* 2012;[Epub ahead of print].

13. Mathian A, Devilliers H, Krivine A, et al. Factors influencing the efficacy of two injections of a pandemic 2009 influenza A (H1N1) nonadjuvanted vaccine in systemic lupus erythematosus. *Arthritis Rheum* 2011;63(11):3502-11.

14. Romanowska M, Banaszkiewicz A, Nowak I, et al. Immunization against influenza during the 2005/2006 epidemic season and the humoral response in children with diagnosed inflammatory bowel disease (IBD). *Med Sci Monit* 2010;16(9):CR433-9.

15. Salles M, Sens Y, Boas L, et al. Influenza virus vaccination in kidney transplant recipients: serum antibody response to different immunosuppressive drugs. *Clin Transplant* 2010;24(1):E17-23.

16. Agarwal N, Ollington K, Kaneshiro M, et al. Are immunosuppressive medications associated with decreased responses to routine immunizations? A systematic review. *Vaccine* 2012;30(8):1413-24.

17. Aikawa N, Campos L, Silva C, et al. Glucocorticoid: major factor for reduced immunogenicity of 2009influenza A (H1N1) vaccine in patients with juvenile autoimmune rheumatic disease. *J Rheumatol* 2012;39(1):167-73.

18. Arad U, Tzadok S, Amir S, et al. The cellular immune response to influenza vaccination is preserved in rheumatoid arthritis patients treated with rituximab. *Vaccine* 2011;29(8):1643-48.

19. Andrews N, Walker W, Finn A, et al. Predictors of immune response and reactogenicity to AS03B-adjuvanted split virion and non-adjuvanted whole virion H1N1 (2009) pandemic influenza vaccines. *Vaccine* 2011;29(45):7913-9.

20. Brydak L, Całbecka M. Immunogenicity of influenza vaccine in patients with hemato-oncological disorders. *Leuk Lymphoma* 1999;32(3-4):369-74.

21. Cooper C, Thorne A, Canadian HIV Trials Network, et al. Vitamin D supplementation does not increase immunogenicity of seasonal influenza vaccine in HIV-infected adults. *HIV Clin Trials* 2011;12(5):275-6.

22. Katz J, Hancock K, Veguilla V, et al. Serum Cross-Reactive Antibody Response to a Novel Influenza A (H1N1) Virus After Vaccination with Seasonal Influenza Vaccine *Morbid Mortal Weekly Rep* 2009;58(19):521-4.

23. Matsushita M. Prevaccination antibody titers can estimate the immune response to influenza vaccine in a rural community-dwelling elderly population. *Vaccine* 2011;Epub ahead of print.

24. Centers for Disease Control and Prevention. Seasonal Influenza - Past Weekly Surveillance Reports. http://www.cdc.gov/flu/weekly/pastreports.htm. (Accessed March 20 2012).

25. Brammer L, Izurieta H, Fukuda K, et al. Surveillance for Influenza --- United States, 1994--95, 1995--96, and 1996--97 Seasons. Atlanta, GA: CDC; 2000. (http://www.cdc.gov/mmwr/preview/mmwrhtml/ss4903a2.htm). (Accessed April 10, 2012).

TABLES

Table 1: Characteristics of Subjects, VA Life Care Facility, 1995-96

	1995	1996
	n=64	n=68
	Mean (Std Dev)	
Age (years)	82.2 (5.3)	81.9 (6.1)
Body mass index	24.1 (4.2)	24.4 (4.4)
Men	24.8 (2.9)	24.6 (3.0)
Women	23.9 (4.5)	24.4 (4.7)
	1995	**1996**
	n=64	**n=68**
	n (%)	
Sex		
Men	12 (18.8)	15 (22.1)
Women	52 (81.2)	53 (77.9)
Number of times received influenza vaccine during past five years		
Vaccinated all five years	54 (85.7)	49 (73.1)
Not vaccinated all five years	9 (14.3)	18 (26.9)
Never vaccinated	*1 (1.6)*	*3 (4.5)*
Vaccinated 1-2 times	*4 (6.4)*	*5 (7.5)*
Vaccinated 3-4 times	*4 (6.4)*	*10 (14.9)*
Physical activity level		
Ambulatory and active	39 (60.9)	18 (26.9)
Not ambulatory and active	25 (39.1)	49 (73.1)
Ambulatory and sedentary	*17 (26.6)*	*14 (20.9)*
Wheelchair and active	*5 (7.8)*	*26 (38.8)*
Wheelchair and inactive	*3 (4.7)*	*6 (9.0)*
Not ambulatory or mobile	*0 (0.0)*	*2 (3.0)*
Bedridden	*0 (0.0)*	*1 (1.5)*
Current smoking status		
Yes	---	3 (4.4)
No	---	65 (95.6)

Smoking data was not available for 1995.

Table 2: Subjects' Seroprotection and Seroconversion to Influenza Vaccine, VA Life Care Facility, 1995-96

	Seroprotection						Seroconversion					
	1995			1996			1995			1996		
	N Sera Drawn	N Titer ≥ 40	%	N Sera Drawn	N Titer ≥ 40	%	N Sera Drawn	N ≥ 4-fold rise	%	N Sera Drawn	N ≥ 4-fold rise	%
Influenza A(H1N1)												
At vaccination	64	27	42.2%	68	36	52.9%	64	---	---	68	---	---
1 week after	35	23	65.7%	57	37	64.9%	35	6	17.1%	57	1	1.8%
2 weeks after	39	27	69.2%	51	36	70.6%	39	11	28.2%	51	5	9.8%
3 weeks after	41	29	70.7%	52	39	75.0%	41	11	26.8%	52	12	23.1%
4 weeks after	47	33	70.2%	67	44	65.7%	47	9	19.1%	67	7	10.4%
Any point during first 4 weeks	64	43	67.2%	68	49	72.1%	64	13	20.3%	68	13	19.1%
12 weeks after	41	17	41.5%	50	34	68.0%	41	2	4.9%	50	3	6.0%
20 weeks after	25	9	36.0%	43	29	67.4%	25	0	0.0%	43	2	4.7%
28 weeks after	43	23	53.5%	46	29	63.0%	43	0	0.0%	46	1	2.2%
Influenza A(H3N2)												
At vaccination	64	10	15.6%	68	9	13.2%	64	---	---	68	---	---
1 week after	35	13	37.1%	57	11	19.3%	35	7	20.0%	57	3	5.3%
2 weeks after	39	21	53.8%	51	18	35.3%	39	19	48.7%	51	17	33.3%
3 weeks after	41	19	46.3%	52	24	46.2%	41	19	46.3%	52	21	40.4%
4 weeks after	47	24	51.1%	67	25	37.3%	47	18	38.3%	67	23	34.3%
Any point during first 4 weeks	64	28	43.8%	68	30	44.1%	64	25	39.1%	68	28	41.2%
12 weeks after	41	15	36.6%	50	17	34.0%	41	9	22.0%	50	13	26.0%
20 weeks after	25	3	12.0%	43	12	27.9%	25	3	12.0%	43	9	20.9%
28 weeks after	43	11	25.6%	46	16	34.8%	43	2	4.7%	46	12	26.1%
Influenza B												
At vaccination	64	27	42.2%	68	37	54.4%	64	---	---	68	---	---
1 week after	35	19	54.3%	57	38	66.7%	35	7	20.0%	57	1	1.8%
2 weeks after	39	31	79.5%	51	39	76.5%	39	15	38.5%	51	10	19.6%
3 weeks after	41	31	75.6%	52	43	82.7%	41	15	36.6%	52	14	26.9%
4 weeks after	47	34	72.3%	67	48	71.6%	47	14	29.8%	67	13	19.4%
Any point during first 4 weeks	64	44	68.8%	68	53	77.9%	64	20	31.3%	68	18	26.5%
12 weeks after	41	23	56.1%	50	37	74.0%	41	6	14.6%	50	10	20.0%
20 weeks after	25	13	52.0%	43	29	67.4%	25	2	8.0%	43	5	11.6%
28 weeks after	43	24	55.8%	46	33	71.7%	43	4	9.3%	46	6	13.0%

Table 3: Subjects' Medication Use, VA Life Care Facility, 1995-96

	1995	1996
	n=64	n=68
Medication Category	n (%)	
Cardiac/antihypertensive	37 (57.8)	42 (61.8)
Anti-inflammatory analgesic	41 (64.1)	41 (60.3)
Steroid	9 (14.1)	10 (14.7)
Bronchodilator	7 (10.9)	8 (11.8)

Refer to Appendix I to see how specific medications were categorized.

Table 4: Subjects' Health Conditions, VA Life Care Facility, 1995-96

	1995	1996
	n=64	n=68
	n (%)	
Arthritis	41 (66.1)	37 (55.2)
Hypertension	22 (34.9)	24 (36.4)
Osteoporosis	12 (18.8)	14 (21.5)
Arteriosclerosis	11 (17.7)	10 (14.9)
Chronic lung disease	9 (14.3)	13 (19.1)
Other heart disease	9 (14.3)	16 (24.2)
Hypothyroidism	9 (14.3)	11 (16.4)
Degenerative joint disease	8 (12.7)	10 (15.4)
Angina	7 (11.1)	10 (14.9)
Mental depression	7 (11.3)	9 (13.2)
Other bone or join disease	6 (10.0)	13 (21.0)
Other neurologic/ neuromuscular disorder	5 (8.2)	6 (9.4)
Anemia	5 (7.8)	7 (10.8)
Myocardial infarction	3 (4.8)	8 (11.9)
Other blood disorder	3 (5.0)	4 (6.4)
History of stroke	3 (4.7)	5 (7.5)
Cancer (under active treatment)	2 (3.2)	4 (5.9)
Diabetes	2 (3.2)	2 (3.0)
Peripheral vascular disease	2 (3.2)	4 (6.0)
Diseases of immune system	1 (1.7)	2 (3.1)
Congestive heart failure	0 (0.0)	2 (3.1)
Kidney disease	0 (0.0)	4 (6.0)
Parkinson's disease	0 (0.0)	2 (3.0)

Table 5: Differences in HI titers in different medication use groups using Kruskal-Wallis test - on day of vaccination, VA Life Care Facility, 1995

		A(H1N1)		A(H3N2)		B	
Medication use	**N**	**Mean Score**	**p-value**	**Mean Score**	**p-value**	**Mean Score**	**p-value**
Cardiac/anti-hypertensives	37	31.3	0.4795	31.4	0.3993	32.2	0.8426
No cardiac/anti-hypertensives	27	34.1		33.9		33.0	
Anti-inflammatory/analgesics	41	32.0	0.7129	32.0	0.6725	34.3	0.2293
No anti-inflammatory/analgesics	23	33.5		33.3		29.3	
Steroids	9	31.8	0.8833	23.3	**0.0108**	24.7	0.1115
No steroids	55	32.6		34.0		33.8	
Bronchodilators	7	23.1	0.0996	28.4	0.3213	32.3	0.9699
No bronchodilators	57	33.6		33		32.5	

Table 6: Differences in HI titers in different medication use groups using Kruskal-Wallis test - two weeks after vaccination, VA Life Care Facility, 1995

		A(H1N1)		A(H3N2)		B	
Medication use	**N**	**Mean Score**	**p-value**	**Mean Score**	**p-value**	**Mean Score**	**p-value**
Cardiac/anti-hypertensives	26	19.3	0.4675	18.5	0.1786	19.0	0.2682
No cardiac/anti-hypertensives	13	21.5		23.0		22.0	
Anti-inflammatory/analgesics	28	18.9	0.2189	19.4	0.5154	20.9	0.2744
No anti-inflammatory/analgesics	11	22.9		21.6		17.8	
Steroids	32	19.6	0.8908	16.6	0.3092	18.8	0.6566
No steroids	7	20.1		20.8		20.3	
Bronchodilators	4	14.0	0.1647	15.9	0.3765	20.9	0.8169
No bronchodilators	35	20.7		20.5		19.9	

Table 7: Differences in HI titers in different medication use groups using Kruskal-Wallis test - three weeks after vaccination, VA Life Care Facility, 1995

		A(H1N1)		A(H3N2)		B	
Medication use	**N**	**Mean Score**	**p-value**	**Mean Score**	**p-value**	**Mean Score**	**p-value**
Cardiac/anti-hypertensives	25	19.1	0.1073	19.8	0.3697	20.1	0.4190
No cardiac/anti-hypertensives	16	24.0		22.8		22.4	
Anti-inflammatory/analgesics	26	19.7	0.2572	20.3	0.5413	23.1	**0.0474**
No anti-inflammatory/analgesics	15	23.2		22.3		17.4	
Steroids	8	17.6	0.2511	15.1	0.0735	18.6	0.3886
No steroids	33	21.8		22.4		21.6	
Bronchodilators	4	15.0	0.1810	15.1	0.2320	26.3	0.2149
No bronchodilators	37	21.6		21.6		20.4	

Table 8: Differences in HI titers in different medication use groups using Kruskal-Wallis test - twelve weeks after vaccination, VA Life Care Facility, 1995

		A(H1N1)		A(H3N2)		B	
Medication use	**N**	**Mean Score**	**p-value**	**Mean Score**	**p-value**	**Mean Score**	**p-value**
Cardiac/anti-hypertensives	24	19.3	0.1929	19.1	0.1491	19.7	0.3323
No cardiac/anti-hypertensives	17	23.5		23.7		22.9	
Anti-inflammatory/analgesics	24	19.3	0.1929	19.1	0.1491	22.3	0.3558
No anti-inflammatory/analgesics	17	23.5		23.7		19.2	
Steroids	9	22.7	0.5799	14.8	**0.0362**	14.3	**0.0267**
No steroids	32	20.5		22.7		22.9	
Bronchodilators	5	21.3	0.9442	16.2	0.2519	16.1	0.2563
No bronchodilators	36	21.0		21.7		21.7	

Table 9: Differences in HI titers in different medication use groups using Kruskal-Wallis test - on day of vaccination, VA Life Care Facility, 1996

		A(H1N1)		A(H3N2)		B	
Medication use	**N**	**Mean Score**	**p-value**	**Mean Score**	**p-value**	**Mean Score**	**p-value**
Cardiac/anti-hypertensives	42	31.5	0.0617	33.3	0.2921	31.1	**0.0392**
No cardiac/anti-hypertensives	26	39.4		36.4		39.9	
Anti-inflammatory/analgesics	41	32.6	0.2582	33.2	0.2533	33.9	0.7328
No anti-inflammatory/analgesics	27	37.4		36.5		35.4	
Steroids	10	25.3	0.0654	32.2	0.4975	29.2	0.2874
No steroids	58	36.1		34.9		35.4	
Bronchodilators	8	27.0	0.1865	30.5	0.2994	31.8	0.6274
No bronchodilators	60	35.5		35.0		34.9	

Table 10: Differences in HI titers in different medication use groups using Kruskal-Wallis test - two weeks after vaccination, VA Life Care Facility, 1996

		A(H1N1)		A(H3N2)		B	
Medication use	N	Mean Score	p-value	Mean Score	p-value	Mean Score	p-value
Cardiac/anti-hypertensives	34	24.5	0.1967	26.8	0.5383	24.5	0.1655
No cardiac/anti-hypertensives	17	29.0		24.5		29.0	
Anti-inflammatory/analgesics	34	26.0	1.0000	25.3	0.5383	26.0	1.0000
No anti-inflammatory/analgesics	17	26.0		27.5		26.0	
Steroids	7	22.1	0.3491	27.7	0.6915	23.6	0.5388
No steroids	44	26.6		25.7		26.4	
Bronchodilators	6	18.5	0.0956	22.3	0.4269	28.5	0.5507
No bronchodilators	45	27.0		26.5		25.7	

Table 11: Differences in HI titers in different medication use groups using Kruskal-Wallis test - three weeks after vaccination, VA Life Care Facility, 1996

		A(H1N1)		A(H3N2)		B	
Medication use	**N**	**Mean Score**	**p-value**	**Mean Score**	**p-value**	**Mean Score**	**p-value**
Cardiac/anti-hypertensives	31	24.2	0.0755	26.8	0.8628	23.7	**0.0128**
No cardiac/anti-hypertensives	21	29.9		26.1		30.7	
Anti-inflammatory/analgesics	32	26.5	1.0000	27.1	0.6631	27.7	0.2755
No anti-inflammatory/analgesics	20	26.5		25.5		24.6	
Steroids	5	25.2	0.7880	22.9	0.5177	27.2	0.8683
No steroids	47	26.6		26.9		26.4	
Bronchodilators	6	20.0	0.1365	21.2	0.2886	26.3	0.9651
No bronchodilators	46	27.3		27.2		26.5	

Table 12: Differences in HI titers in different medication use groups using Kruskal-Wallis test - twelve weeks after vaccination, VA Life Care Facility, 1996

		A(H1N1)		A(H3N2)		B	
Medication use	N	Mean Score	p-value	Mean Score	p-value	Mean Score	p-value
Cardiac/anti-hypertensives	30	23.1	0.0710	23.7	0.6128	22.3	**0.0239**
No cardiac/anti-hypertensives	19	29.3		22.0		29.3	
Anti-inflammatory/analgesics	31	24.0	0.2352	23.7	0.5439	23.7	0.2776
No anti-inflammatory/analgesics	18	28.0		21.7		27.2	
Steroids	8	23.8	0.6466	21.9	0.7655	25.1	0.9710
No steroids	41	25.8		23.2		25.0	
Bronchodilators	6	17.5	0.0762	22.5	0.9144	19	0.1405
No bronchodilators	43	26.6		23.1		25.8	

Table 13: Effect of cardiac/anti-hypertensive medication use on immune response to influenza vaccine in a VA Life Care Facility, 1995

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	64	37 (57.8)	27 (42.2)	1.45	(0.52, 3.98)	0.4795	1.34	(0.44, 4.12)	0.6016	---	---	---	---	---	---
2 weeks after	39	26 (66.7)	13 (33.3)	1.70	(0.41, 6.97)	0.4675	1.31	(0.24, 7.25)	0.7569	2.91	(0.53, 16.10)	0.2760	7.48	(0.86, 64.75)	0.0677
3 weeks after	41	25 (61.0)	16 (39.0)	3.11	(0.77, 12.51)	0.1073	3.30	(0.47, 23.10)	0.2288	2.04	(0.45, 9.24)	0.4783	3.35	(0.50, 22.43)	0.2133
12 weeks after	41	24 (58.5)	17 (41.5)	2.40	(0.64, 8.94)	0.1929	1.81	(0.33, 9.87)	0.4923	3.89	(0.17, 86.32)	0.5024	---	---	---
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	64	37 (57.8)	27 (42.2)	1.87	(0.44, 8.00)	0.4983	2.70	(0.49, 14.84)	0.2526	---	---	---	---	---	---
2 weeks after	39	26 (66.7)	13 (33.3)	2.56	(0.65, 10.06)	0.1786	0.91	(0.12, 6.82)	0.9273	1.17	(0.31, 4.43)	0.8231	0.92	(0.21, 4.01)	0.9127
3 weeks after	41	25 (61.0)	16 (39.0)	1.81	(0.50, 6.50)	0.3697	0.13	(0.01, 2.43)	0.1739	0.52	(0.15, 1.85)	0.3147	0.31	(0.07, 1.37)	0.4523
12 weeks after	41	24 (58.5)	17 (41.5)	2.75	(0.69, 10.91)	0.1954	1.60	(0.19, 13.51)	0.6463	0.86	(0.19, 3.80)	1.0000	0.80	(0.15, 4.18)	0.7895
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	64	37 (57.8)	27 (42.2)	1.11	(0.41, 3.03)	0.8426	1.49	(0.49, 4.52)	0.4791	---	---	---	---	---	---
2 weeks after	39	26 (66.7)	13 (33.3)	2.44	(0.50, 11.96)	0.4023	2.02	(0.30, 13.53)	0.4705	2.86	(0.64, 12.84)	0.2951	7.89	(1.07, 58.30)	**0.0431**
3 weeks after	41	25 (61.0)	16 (39.0)	1.82	(0.43, 7.69)	0.4190	4.19	(0.52, 33.83)	0.1786	2.36	(0.59, 9.37)	0.3219	2.71	(0.57, 12.82)	0.2092
12 weeks after	41	24 (58.5)	17 (41.5)	1.88	(0.53, 6.62)	0.3323	4.55	(0.35, 59.34)	0.2472	1.50	(0.24, 9.30)	1.0000	1.55	(0.23, 10.38)	0.6541

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 14: Effect of anti-inflammatory analgesic medication use on immune response to influenza vaccine in a VA Life Care Facility, 1995

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	64	41 (64.1)	23 (35.9)	1.22	(0.43, 3.44)	0.7129	0.85	(0.26, 2.74)	0.7801	---	---	---	---	---	---
2 weeks after	39	28 (71.8)	11 (28.2)	2.50	(0.57, 10.80)	0.2189	5.08	(0.68, 38.65)	0.1167	0.58	(0.13, 2.61)	0.6940	0.66	(0.09, 4.65)	0.6771
3 weeks after	41	26 (63.4)	15 (36.6)	2.22	(0.56, 8.82)	0.2572	2.18	(0.36, 13.21)	0.3956	1.78	(0.39, 8.09)	0.7158	2.33	(0.39, 13.77)	0.3511
12 weeks after	41	24 (58.5)	17 (41.5)	2.40	(0.64, 8.94)	0.1929	2.27	(0.42, 12.26)	0.3407	0.70	(0.04, 11.95)	1.0000	1.20	(0.06, 24.47)	0.9057
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	64	41 (64.1)	23 (35.9)	1.37	(0.32, 5.92)	1.0000	1.06	(0.22, 5.10)	0.9424	---	---	---	---	---	---
2 weeks after	39	28 (71.8)	11 (28.2)	1.60	(0.39, 6.51)	0.5154	1.23	(0.15, 9.88)	0.6241	1.20	(0.30, 4.86)	0.8008	0.86	(0.18, 4.05)	0.8510
3 weeks after	41	26 (63.4)	15 (36.6)	1.50	(0.41, 5.44)	0.5413	0.53	(0.06, 4.73)	0.5665	0.64	(0.18, 2.30)	0.5006	0.50	(0.12, 1.98)	0.3214
12 weeks after	41	24 (58.5)	17 (41.5)	2.75	(0.69, 10.92)	0.1954	3.80	(0.44, 33.21)	0.2269	1.56	(0.33, 7.34)	0.7113	1.73	(0.32, 9.54)	0.5274
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	64	41 (64.1)	23 (35.9)	0.53	(0.19, 1.49)	0.2293	0.54	(0.17, 1.69)	0.2878	---	---	---	---	---	---
2 weeks after	39	28 (71.8)	11 (28.2)	0.30	(0.03, 2.78)	0.3996	0.25	(0.01, 5.94)	0.3880	2.00	(0.44, 9.18)	0.4770	1.06	(0.12, 9.20)	0.9600
3 weeks after	41	26 (63.4)	15 (36.6)	0.13	(0.02, 1.20)	0.0635	0.31	(0.03, 3.64)	0.3501	2.02	(0.51, 8.05)	0.5020	1.22	(0.26, 5.73)	0.8023
12 weeks after	41	24 (58.5)	17 (41.5)	0.55	(0.15, 1.96)	0.3558	0.24	(0.02, 3.60)	0.3028	1.50	(0.24, 9.30)	1.0000	1.39	(0.19, 10.17)	0.7451

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 15: Effect of steroidal medication use on immune response to influenza vaccine in a VA Life Care Facility, 1995

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	64	9 (14.1)	55 (85.9)	1.11	(0.27, 4.60)	1.0000	1.16	(0.27, 5.11)	0.8401	---	---	---	---	---	---
2 weeks after	39	7 (18.0)	32 (82.0)	1.14	(0.19, 6.89)	1.0000	1.40	(0.17, 11.32)	0.7535	10.83	(1.68, 69.92)	**0.0122**	---	---	---
3 weeks after	41	8 (19.5)	33 (80.5)	3.50	(0.38, 32.12)	0.3984	5.60	(0.51, 62.14)	0.1604	16.80	(2.61, 108.12)	**0.0022**	---	---	---
12 weeks after	41	9 (22.0)	32 (78.0)	0.64	(0.14, 3.03)	0.7113	0.47	(0.06, 3.53)	0.4599	3.88	(0.22, 68.94)	0.3951	10.00	(0.32, 315.24)	0.1909
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	64	9 (14.1)	55 (85.9)	6.53	(1.37, 31.23)	**0.0272**	7.62	(1.49, 38.94)	**0.0148**	---	---	---	---	---	---
2 weeks after	39	7 (18.0)	32 (82.0)	2.50	(0.42, 14.83)	0.4179	1.71	(0.15, 19.09)	0.6648	0.35	(0.06, 2.09)	0.4075	0.31	(0.05, 1.95)	0.2127
3 weeks after	41	8 (19.5)	33 (80.5)	4.62	(0.81, 26.45)	0.1152	8.61	(0.38, 198.04)	0.1783	0.64	(0.13, 3.11)	0.7033	0.58	(0.11, 3.06)	0.5224
12 weeks after	41	9 (22.0)	32 (78.0)	5.11	(1.05, 24.96)	0.0525	5.71	(0.41, 79.53)	0.1949	0.38	(0.04, 3.48)	0.6541	0.22	(0.02, 2.43)	0.2163
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	64	9 (14.1)	55 (85.9)	3.24	(0.73, 14.35)	0.1505	4.22	(0.92, 19.38)	0.0639	---	---	---	---	---	---
2 weeks after	39	7 (18.0)	32 (82.0)	1.68	(0.17, 16.37)	1.0000	0.58	(0.03, 13.49)	0.7334	0.21	(0.02, 1.99)	0.2159	0.38	(0.03, 5.82)	0.4894
3 weeks after	41	8 (19.5)	33 (80.5)	2.63	(0.28, 24.44)	0.6532	1.00	(0.05, 19.37)	1.0000	1.05	(0.21, 5.19)	1.0000	2.32	(0.32, 17.02)	0.4073
12 weeks after	41	9 (22.0)	32 (78.0)	9.07	(1.01, 81.15)	0.0535	4.33	(0.24, 78.13)	0.3203	0.21	(0.01, 4.18)	0.3090	---	---	---

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 16: Effect of bronchodilator medication use on immune response to influenza vaccine in a VA Life Care Facility, 1995

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	64	7 (10.9)	57 (89.1)	3.98	(0.71, 22.31)	0.1222	3.67	(0.64, 21.19)	0.1434	---	---	---	---	---	---
2 weeks after	39	4 (10.3)	35 (89.7)	4.78	(0.24, 96.27)	0.2916	---	---	---	0.24	(0.01, 4.78)	0.3091	---	---	---
3 weeks after	41	4 (9.8)	37 (90.2)	4.41	(0.22, 88.53)	0.3024	---	---	---	0.90	(0.08, 9.69)	1.0000	2.69	(0.12, 59.22)	0.5314
12 weeks after	41	5 (12.2)	36 (87.8)	0.93	(0.14, 6.29)	1.0000	0.19	(0.03, 2.74)	0.1866	1.25	(0.05, 29.78)	1.0000	---	---	---
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	64	7 (10.9)	57 (89.1)	2.45	(0.40, 14.85)	0.2992	2.62	(0.41, 16.77)	0.3102	---	---	---	---	---	---
2 weeks after	39	4 (10.3)	35 (89.7)	2.83	(0.27, 29.96)	0.6094	9.81	(0.36, 264.41)	0.1744	0.31	(0.03, 3.33)	0.6050	0.28	(0.03, 3.02)	0.2916
3 weeks after	41	4 (9.8)	37 (90.2)	3.94	(0.37, 41.48)	0.3210	3.27	(0.17, 64.23)	0.4351	0.35	(0.03, 3.70)	0.6099	0.26	(0.02, 2.84)	0.2673
12 weeks after	41	5 (12.2)	36 (87.8)	3.00	(0.44, 20.44)	0.3365	---	---	---	0.88	(0.08, 8.97)	1.0000	0.77	(0.06, 9.40)	0.8409
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	64	7 (10.9)	57 (89.1)	1.03	(0.21, 5.04)	1.0000	1.26	(0.25, 6.38)	0.7776	---	---	---	---	---	---
2 weeks after	39	4 (10.3)	35 (89.7)	0.75	(0.07, 8.35)	1.0000	0.58	(0.03, 13.49)	0.7334	0.50	(0.05, 5.31)	1.0000	0.45	(0.03, 7.88)	0.5878
3 weeks after	41	4 (9.8)	37 (90.2)	0.28	(0.03, 2.28)	0.2454	---	---	---	0.55	(0.05, 5.79)	1.0000	0.50	(0.04, 6.13)	0.5902
12 weeks after	41	5 (12.2)	36 (87.8)	3.58	(0.36, 35.23)	0.3629	0.67	(0.03, 18.06)	0.8096	1.55	(0.14, 16.85)	0.5568	5.24	(0.24, 116.63)	0.2953

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 17: Effect of cardiac/anti-hypertensive medication use on immune response to influenza vaccine in a VA Life Care Facility, 1996

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	68	42 (61.8)	26 (38.2)	2.60	(0.95, 7.11)	0.0617	2.47	(0.85, 7.20)	0.0979	---	---	---	---	---	---
2 weeks after	51	34 (66.7)	17 (33.3)	2.28	(0.65, 7.94)	0.1967	2.04	(0.45, 9.23)	0.3547	2.13	(0.21, 20.72)	0.6536	2.84	(0.27, 30.27)	0.3872
3 weeks after	52	31 (59.6)	21 (40.4)	3.20	(0.87, 11.75)	0.0755	3.34	(0.65, 17.10)	0.1487	0.93	(0.25, 3.46)	0.9186	1.14	(0.24, 5.33)	0.8717
12 weeks after	50	31 (62.0)	19 (38.0)	3.09	(0.89, 10.59)	0.0710	2.35	(0.55, 10.01)	0.2489	0.28	(0.02, 3.36)	0.5492	---	---	---
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	68	42 (61.8)	26 (38.2)	2.40	(0.46, 12.56)	0.4652	1.79	(0.31, 10.34)	0.5171	---	---	---	---	---	---
2 weeks after	51	34 (66.7)	17 (33.3)	0.68	(0.21, 2.27)	0.5383	0.81	(0.19, 3.40)	0.7738	3.27	(0.79, 13.54)	0.1221	4.90	(0.85. 28.22)	0.0750
3 weeks after	52	31 (59.6)	21 (40.4)	0.91	(0.30, 2.75)	0.8628	0.58	(0.13, 2.65)	0.4830	1.65	(0.52, 5.20)	0.3983	1.02	(0.26, 4.00)	0.9761
12 weeks after	50	31 (62.0)	19 (38.0)	0.73	(0.22, 2.46)	0.6128	0.49	(0.09, 2.56)	0.3967	1.99	(0.51, 7.79)	0.3269	3.10	(0.60, 15.92)	0.1750
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	68	42 (61.8)	26 (38.2)	2.88	(1.05, 7.92)	**0.0392**	2.56	(0.87, 7.53)	0.0878	---	---	---	---	---	---
2 weeks after	51	34 (66.7)	17 (33.3)	2.55	(0.67, 9.62)	0.1655	1.55	(0.30, 7.94)	0.5982	0.70	(0.17, 2.90)	0.7137	0.99	(0.20, 4.96)	0.9908
3 weeks after	52	31 (59.6)	21 (40.4)	7.25	(1.33, 39.53)	**0.0220**	3.42	(0.49, 23.86)	0.2141	0.87	(0.25, 3.01)	0.8271	2.22	(0.44, 11.23)	0.3331
12 weeks after	50	31 (62.0)	19 (38.0)	4.73	(1.18, 19.02)	**0.0392**	3.34	(0.70, 16.07)	0.1317	1.62	(0.36, 7.24)	0.5326	3.19	(0.53, 19.30)	0.2072

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 18: Effect of anti-inflammatory analgesic medication use on immune response to influenza vaccine in a VA Life Care Facility, 1996

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	68	41 (60.3)	27 (39.7)	1.76	(0.66, 4.71)	0.2582	1.59	(0.56, 4.50)	0.3821	---	---	---	---	---	---
2 weeks after	51	34 (66.7)	17 (33.3)	1.00	(0.28, 3.59)	1.0000	0.62	(0.14, 2.79)	0.5376	0.73	(0.11, 4.82)	1.0000	0.87	(0.12, 6.07)	0.8849
3 weeks after	52	32 (61.5)	20 (38.5)	1.00	(0.28, 3.63)	1.0000	0.66	(0.15, 3.00)	0.5951	0.84	(0.23, 3.13)	0.7966	1.43	(0.32, 6.43)	0.6400
12 weeks after	50	31 (62.0)	19 (38.0)	2.09	(0.62, 7.05)	0.2352	1.70	(0.38, 7.72)	0.4910	1.24	(0.10, 14.70)	1.0000	1.00	(0.03, 38.56)	1.0000
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	68	41 (60.3)	27 (39.7)	2.57	(0.49, 13.46)	0.3003	3.38	(0.55, 20.69)	0.1871	---	---	---	---	---	---
2 weeks after	51	34 (66.7)	17 (33.3)	1.49	(0.42, 5.19)	0.5383	1.72	(0.41, 7.30)	0.4601	2.01	(0.54, 7.51)	0.3579	1.97	(0.48, 8.16)	0.3459
3 weeks after	52	32 (61.5)	20 (38.5)	0.78	(0.25, 2.39)	0.6631	0.97	(0.24, 3.94)	0.9678	0.73	(0.24, 2.28)	0.5954	0.93	(0.25, 3.43)	0.9161
12 weeks after	50	31 (62.0)	19 (38.0)	0.68	(0.19, 2.36)	0.5439	0.48	(0.08, 2.77)	0.4120	1.35	(0.34, 5.36)	0.7432	1.20	(0.25, 5.93)	0.8190
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	68	41 (60.3)	27 (39.7)	1.19	(0.45, 3.15)	0.7328	1.29	(0.45, 3.64)	0.6353	---	---	---	---	---	---
2 weeks after	51	34 (66.7)	17 (33.3)	1.00	(0.26, 3.94)	1.0000	1.09	(0.21, 5.76)	0.9201	0.70	(0.17, 2.90)	0.7137	0.66	(0.13, 3.22)	0.6024
3 weeks after	52	32 (61.5)	20 (38.5)	0.40	(0.07, 2.14)	0.4540	0.27	(0.04, 1.97)	0.1949	0.35	(0.10, 1.22)	0.0960	0.27	(0.05, 1.35)	0.1113
12 weeks after	50	31 (62.0)	19 (38.0)	2.08	(0.55, 7.83)	0.3159	1.92	(0.41, 8.99)	0.4102	1.46	(0.33, 6.53)	0.7258	1.45	(0.27, 7.84)	0.6638

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 19: Effect of steroidal medication use on immune response to influenza vaccine in a VA Life Care Facility, 1996

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	68	10 (14.7)	58 (85.3)	4.29	(0.84, 21.94)	0.0893	5.07	(0.95, 26.94)	0.0569	---	---	---	---	---	---
2 weeks after	51	7 (13.7)	44 (86.3)	2.80	(0.31, 25.52)	0.6581	2.95	(0.28, 31.36)	0.3688	0.48	(0.02, 9.60)	1.0000	---	---	---
3 weeks after	52	5 (9.6)	47 (90.4)	1.37	(0.14, 13.51)	1.0000	1.77	(0.16, 19.30)	0.9536	2.47	(0.36, 16.84)	0.3248	2.56	(0.30, 21.62)	0.3867
12 weeks after	50	8 (16.0)	42 (84.0)	1.50	(0.27, 8.41)	1.0000	1.26	(0.18, 8.70)	0.8123	0.66	(0.03, 14.08)	1.0000	---	---	---
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	68	10 (14.7)	58 (85.3)	1.82	(0.32, 10.37)	0.6110	2.10	(0.34, 13.02)	0.4264	---	---	---	---	---	---
2 weeks after	51	7 (13.7)	44 (86.3)	0.70	(0.12, 4.03)	1.0000	1.13	(0.16, 7.84)	0.8999	0.77	(0.14, 4.47)	1.0000	0.65	(0.10, 4.36)	0.6592
3 weeks after	52	5 (9.6)	47 (90.4)	1.86	(0.28, 12.16)	0.6521	2.55	(0.27, 24.04)	0.4133	0.34	(0.04, 3.25)	0.6368	0.37	(0.03, 4.71)	0.4463
12 weeks after	50	8 (16.0)	42 (84.0)	1.29	(0.25, 6.60)	1.0000	1.44	(0.19, 11.16)	0.7266	0.36	(0.04, 3.34)	0.6539	0.36	(0.04, 3.75)	0.3956
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	68	10 (14.7)	58 (85.3)	2.18	(0.51, 9.26)	0.3264	2.38	(0.54, 10.47)	0.2513	---	---	---	---	---	---
2 weeks after	51	7 (13.7)	44 (86.3)	2.00	(0.22, 18.49)	1.0000	1.36	(0.11, 16.60)	0.8108	0.65	(0.07, 6.09)	1.0000	0.58	(0.05, 6.80)	0.6671
3 weeks after	52	5 (9.6)	47 (90.4)	0.82	(0.08, 8.35)	1.0000	0.24	(0.01, 7.92)	0.4240	0.65	(0.07, 6.41)	1.0000	1.29	(0.06, 26.30)	0.8666
12 weeks after	50	8 (16.0)	42 (84.0)	0.97	(0.17, 5.58)	1.0000	0.65	(0.09, 4.80)	0.6735	0.51	(0.05, 4.69)	1.0000	0.47	(0.04, 5.51)	0.5468

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

Table 20: Effect of bronchodilator medication use on immune response to influenza vaccine in a VA Life Care Facility, 1996

				Seroprotection						Seroconversion					
		Used Medication?		Bivariate Chi-square Analysis			Multivariate Log Reg Analysis			Bivariate Chi-square Analysis			Multivariate Log Reg Analysis		
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H1N1)															
At vaccination	68	8 (11.8)	60 (88.2)	3.00	(0.56, 16.07)	0.2660	3.22	(0.58, 17.93)	0.1818	---	---	---	---	---	---
2 weeks after	51	6 (11.8)	45 (88.2)	6.61	(0.35, 125.06)	0.1622	---	---	---	2.05	(0.19, 22.15)	0.4799	2.06	(0.17, 24.85)	0.5709
3 weeks after	52	6 (11.5)	46 (88.5)	5.23	(0.28, 99.60)	0.3172	---	---	---	4.11	(0.71, 23.86)	0.1273	4.43	(0.64, 30.85)	0.1328
12 weeks after	50	6 (12.0)	44 (88.0)	7.53	(0.40, 142.32)	0.1587	---	---	---	4.20	(0.32, 55.06)	0.3243	8.67	(0.34, 222.18)	0.1919
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza A(H3N2)															
At vaccination	68	8 (11.8)	60 (88.2)	2.52	(0.42, 15.02)	0.2847	2.72	(0.41, 18.04)	0.3002	---	---	---	---	---	---
2 weeks after	51	6 (11.8)	45 (88.2)	2.00	(0.36, 11.13)	0.6524	2.18	(0.32, 14.94)	0.4269	1.00	(0.16, 6.09)	1.0000	1.02	(0.14, 7.65)	0.6512
3 weeks after	52	6 (11.5)	46 (88.5)	2.60	(0.43, 15.65)	0.3967	1.67	(0.19, 14.77)	0.6434	0.26	(0.03, 2.41)	0.3818	0.40	(0.03, 5.05)	0.4780
12 weeks after	50	6 (12.0)	44 (88.0)	1.11	(0.17, 7.43)	1.0000	0.44	(0.03, 5.78)	0.5292	0.19	(0.01, 3.60)	0.3007	---	---	---
Association	N	Yes (%)	No (%)	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value	Odds Ratio	95% Conf Interval	p-value
Influenza B															
At vaccination	68	8 (11.8)	60 (88.2)	1.46	(0.32, 6.66)	0.7189	1.02	(0.13, 7.91)	0.9850	---	---	---	---	---	---
2 weeks after	51	6 (11.8)	45 (88.2)	0.58	(0.09, 3.59)	0.6185	0.49	(0.06, 4.36)	0.5242	0.80	(0.08, 7.73)	1.0000	0.39	(0.04, 4.43)	0.4506
3 weeks after	52	6 (11.5)	46 (88.5)	1.05	(0.11, 10.27)	1.0000	0.38	(0.02, 8.65)	0.5429	1.42	(0.23, 8.75)	0.6548	2.44	(0.18, 34.07)	0.5063
12 weeks after	50	6 (12.0)	44 (88.0)	5.16	(0.27, 98.57)	0.3142	---	---	---	2.19	(0.34, 14.10)	0.5883	2.61	(0.27, 25.02)	0.4054

Relevant confounders included in logistic regression: HI titers at vaccination, body mass index, and recent influenza vaccination history.

FIGURES

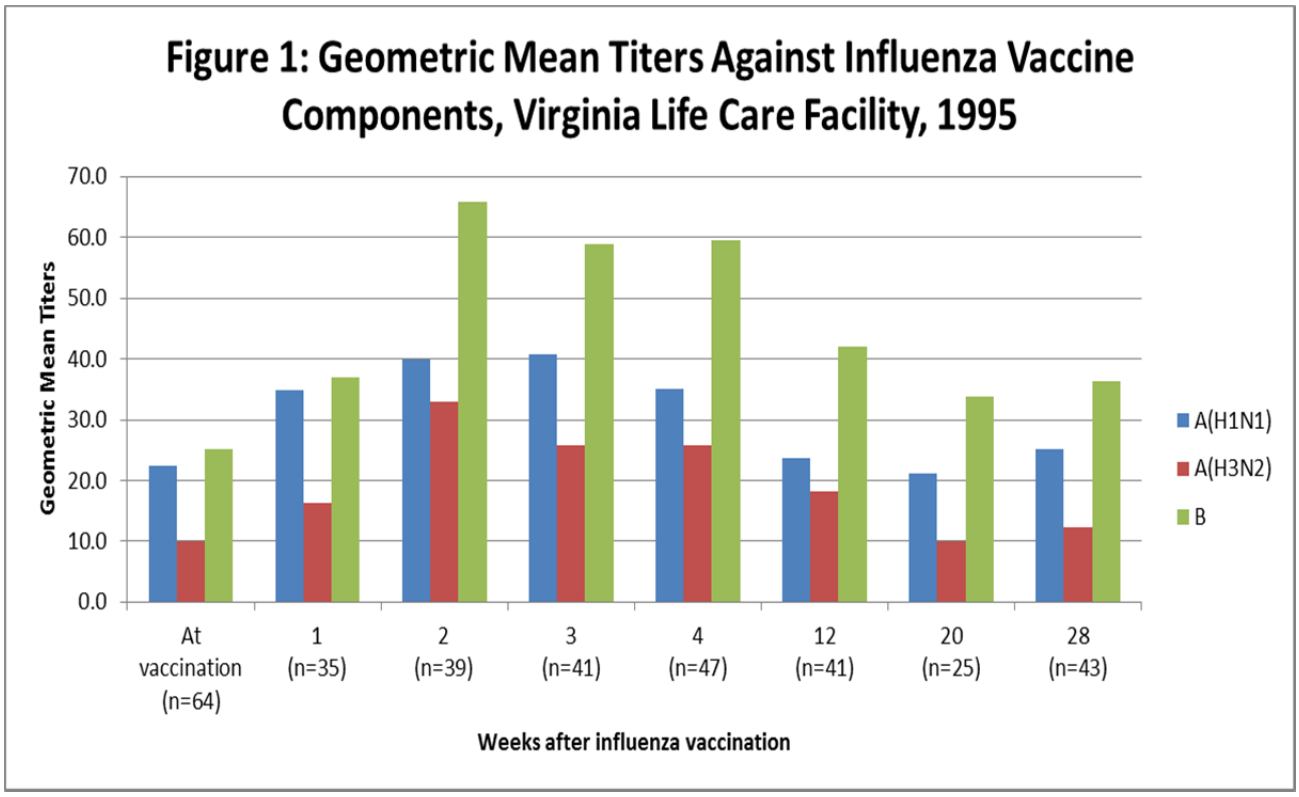


Figure 1: Geometric Mean Titers Against Influenza Vaccine Components, Virginia Life Care Facility, 1995
Geometric Mean Titers
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
At vaccination (n=64)
1 (n=35)
2 (n=39)
3 (n=41)
4 (n=47)
12 (n=41)
20 (n=25)
28 (n=43)
Weeks after influenza vaccination
A(H1N1)
A(H3N2)
B

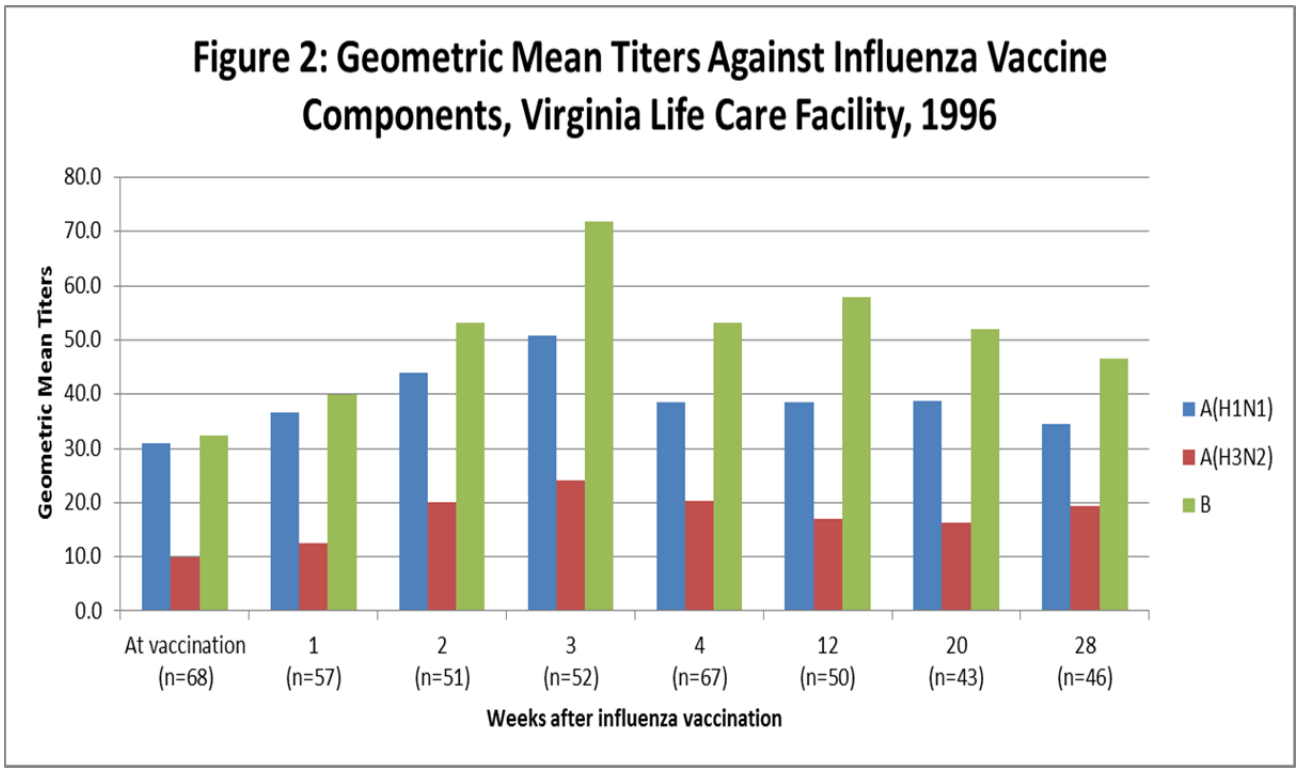
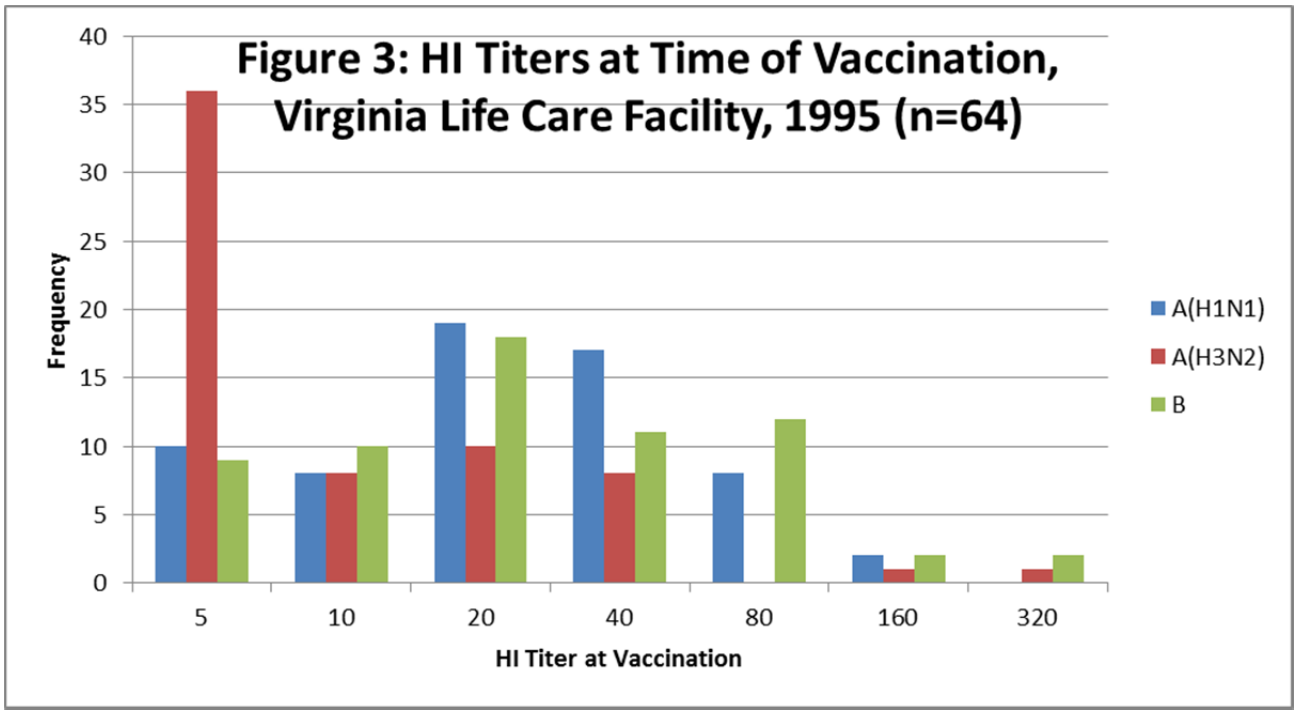
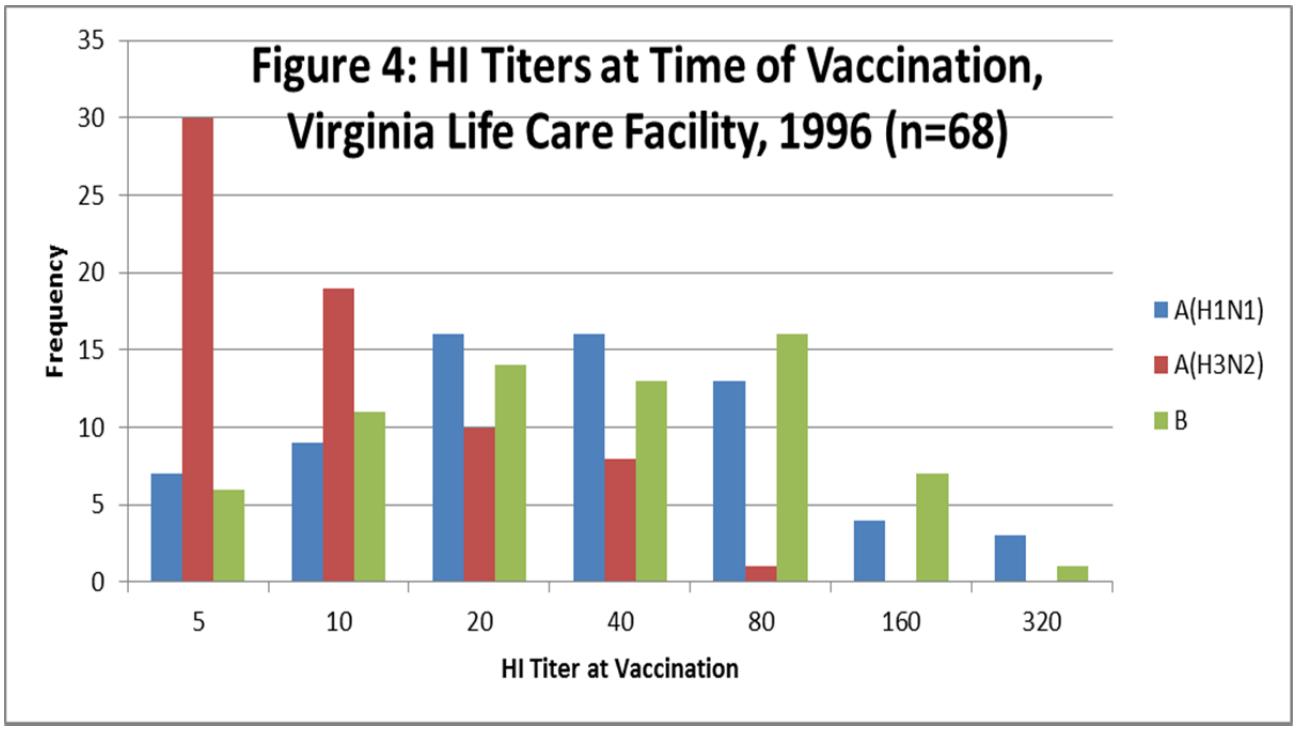


Figure 2: Geometric Mean Titers Against Influenza Vaccine Components, Virginia Life Care Facility, 1996
Geometric Mean Titers
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
At vaccination (n=68)
1 (n=57)
2 (n=51)
3 (n=52)
4 (n=67)
12 (n=50)
20 (n=43)
28 (n=46)
Weeks after influenza vaccination
A(H1N1)
A(H3N2)
B



Note: "HI Titers" refers to hemagglutination inhibition (HI) antibody titers.



Note: "HI Titers" refers to hemagglutination inhibition (HI) antibody titers.

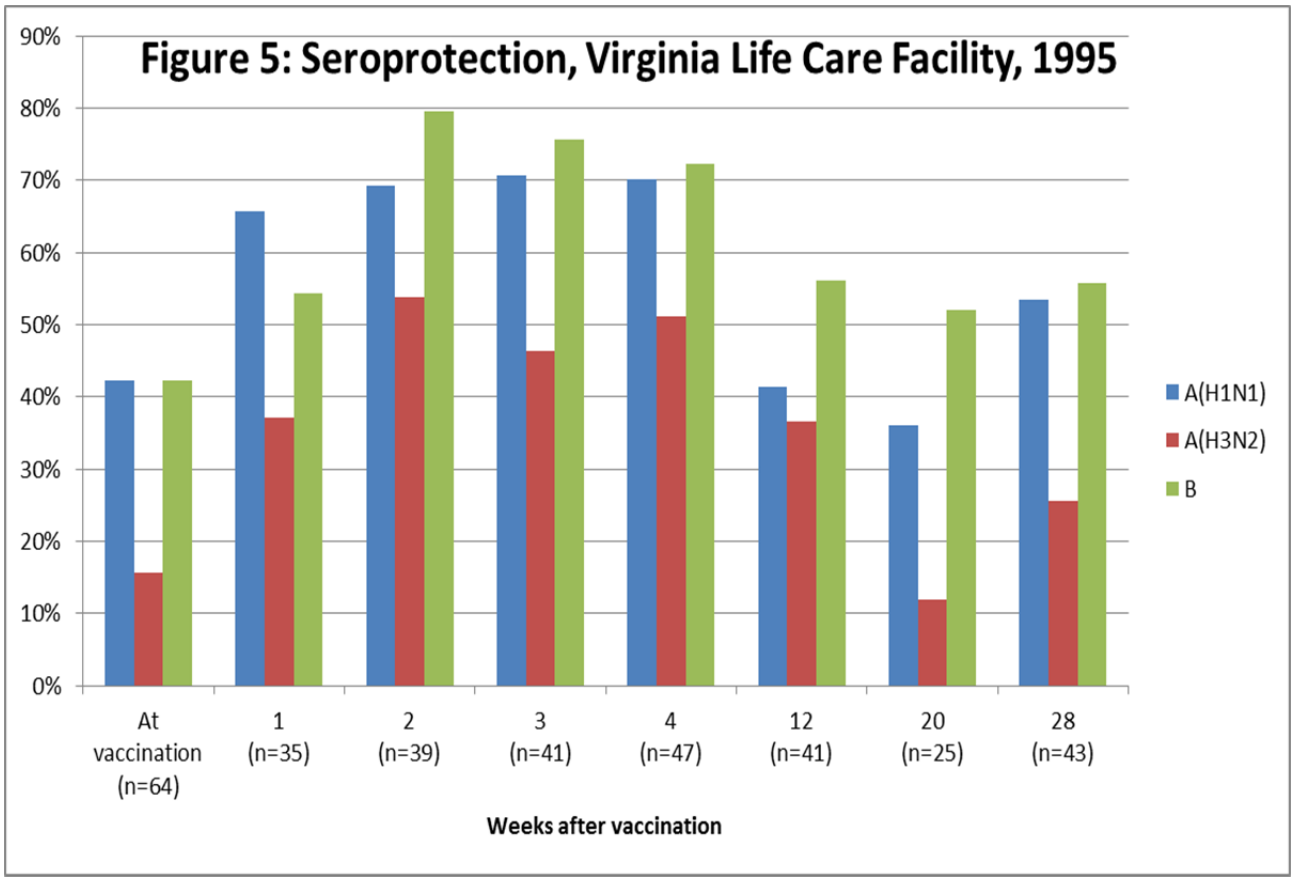
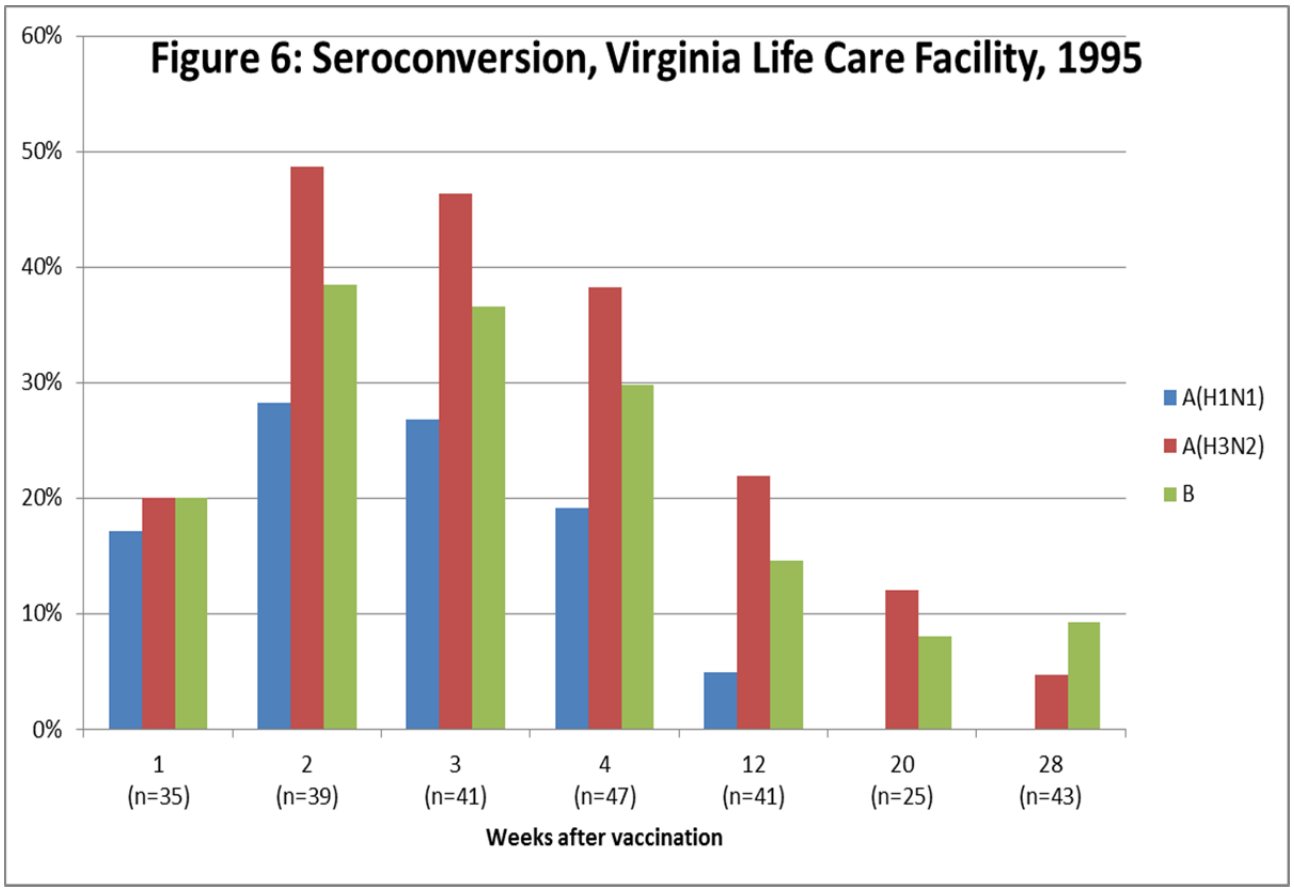


Figure 5: Seroprotection, Virginia Life Care Facility, 1995
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
At vaccination (n=64)
1 (n=35)
2 (n=39)
3 (n=41)
4 (n=47)
12 (n=41)
20 (n=25)
28 (n=43)
Weeks after vaccination
A(H1N1)
A(H3N2)
B



Note: Bars indicate percentage of subjects who experienced at least a 4-fold increase over baseline HI titers during that week.

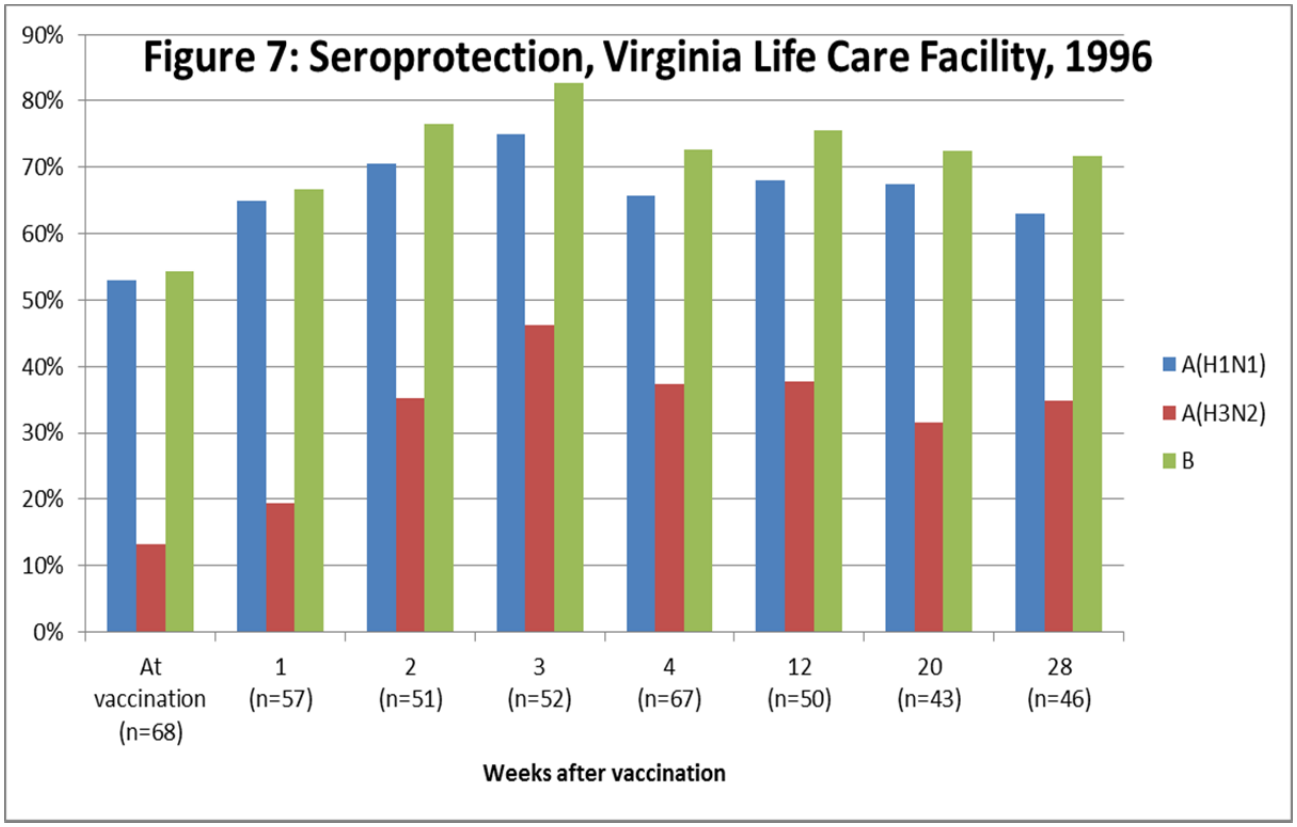
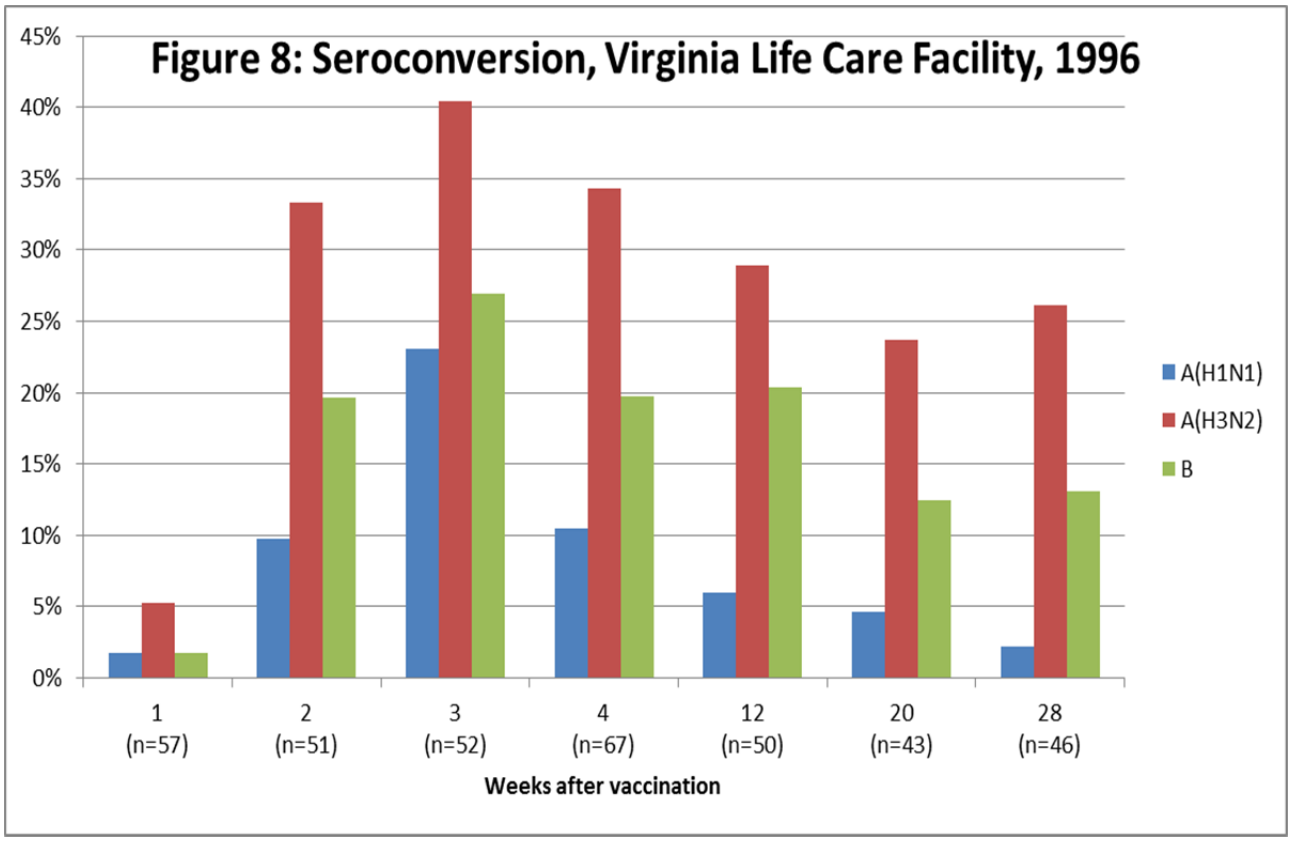


Figure 7: Seroprotection, Virginia Life Care Facility, 1996
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
At vaccination (n=68)
1 (n=57)
2 (n=51)
3 (n=52)
4 (n=67)
12 (n=50)
20 (n=43)
28 (n=46)
Weeks after vaccination
A(H1N1)
A(H3N2)
B



Note: Bars indicate percentage of subjects who experienced at least a 4-fold increase over baseline HI titers during that week.

APPENDIX I

Cardiac anti-hypertensive medications

1995

- Aldactone
- Amiodarone
- Atenolol
- Calan SR
- Cardizem
- Clonidine
- Dilacor
- Dilantin
- Diltizaem
- Dyazide
- Furosemide
- Hydrochlorot
- Hydroiuril
- Hytrin
- Isosorbide Dinitrate
- Lanoxin
- Lasix
- Lotensin
- Lozol
- Maxzide
- Metoprolol
- Mevacor
- Neptazane
- Nifedipine
- Nitro-Dur
- Nitroglycerine
- Nitrostat
- Normadyne
- Norvasc
- NTG
- Ocupress
- Papaverine
- Pravachol
- Prinivil
- Procardia
- Quinadine Gluconate
- Spirolactone
- Tenormin
- Timoptic
- Triamterene/HCT
- Vasocor
- Vasotec
- Zaroxolyn
- Zestril
- Ziac

1996

- Aldactone
- Altace
- Amlodipine
- Atenolol
- Cardizem
- Clonidine
- Corgard
- Cozaar
- Dilantin
- Diltiazem
- Dyazide
- Furosemide
- Iescol
- Isosorbide Dinitrate
- Hydralazine
- Hytrin
- Lanoxin
- Lasix
- Lotensin
- Lopid

- Lozol
- Metoprolol
- Mevacor
- Neptazane
- Nitro-Dur
- Nitrostat
- Norvasc
- Papaverine
- Prinivil
- Procardia
- Tiazac
- Tenormin
- Timoptic
- Triamterene/HCT
- Vasotec
- Verelan
- Zaroxolyn
- Zestril
- Ziac
- Zocor

Anti-inflammatory analgesic medications

1995

- Acetaminophen
- Aspirin
- Clinoril
- Darvocet
- Fioricet
- Ibuprofen
- Methocarbamol
- Naprosyn
- Paregoric
- Percodan
- Relafen
- Soma
- Tilade
- Voltaren

1996

- Acetaminophen
- Aspirin
- Butalbital
- Carisoprodol
- Fioricet
- Ibuprofen
- Methocarbamol
- Propacet
- Propoxy-n
- Relafen
- Robasisal
- Roxicet
- Soma
- Sulindac
- Ultram
- Unisom
- Voltaren

Steroidal medications

1995

- Azmacort
- Beclovent
- Beconase
- Betamethasone
- Flonase
- Meticorten
- Prednisone
- Vancenase

1996

- Azmacort
- Beconase
- Cordisone
- Flonase
- Omnaris
- Prednisone

- Vancenase
- Vanceril

Bronchodilator medications

1995

- Proventil
- Serevent
- Theodur
- T-Phyl

1996

- Albuterol
- Atrovent
- Serevent
- Theodur
- Theophylline
- Ventolin

APPENDIX II

[NAME OF VIRGINIA LIFE CARE FACILITY REDACTED]/CDC INFLUENZA STUDY DATA COLLECTION FORM 1995/1996

Name: _____
Sex: Male ___ Female ___
Race/Ethnicity:
American Indian or Alaskan Native ____
Asian or Pacific Islander ____
Black, not of Hispanic Origin ____
Hispanic ____
White, not of Hispanic Origin ____
Month/Year of birth: __ / ____ (month/4-digit year)
Year you moved to [NAME OF FACILITY REDACTED]: _____
Height: _____
Weight: _____
In which section do you currently reside? (Check one)
Independent Living ____
Assisted Living ____
Health Care Unit ____
Do you currently smoke cigarettes?(Check one)
Yes ___
No ___
If yes, number of cigarettes per day ___, or number of packs per week ___.

Chronic Conditions:

Have you been diagnosed with the following health conditions?

		Yes	No
1.	Arteriosclerotic Cardiovascular Disease (hardening of the arteries)	____	____
2.	Angina	____	____
3.	Myocardial infarction (heart attack)	____	____
4.	Congestive heart failure	____	____
5.	Hypertension (high blood pressure)	____	____
6.	Peripheral vascular disease (venous insufficiency)	____	____
7.	Other heart disease	____	____
	If yes, specify _____		
8.	Chronic lung disease	____	____
9.	Diabetes	____	____
	If yes, do you use insulin?	____	____
10.	Hypothyroidism	____	____
11.	Kidney disease	____	____
	If yes, specify _____		
12.	Do you currently receive dialysis?	____	____
13.	Cancer (under active treatment)	____	____

Chronic Conditions Continued:

Have you been diagnosed with the following health conditions?

	Yes	No
14. Diseases of the immune system (does not include a depressed immune system caused by medication)	____	____
If yes, please list disease(s) _____		
15. Mental depression	____	____
16. Arthritis	____	____
17. Osteoporosis	____	____
18. Degenerative joint disease	____	____
19. Other bone or joint disease	____	____
If yes, specify _____		
20. History of stroke	____	____
21. Parkinson's disease	____	____
22. Multiple sclerosis	____	____
23. Other neurologic/neuromuscular disorder	____	____
If yes, specify _____		
24. Anemia	____	____
25. Other blood disorder	____	____
If yes, specify _____		

Vaccination History

During how many of the past 5 years have you received influenza vaccine?

Never ____

1-2 times ____

3-4 times ____

Every year ____

Have your ever received pneumococcal vaccine?

Yes ____ No ____

If yes, in what year did you receive pneumococcal vaccine? _____

Please list below all medications, both prescription and over the counter that you are currently taking.

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

APPENDIX 3

CONSENT AGREEMENT FOR A STUDY OF THE IMMUNE RESPONSE TO FLU SHOTS AMONG OLDER PEOPLE

Researchers at the Centers for Disease Control and Prevention (CDC**)** and [NAME OF VIRGINIA LIFE CARE FACILITY REDACTED] are doing a five-year study to look at how age, health problems, certain drugs, overall level of health, and past influenza vaccinations ("flu shots") affect the level of protection given by the current flu shot in people age 65 and older.

A yearly flu shot is recommended for all people age 65 and older. The flu shot should be taken every year because the antibody levels (protection given by the vaccine) decline over time. Also, flu viruses are slowly changing all the time so one or two of the viruses in the vaccine are changed each year. Flu shots are about 30% to 70% effective in keeping older people from having to go to the hospital for flu or pneumonia, and are even better at keeping people from dying from flu or other health problems made worse by the flu. The rate at which antibody levels drop varies from person to person, but may happen more quickly among older people. Because older people also generally have a lower level of antibody after a flu shot, antibody levels may fall below what is needed to keep them from getting the flu before the flu season has ended. Although a second flu shot given in the middle of the flu season has not been recommended, it is thought that it could help some people. Studies have not been done yet to see who would be helped and by how much. This study will seek to answer the questions below.

1.) What is the level of antibody made after the flu shot?

2.) How long does it last during the flu season?

3.) What level of antibody protects against severe illness?

4.) What characteristics affect antibody levels after a flu shot?

5.) Will a second flu shot in the middle of the flu season provide extra protection?

6.) Will a second flu shot have more side effects?

You are being asked to be in this study because you live at [NAME OF VIRGINIA LIFE CARE FACILITY REDACTED]. People who have gotten flu shots during the past five years, as well as those who have not gotten flu shots, are being asked to be in the study. We hope to have 100 people join the study.

If you agree to take part in this study, you will be asked to receive a flu shot, answer some questions about yourself and your health, and to donate a small amount of blood (less than one teaspoon) at 5 scheduled times between October 1997 and May 1998. Also, you may be chosen at random to receive a second flu shot during the winter. You don't have to do anything special, such as not eating or drinking, before you get your flu shot or give

a blood sample. If an outbreak of flu-like illness occurs at [NAME OF VIRGINIA LIFE CARE FACILITY REDACTED] and you become ill, you may be asked to allow [NAME OF VIRGINIA LIFE CARE FACILITY REDACTED] staff members to swab your throat so that the people doing the study at CDC can find out what virus is causing the illness.

If you take part in this study, a study code number, your month and year of birth, and medical information will be written on a form or entered into a computer and will be given to the people doing the study at CDC. Only staff members of [NAME OF VIRGINIA LIFE CARE FACILITY REDACTED] who are working on the study will be able to link your name and study code number. This information will be kept private to the fullest extent allowed by law. You will never be named in any report that may come from this study.

Benefits and Discomforts

There is no direct benefit to you as a result of taking part in this study, but your being in the study may help the people doing the study to learn more about how people respond to flu shots, and improve ways to protect older people from the flu and its complications. Having blood taken may be uncomfortable and may sometimes leave a bruise around the vein it was taken from. Some people may feel like gagging when their throat is swabbed. People who have a sore throat may also find it uncomfortable during the short time that a throat swab is taken. The risks and benefits of flu shots are discussed in the attached document "Influenza Vaccine: What You Need to Know". Earlier studies do not show any more side effects after a second flu shot compared to the first flu shot during a single flu season.

You do not have to be in this study if you do not want to join. You do not have to be in the study to get a flu shot. A flu shot is recommended for all people age 65 and older. Those who do agree to take part in the study may change their mind and leave the study at any time. If you do not want to be in the study or decide to leave the study, it will not affect the care you receive. People in the study will be able to contact the people doing the study at CDC or at [NAME OF VIRGINIA LIFE CARE FACILITY REDACTED] at any time if they have questions about the study.

* * * * * * * *

The proposed study has been explained to me, and I have been given the chance to ask questions. I understand that if I have further questions I may contact [NAMES AND CONTACT INFO REDACTED] My signature below indicates my agreement to be in this study.

_____ _____

Date Signature of participant

_____ _____

Date Signature of guardian/responsible party

_____ _____

Date Signature of witness

Consent for Storage and Use of Left Over Specimens

I agree to allow any portion of my blood specimen which remains at the end of this study to be stored at CDC for possible use in future research studies of respiratory viruses. If any findings of clinical significance to my health are obtained as a result of these studies, CDC will make every effort to contact me and inform me of the results. Refusal to allow CDC to store the unused portion of my blood specimen will not affect my ability to be in the influenza vaccine study. My signature below indicates my agreement to allow storage of my blood samples.

_____ _____

Date Signature of participant

_____ _____

Date Signature of guardian/responsible party

_____ _____

Date Signature of witness

APPENDIX 4

Below is the SAS code and output for a logistic regression model with the outcome of seroprotection against the 1995 A(H3N2) antigen two weeks after vaccination. The predictor of interest is use of cardiac anti-hypertensive medication.

Potential confounding variables are: age, A(H3N2) antibody titers at vaccination, physical activity level, body mass index, and previous vaccination history, all dichotomized as described in the Methods section.

Interaction terms are two-terms: the predictor of interest multiplied by each potential confounder.

The hierarchical backward elimination approach was used to reduce the initial model containing all possible confounders and interaction terms to a final model containing only statistically significant interaction terms, their lower order components, relevant confounders, and the predictor of interest.

Here is the SAS code for the initial model:

```
proc logistic data = work.pfg95dichot desc;
  model pjos3 = ca agedi basejos1di activitydi bmidi vacxdi ca*agedi
  ca*basetxs1di ca*activitydi ca*bmidi ca*vacxdi / expb;
run;
```

The output is below. All interaction terms are highly non-significant, and continue to be so when removed one at a time by highest p-value, as described in the Methods section:

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq	Exp(Est)
Intercept	1	-59.5374	240.7	0.0612	0.8046	0.000
CA	1	56.3933	240.6	0.0549	0.8147	3.099E24
agedi	1	13.8858	66.7240	0.0433	0.8351	1072780
basejos1di	1	-4.2908	1.5742	7.4292	0.0064	0.014
activitydi	1	8.9372	101.0	0.0078	0.9295	7609.850
bmidi	1	14.5775	66.7175	0.0477	0.8270	2142632
vacxdi	1	13.9251	88.1112	0.0250	0.8744	1115874
CA*agedi	1	-13.1035	66.6913	0.0386	0.8442	0.000
CA*basetxs1di	1	0.8873	1.1639	0.5812	0.4458	2.429
CA*activitydi	1	-7.5665	101.0	0.0056	0.9403	0.001
CA*bmidi	1	-13.0057	66.6542	0.0381	0.8453	0.000

Analysis of Maximum Likelihood Estimates						
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**	**Exp(Est)**
CA*vacxdi	1	-11.0075	88.0416	0.0156	0.9005	0.000

Next, all potential confounders were evaluated for statistical significance, while keeping in mind biological relevance.

```
proc logistic data = work.pfg95dichot;
  model pjos3 = ca agedi basejos1di activitydi bmidi vacxdi  / expb;
run;
```

The output is below. Age was the potential confounder with the highest p-value, suggesting it was not an important predictor of the outcome. Considering biological relevance, subjects ranged in age between 72 and 96 years, with 61% between 80-89 years. Thus, age was considered to be relatively homogenous and non-informative. As such, age was removed as a potential confounder. This pattern was followed in all other logistic regression modeling.

Analysis of Maximum Likelihood Estimates						
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**	**Exp(Est)**
Intercept	1	4.1660	3.0046	1.9225	0.1656	64.455
CA	1	0.1405	1.0542	0.0178	0.8940	1.151
agedi	1	0.1151	0.9040	0.0162	0.8987	1.122
basejos1di	1	-3.4414	1.0504	10.7339	0.0011	0.032
activitydi	1	0.1483	1.0694	0.0192	0.8897	1.160
bmidi	1	0.1900	1.0020	0.0359	0.8496	1.209
vacxdi	1	0.2706	1.4559	0.0346	0.8525	1.311

The model, without age, was re-run. As with interaction terms, potential confounders were removed one at a time based on highest p-value, keeping in mind biological relevance.

Analysis of Maximum Likelihood Estimates						
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**	**Exp(Est)**
Intercept	1	4.3344	2.7050	2.5677	0.1091	76.281
CA	1	0.1255	1.0506	0.0143	0.9049	1.134
basejos1di	1	-3.4471	1.0507	10.7635	0.0010	0.032
activitydi	1	0.1468	1.0701	0.0188	0.8909	1.158
bmidi	1	0.1963	1.0007	0.0385	0.8445	1.217
vacxdi	1	0.2931	1.4443	0.0412	0.8392	1.341

Physical activity level was removed next and the model was re-run.

Analysis of Maximum Likelihood Estimates						
Parameter	**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**	**Exp(Est)**
Intercept	1	4.5466	2.2305	4.1551	0.0415	94.313
CA	1	0.0938	1.0276	0.0083	0.9273	1.098
basejos1di	1	-3.4223	1.0310	11.0181	0.0009	0.033
bmidi	1	0.2327	0.9681	0.0578	0.8100	1.262
vacxdi	1	0.2391	1.3849	0.0298	0.8629	1.270

Though they have high p-values, body mass index and previous vaccination history were considered to be biologically relevant predictors, so kept in the model. The SAS code for the final model is as follows:

```
proc logistic data = work.pfg95dichot desc;
  model pjos3 = ca basejos1di bmidi vacxdi / expb;
run;
```

Below are the odds ratio estimates:

Odds Ratio Estimates			
Effect	**Point Estimate**	**95% Wald Confidence Limits**	
CA	0.911	0.122	6.822
basejos1di	30.641	4.062	231.156
bmidi	0.792	0.119	5.285
vacxdi	0.787	0.052	11.884

The output can be interpreted as follows: the odds of seroprotection against the 1995 A(H3N2) antigen two weeks after influenza vaccination for a cardiac anti-hypertensive medication user versus a non-user were 0.91 (95% CI: 0.12, 6.82). However, this association was not statistically significant (p=0.