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Caroline Q. Pratt	Date

Risk Factors for Failure to Respond to Influenza Vaccination Among Adults Hospitalized with Community-Acquired Pneumonia in the CDC Etiology of Pneumonia in the Community (EPIC) Study

By

Caroline Q. Pratt

Master of Public Health

Global Epidemiology

_____

John E. McGowan, MD
Faculty Thesis Advisor

_____

Evan J. Anderson, MD
Thesis Field Advisor

Abstract

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By Caroline Q. Pratt

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

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Abstract

Background: Influenza is a significant cause of morbidity and mortality among adults. Despite influenza vaccination being recommended for adults, many adults remain unvaccinated and strategies to improve influenza vaccination are needed. Administration of influenza vaccine during a non-influenza associated hospital admission may improve influenza vaccination rates, but the serological response to vaccination might be impaired due to illness.

Methods: Adults with radiologically-confirmed pneumonia requiring hospitalization were prospectively enrolled into the CDC-sponsored Etiology of Pneumonia in the Community (EPIC) study. A subset of these enrolled adults without evidence of influenza infection received seasonal influenza vaccination in between the obtaining of acute and convalescent serologies. Seroconversion was defined as those with ≥4–fold rise in hemagglutination inhibition (HAI) titer between acute and convalescent serology for influenza A (H3N2 or H1N1) and/or B (Yamagata or Victoria strain). Seroconversion and non-seroconversion populations were compared and risk factors associated with failure to seroconvert to influenza vaccination were identified using univariate analysis. Multivariate stepwise analysis was conducted using variables potentially associated with failure to seroconvert identified on univariate analysis ($p<0.20$).

Results: Of the 95 patients who met the inclusion criteria, 66 (69.5%) seroconverted after receipt of seasonal influenza vaccination to one or more strains of influenza A and/or B. In univariate analysis, failure to seroconvert was associated with diabetes ($p=0.03$) and an elevated baseline HAI titer for influenza B (Yamagata) strain ($p=0.03$). Other variables approaching significance that were included in the stepwise multivariate model were gender ($p=0.15$), receipt of influenza vaccine in prior season ($p=0.05$), liver disease ($p=0.07$), identification of a bacterial pathogen ($p=0.09$), and elevated baseline HAI titer for influenza A (H3N2) ($p=0.17$). Markers of CAP severity (e.g., PSI score, ICU admission, and duration of hospitalization) did not correlate with a failure to seroconvert. On multivariate analysis, failure to seroconvert was predicted by diabetes ($p=0.02$), receipt of the influenza vaccine in the previous season ($p=0.03$), presence of positively identified bacterial infection ($p=0.05$), and elevated influenza B (Yamagata) HAI titer at baseline ($p=0.03$). It inversely correlated with the presence of liver disease ($p=0.05$).

Conclusions: About one-third of patients who received seasonal influenza vaccination during hospitalization for community-acquired pneumonia did not seroconvert. Factors associated with a failure to seroconvert to either influenza A or B were diabetes, receipt of influenza vaccine in the prior season, presence of positively identified bacterial infection, and elevated influenza B HAI baseline titers. Certain groups may be at risk for failure to seroconvert after receiving seasonal influenza vaccine while hospitalized for community-acquired pneumonia.

Risk Factors for Failure to Respond to Influenza Vaccination Among Adults Hospitalized with Community-Acquired Pneumonia in the CDC Etiology of Pneumonia in the Community (EPIC) Study

By

Caroline Q. Pratt

B.S.N.
Georgetown University
2010

B.A.
University of Virginia
2008

Faculty Thesis Advisor: John E. McGowan, MD

Thesis Field Advisor: Evan J. Anderson, MD

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Chapter I: Background

Influenza and Vaccination

Worldwide each year, there are between three and five million severe cases of influenza and between 250,000 and 500,000 deaths (1). During an epidemic, 5-15% of the world's population is thought to be infected (2). In 2011 (the most recent data available), 53,667 deaths were attributed to influenza and pneumonia in the US, a 4% increase in the death rate from 2010 (3).

Annual influenza vaccination is the best method for preventing influenza (4). Healthy People 2020 set a goal of 80% influenza vaccination coverage in the US for adults aged 18 to 64 and 90% coverage for individuals 65 and older (5) in an attempt to reach "herd protection." In 2008, however, only 29.5% of community-dwelling adults aged 18-64 and only 66% of community-dwelling adults 65 and older received the vaccine (5).

Since January 1, 2012, the Joint Commission has monitored influenza vaccination rates as a national inpatient quality measure in an attempt to increase influenza vaccination rates in the United States (6). The Joint Commission currently recommends influenza vaccination for any unvaccinated patient discharged from the hospital during October through March (7). As a result, many hospitals employ standard order sets and physician reminders to offer influenza vaccine to every patient at time of discharge (8, 9).

Many patients that receive influenza vaccination while hospitalized are currently, or are very recently, critically ill. This population is therefore different from relatively healthy individuals receiving the vaccine as outpatients in providers' office, clinics, and vaccine drives. This leads to the question, among patients receiving the vaccine while

hospitalized, are there differences (e.g. risk factors) between patients who seroconvert to the vaccine and those that do not? Additionally, are there specific risk factors associated with a failure to seroconvert after receiving the vaccine? Looking towards future studies, are there specific populations receiving influenza vaccination while hospitalized that would benefit from an adjuvanted vaccine, a high-dose influenza vaccine, or from delaying vaccination until they have recovered?

Past Studies

Past studies in vulnerable adult populations have identified chronic obstructive pulmonary disease (COPD), dementia, malnutrition, diabetes, older age, chronic lung disease, low pre-vaccine HAI titer, and residing in a long term care facility as potential risk factors for failure to seroconvert to the influenza vaccine (10, 11, 12, 13, 14, 15, 16). The majority of studies examining adult vulnerable populations have focused on elderly living in long-term care facilities rather than among individuals residing in the community (10, 11, 15). In addition, while some studies examine potential risk factors, data about potential risk factors for failure to seroconvert after influenza vaccination administered while hospitalized are limited, particularly as it relates to pre-existing conditions, disease severity, and biometric measures. This study investigates this issue through a secondary review of de-identified data from the Etiology of Pneumonia in the Community (EPIC) study.

Description of Dataset

Data on serologic response to influenza vaccination was obtained from the Centers for Disease Control and Prevention's EPIC study. This included data from adults admitted to five Chicago and Nashville-area hospitals between January 1, 2010 and June

30, 2012 (17). The EPIC study was a population-based study on the incidence and etiology of radiologically confirmed community-acquired pneumonia (CAP). Of the 2,481 adults originally enrolled in the EPIC study, 373 adults (15%) met radiographic criteria for community acquired pneumonia, did not have influenza identified by polymerase chain reaction (PCR), and received the influenza vaccine during hospitalization. Among this group, 101 patients received the influenza vaccine, had not received the vaccine previously in the current influenza season, and received the vaccine after acute serology was obtained but at least two weeks before convalescent serology was obtained. Four patients who received only the 2009 H1N1 vaccine during the fall/winter of 2009-10—but not the standard inactivated influenza vaccine containing influenza A (H1N1 and H3N2 strains) and influenza B (Victoria and Yamagata lineage)—were excluded. Two patients that received two doses of influenza vaccine during their admission were also excluded. Of the 95 patients meeting the final study criteria, 66 individuals seroconverted (defined as a greater than four-fold rise in hemagglutination inhibition [HAI] titer between acute and convalescent serology to either influenza A or B) while 29 did not seroconvert (defined as a less than four-fold rise in HAI titer between acute and convalescent serology).

Hypothesis

Following a review of the existing literature, one can hypothesize that likely risk factors for failure to seroconvert to at least one influenza strain are older age, chronic medical conditions, and more severe current illness. Data on chronic conditions that were available for review included COPD, asthma, chronic kidney disease, coronary artery disease or heart failure, type-II diabetes, liver disease, and HIV. Other potential risk

factors examined included: gender, race/ethnicity, smoking status, receipt of influenza vaccine in previous season, receipt of both influenza and pneumococcal vaccines, oral steroid use, identification of a bacterial pathogen, identification of a viral pathogen, specific identification of rhinovirus, history of stroke, previous pneumonia admission, history of non-skin cancer, immunosuppression, baseline influenza A (H1N1) HAI titer ≥40, baseline influenza A (H3N2) HAI titer ≥40, baseline influenza B (Victoria lineage) ≥40, baseline influenza B (Yamagata lineage) ≥40, and body mass index (BMI).

Outcome of Interest

Because it is difficult, and potentially dangerous, to perform large randomized clinical trials that directly measure a vaccine's protection against a specific disease, correlates of protection against disease are often used instead. A correlate is defined as "a specific immune response to a vaccine that is closely related to protection against infection, disease, or other defined end point" (18). Previous studies have shown that four-fold rise in HAI titer correlates with prevention of influenza disease in 50% of those who receive the vaccine (19). Immunological protection against influenza correlates to an HAI titer dilution of 1:40 or greater (18). Additionally, meta-analysis studies reported a 50% reduction in all-cause mortality among those 65-years-old and older who receive influenza vaccination (20).

Each of the 95 subjects meeting the final study criteria was placed into two groups divided by the outcome of interest—the dichotomous variable of seroconversion. HAI titers are determined using serial dilution of HAI assays. Samples of influenza virus and red blood cells are mixed together. As the virus binds to the red blood cells, a lattice is formed. The presence of influenza virus antibodies in the patient sample prevents

hemagglutination and the subsequent formation of a lattice. The higher the dilution in which patient antibodies prevent hemagglutination, the greater the correlate protection against influenza virus (21). An HAI titer dilution of 1:40 or a ≥ four-fold rise from acute to convalescent titer are generally accepted as indicative of preventing 50% of influenza cases in a given population (22). Seroconversion versus non-seroconversion was determined by dividing each patient's convalescent titer by his or her acute titer. Those with a four-fold rise or higher were placed in the seroconversion group (66 subjects) while those with a less than four-fold rise were placed in the non-seroconversion group (29 subjects).

Several studies, however, have questioned the 50% mortality prevention rate of vaccinating seniors. One study points out that only approximately 5% of all winter deaths are on average related to influenza and that the largest differences in mortality between those who are and are not vaccinated actually occur before influenza season begins, making it extremely unlikely that the majority of mortality prevention can be attributed to the influenza vaccine (23). An additional potential flaw in the 50% mortality reduction statistic is that more frail elderly individuals who die early in the influenza season (and therefore before receiving a vaccine) artificially raise the mortality rate of the unvaccinated when their deaths were not necessarily related to influenza infection (23, 24). One 2009 study points out "there is no 'gold-standard' randomized clinical trials to document influenza vaccine benefits in seniors aged 70 and older" (23). The high reduction in mortality may therefore be due to a selection bias (25).

Variables of Interest

To determine whether older age, severity of illness, and presence of pre-existing conditions are risk factors for failure to seroconvert to influenza vaccination, the following variables were compared between the two groups: age (in years), pneumonia severity index (PSI) (26), length of stay (in days), admission to intensive care unit, and past history of COPD, asthma, chronic kidney disease, coronary artery disease or heart failure, diabetes, chronic liver disease, stroke, HIV, immunosuppression, obesity, and non-skin cancer. History of the pre-existing conditions was obtained through patient interview and medical chart abstraction. To consider the effect of baseline HAI titers, HAI for influenza A strains H1N1 and H3N2 and influenza B Victoria and Yamagata lineages, influenza vaccination in the previous season was also compared between the seroconversion and non-seroconversion groups.

Age was chosen as a potential covariate due to the concept of immunosenescence, the loss of ability to mount an immune response that increases with age (27, 28). Previous studies of, for instance, pneumococcal vaccine, have shown that, "the ability to elicit a functional antibody response is distinctly reduced with advanced age" (27).

Severity of illness was also chosen as a potential covariate due to the fact that an immune system already fighting one serious illness may not be able to simultaneously mount a sufficient immune response for the influenza vaccine to be effective (29). The Centers for Disease Control even lists "moderate or severe acute illness with or without fever" as a "precaution" for giving the influenza vaccine (30). Anyone receiving the vaccine while admitted to the hospital likely fits this description. The PSI is a tool used by providers to predict mortality rates in individuals with CAP. The tool uses patient factors including age, sex, past medical history, vital signs, laboratory and radiology

results to place patients in one of five classes of mortality rates (26). PSI can also be used as a surrogate variable for severity of illness (31). No studies specifically examine the effectiveness of influenza vaccination in individuals with a current illness, such as pneumonia, severe enough to require hospitalization.

Past studies have also shown that sex has an effect on vaccination response (32). Specifically, females have been found to have more robust immune responses to influenza vaccination. Sex, therefore, was also included in the analysis in order to identify potential population differences.

Lastly, though not ultimately included in the final manuscript, presence of multiple pre-existing conditions, defined as individuals with two or more of the following conditions: asthma, chronic obstructive pulmonary disease, chronic kidney disease, coronary artery disease, diabetes mellitus, chronic liver disease, HIV, and any sort of chronic immunosuppression, was explored to determine whether having multiple chronic conditions could influence the immune system's ability to mount a sufficient immune response (12).

Chapter II: Manuscript

Title

Risk Factors for Failure to Respond to Influenza Vaccination Among Adults Hospitalized with Community Acquired Pneumonia in the CDC Etiology of Pneumonia in the Community (EPIC) Study

Abstract

Background: Influenza is a significant cause of morbidity and mortality among adults. Despite influenza vaccination being recommended for adults, many adults remain unvaccinated and strategies to improve influenza vaccination are needed. Administration of influenza vaccine during a non-influenza associated hospital admission may improve influenza vaccination rates, but the serological response to vaccination might be impaired due to illness.

Methods: Adults with radiologically-confirmed pneumonia requiring hospitalization were prospectively enrolled into the CDC-sponsored Etiology of Pneumonia in the Community (EPIC) study. A subset of enrolled adults without evidence of influenza infection received seasonal influenza vaccination in between the obtaining of acute and convalescent serologies. Seroconversion was defined as those with ≥4–fold rise in hemagglutination inhibition (HAI) titer between acute and convalescent serology for influenza A (H3N2 or H1N1) and/or B (Yamagata or Victoria strain). Seroconversion and non-seroconversion populations were compared and risk factors associated with failure to seroconvert to influenza vaccination were identified using univariate analysis.

Multivariate stepwise analysis was conducted using variables potentially associated with failure to seroconvert identified on univariate analysis ($p<0.20$).

Results: Of the 95 patients who met the inclusion criteria, 66 (69.5%) seroconverted after receipt of seasonal influenza vaccination to one or more strains of influenza A and/or B. In univariate analysis, failure to seroconvert was associated with diabetes ($p=0.03$) and an elevated baseline HAI titer for influenza B (Yamagata) strain ($p=0.03$). Other variables approaching significance that were included in the stepwise multivariate model were gender ($p=0.15$), receipt of influenza vaccine in prior season ($p=0.05$), liver disease ($p=0.07$), identification of a bacterial pathogen ($p=0.09$), and elevated baseline HAI titer for influenza A (H3N2) ($p=0.17$). Markers of CAP severity (e.g., PSI score, ICU admission, and duration of hospitalization) did not correlate with a failure to seroconvert. On multivariate analysis, failure to seroconvert was predicted by diabetes ($p=0.02$), receipt of the influenza vaccine in the previous season ($p=0.03$), presence of a bacterial pathogen ($p=0.05$), and elevated influenza B (Yamagata) HAI titer at baseline ($p=0.03$). Seroconversion inversely correlated with the presence of liver disease ($p=0.05$).

Conclusions: About one-third of patients who received seasonal influenza vaccination during hospitalization for community acquired pneumonia did not seroconvert. Factors associated with a failure to seroconvert to either influenza A or B were diabetes, receipt of influenza vaccine in the prior season, presence of a bacterial pathogen, and elevated influenza B HAI baseline titers. Certain groups may be at risk for failure to seroconvert

after receiving seasonal influenza vaccine while hospitalized for community-acquired pneumonia.

Introduction

Worldwide each year, there are between three and five million severe cases of influenza and between 250,000 and 500,000 deaths (1). During an epidemic, 5-15% of the world's population is thought to be infected (2). In 2011, 53,667 deaths were attributed to influenza and pneumonia in the US, a 4% increase in the death rate from 2010 (3). Annual influenza vaccination is the best method for preventing infection (4). Healthy People 2020 set a goal of 80% influenza vaccination coverage for adults aged 18 to 64 and 90% coverage for individuals 65 and older (5). In 2008, however, only 29.5% of community-dwelling adults aged 18-64 and only 66% of community-dwelling adults 65 and older received the vaccine (5).

Hospitalization provides an opportunity to administer influenza vaccine to patients who might otherwise not have a medical encounter in which influenza vaccine could be given. Patients with recent hospitalization also have a heightened risk of hospital readmission within the months immediately after hospital discharge (6).

The Joint Commission monitors influenza vaccination rates as a national inpatient quality measure in an attempt to increase influenza vaccination rates in the United States (7). The Joint Commission currently recommends influenza vaccination for any unvaccinated patient discharged from the hospital during October through March (8). Standardized orders and electronic reminder systems have been shown in several studies to increase influenza vaccination rates of admitted patients (9, 10). Although providing an opportunity for influenza vaccine administration, it is possible that adults receiving seasonal influenza vaccination while hospitalized may not have an optimal serological response due to acute illness or other comorbidities.

Past studies of influenza vaccination in adult populations have identified chronic obstructive pulmonary disease (COPD), dementia, malnutrition, diabetes, older age, chronic lung disease, high pre-vaccine hemagglutination-inhibition (HAI) antibody titer, and living in long-term care facility as potential risk factors for failure to seroconvert to influenza vaccination (11, 12, 13, 14, 15, 16, 17, 18). Many studies have focused on the elderly living in long-term care facilities rather than on community-dwelling elderly (11, 12, 16). Data are limited regarding influenza rates of seroconversion and risk factors for failure to seroconvert after vaccination in adults with a comprehensive list of pre-existing conditions and acute illness.

The CDC-sponsored Etiology of Pneumonia in the Community (EPIC) study was a large multicenter prospective study of radiologically-confirmed community-acquired pneumonia (19). Acute and convalescent serology was obtained on a number of patients and a subset received influenza vaccination while hospitalized providing a unique opportunity to retrospectively identify risk factors associated with failure to seroconvert after influenza vaccination.

Methods

Patient Population and Study Design: EPIC was a prospective study of the incidence and etiology of community-acquired pneumonia (CAP) in adults requiring hospitalization that was conducted at five Chicago and Nashville-area hospitals between January 1, 2010 and June 30, 2012 (19). Written informed consent was obtained from all participants, their legally authorized representatives, or both. Institutional review boards at the research sites and the CDC approved the study. Subjects were enrolled prospectively, a detailed questionnaire was administered, and data on medications and epidemiological and demographic data were collected using medical chart review. The full methods for this study are published elsewhere (19). Using data collected in the EPIC study, *post-hoc* analysis of the data was performed.

Among those enrolled in EPIC with radiologically-confirmed pneumonia, we required that they have a negative influenza NP/OP swab by PCR and to have acute and convalescent (obtained 2 – 10 weeks after enrollment into EPIC) serology available for testing (19). We also required that patients have received influenza vaccination only once while hospitalized and that this occurred at least 2 weeks prior to the convalescent serology date (see **Figure 1**).

The four HAI antibody titers examined were influenza A H1N1, influenza A H2N3, influenza B Victoria lineage, and Influenza B Yamagata lineage. In this study, seroconversion was defined as a four-fold or greater rise in HAI antibody titer between acute and convalescent serology. Those with ≥4-fold rise in ≥1 HAI titer(s) were considered to have seroconverted while those with a <4-fold rise in all influenza HAI titers were considered to have not seroconverted (20).

Statistical Methods: SAS Software version 9.4 was used to for data analysis. Patients were divided into the dichotomous categories of seroconversion or non-seroconversion. Categorical risk factors for failure to seroconvert were analyzed using percentages and number of subjects per group. Categorical risk factors included the following: sex, race/ethnicity, smoking status, receipt of influenza vaccine in the previous season, receipt of both influenza and pneumococcal vaccines, patient co-morbidities (e.g., COPD, asthma, previous pneumonia admission, chronic kidney disease, coronary artery disease or heart failure, type-II diabetes, liver disease, history of stroke, history of non-skin cancer, oral steroid use, HIV, immunosuppression), ICU admission, identification of a bacterial pathogen, identification of a viral pathogen including identification of rhinovirus, baseline influenza A (H1N1) HAI titer ≥40, baseline influenza A (H3N2) HAI titer ≥40, baseline influenza B (Victoria lineage) ≥40, and baseline influenza B (Yamagata lineage) ≥40. In order to detect potential bias within the study, study hospital and year of enrollment were also compared between the seroconversion and non-seroconversion groups. Continuous variables examined included age in years, body mass index (BMI), Pneumonia Severity Index (PSI), and duration of hospitalization in days.

The chi-square test was used to test for significant dichotomous variable differences between the seroconversion and non-seroconversion groups. Analysis of variance was used to examine potential differences between the two groups for categorical variables with >2 categories. Continuous variables were analyzed using median and interquartile range. The Wilcoxon rank test was used to test for significant differences between the two groups among continuous variables. Redundancy among the variables was assessed by testing for multicollinearity. Variables with a p-value <0.20 on

univariate analysis were then entered into a logistic regression model with manual stepwise selection (entry alpha=0.20, exit alpha=0.20). Risk ratios with 95% confidence intervals were calculated for each risk factor with p-value <0.20.

In order to assess for potential confounding, several covariates were stratified for age using three categories (18-49 years, 50-64 years, and >65 years old). Unadjusted risk ratios for non-seroconversion were compared to risk ratios adjusted for age. Smoking was also examined as a potential confounder for having received the influenza vaccine in the previous season. Additionally, having received the influenza vaccine in the previous season was considered as a potential confounder for responses to each influenza strain (influenza A H1N1 and H3N2 strains and influenza B Victoria and Yamagata lineage). Risk ratios for non-seroconversion unadjusted and adjusted for receipt of vaccine in the previous season were compared.

Results

Subject Distributions: Of the 2,481 adults originally enrolled in the EPIC study, 2320 (93%) met the final study definition of radiographically confirmed pneumonia. Of these 2320 enrolled adults, 373 adults (16.1%) did not have influenza identified by PCR but did receive influenza vaccine once while hospitalized (**Figure 1**). Of these 373, 95 patients (25.5%) had both acute and convalescent serology and received influenza vaccine at least two weeks before convalescent serology. Of the 95 patients meeting criteria for analysis, 66 (69%) seroconverted to one or more influenza strains contained in the vaccine while 29 (31%) did not seroconvert to any influenza strain in the vaccine (**Table 1**).

Overall, 44% of subjects in this substudy had a ≥4-fold rise in HAI for H1N1, 46% had a ≥4-fold rise in HAI for H3N2, 34% of subjects for influenza B (Victoria), and 22% had a ≥4 fold rise in HAI for influenza B (Yamagata).

Univariate Analysis: Univariate comparisons between those that seroconverted and those that did not seroconvert are shown in **Table 1**. The median age of those that seroconverted against any influenza strain was 54 (IQR=42-65) versus 55 (IQR=49-71) in those that did not seroconvert (p=0.5). The median PSI score among those that seroconverted was 68 (IQR=48-101) versus 71 (IQR=48-106) in those that did not seroconvert (p=0.6). The median duration of hospitalization was similar between those that seroconverted and those that did not [3.5 (IQR=2-5) days and 3.0 (IQR=2-5) days respectively (p=0.7)].

Thirty-one of those that seroconverted were female (47%) versus 9 (31%) in the non-seroconversion group (p=0.15). Eleven (17%) in the seroconversion group and 10

(34%) in the non-seroconversion group had received the influenza vaccine in the previous season (p=0.05). Ten patients (15%) in those that seroconverted versus 10 (34%) in those that did not seroconvert had a history of diabetes mellitus (p=0.03). All seven patients (11%) with a history of chronic liver disease seroconverted (p=0.07). No significant difference was observed in the rate of seroconversion among those patients who were immunosuppressed (6% versus 13.8%, p = 0.21). A bacterial pathogen was identified in 7 patients in those that seroconverted (11%) and in 7 (28%) of those that did not seroconvert (p=0.09). Among those that seroconverted, 14 (21%) had a baseline HAI titer ≥ 40 for influenza A (H3N2) versus 10 (34%) in those that did not seroconvert (p=0.17). Thirty-four (51%) of those that seroconverted versus 22 (76%, p=0.03) of those that did not seroconvert had a baseline HAI titer ≥ 40 for influenza B (Yamagata).

Multivariate Analysis: Multivariate analysis showed the following variables to be significant in the model for predicting seroconversion versus non-seroconversion: identification of a bacterial pathogen (p=0.05), history of diabetes (p=0.02), receipt of the influenza vaccine in the previous season (p=0.03), and elevated influenza B (Yamagata) HAI titer at baseline (p=0.03). Liver disease (p=0.05) was found to be inversely correlated with failure to seroconvert. Together, these five variables explain 22.2% of the result of seroconversion versus non-seroconversion.

Several additional calculations were performed to adjust for potential confounding. Adjusting for age as a categorical variable (using the categories of 18-49 years old, 50-64 years old, and 65 or older) did not alter the risk ratio for non-seroconversion in any variable by more than 6% (**Table 2**). Additionally, the majority of the adjusted risk ratios were closer to the null than the corresponding unadjusted risk

ratio. When adjusted for smoking status, the risk for non-seroconversion between those who did and did not receive the influenza vaccine in the previous season decreased by almost 10%. Adjusting for receipt of vaccine in the previous season on the risk of non-seroconversion for those who did and did not have elevated HAI antibody titers at baseline decreased the risk ratio by 8% for influenza A (H1N1) and by 5% for influenza A (H3N2) but did not alter either of the influenza B risk ratios.

Discussion

While 69% of the 95 individuals who received the influenza vaccine during hospitalization seroconverted to one or more influenza strains, 31% did not seroconvert to any of the vaccine strains. The extensive amount of data available on each subject as part of the EPIC study allowed for a careful examination of a varied and large list of potential covariates. Risk factors for failure to seroconvert included presence of diabetes, receipt of influenza vaccine in the previous season, identification of a bacterial pathogen, and elevated influenza B (Yamagata) HAI titer at baseline (**Table 2**). Notably, chronic liver disease was associated with a better response to influenza vaccination.

An elevated influenza B (Yamagata) baseline titer logically leads to an increased likelihood of an individual not having a four-fold or greater rise in HAI titer from baseline to convalescence. Among the 95 total individuals in the study, 59% had an elevated influenza B (Yamagata) antibody titer greater than 40 at baseline. The titer was high originally in these subjects and development of a four-fold increase after vaccination might have been impaired as has been described in other studies (12) Since it was possible that these high baseline Yamagata serologies were confounding the data analysis, we repeated the multivariate analysis after excluding the baseline Yamagata serology data. The results of this analysis were similar in that diabetes (p=0.03), liver disease (p=0.05), and receipt of previous season of influenza vaccine (p=0.03) remained statistically significant while identification of a bacterial pathogen (p=0.06) and sex (p=0.08) approached significance (overall R-squared=19.7%). Since those that had received an influenza vaccine in the prior season might also have higher baseline antibodies, this analysis was also repeated after removing the variable of prior influenza

vaccine receipt from multivariate analysis, and similar results were obtained. Diabetes, elevated baseline Yamagata titer (p=0.03), and liver disease (p=0.05) remained significant while bacterial infection approached significance (p=0.11). These data are also similar to prior data that demonstrate that influenza seroconversion is impaired after prior influenza vaccine receipt (13).

Importantly, we identified diabetes as a risk factor for failure to seroconvert after influenza vaccination. These data confirm the findings of others that have observed impaired seroconversion in those with diabetes (12). The etiology for this impaired response and whether tighter glucose control might improve the seroresponses observed after vaccination, remain uncertain. The identification of a bacterial pathogen supports the evidence from prior studies that individuals with immune systems already fighting other pathogens may not mount an immune response to the vaccine sufficient enough to produce seroconversion (12, 13, 21).

Other authors have found responses to influenza vaccination to correlate with age (16, 22). In this study, age, tested as both a categorical and continuous variable, was not found to differ significantly between the seroconversion and non-seroconversion groups. This could be due to the fact that average age of patients included was 56.9 years. If more adults between 18 and 49 had been found in the patient population, a significant difference in ages may have emerged. The small number of subjects included in the study could also play a role in the results. After adjusting for age as a potential confounder, the variable of elevated influenza B (Yamagata) baseline titer risk ratio increased from 2.19 to 2.15, having received the influenza vaccine in the previous season decreased from 1.85 to 1.76, and the risk ratio for the diabetes variable decreased slightly from 1.97 to 1.86

(**Table 2**). Each of these differences between adjusted and unadjusted risk ratios was less than 6% change.

Measures of severity of illness (e.g., PSI score, duration of hospitalization, and ICU admission) were not found to be significantly different between those who seroconverted and those who did not. The median PSI score of 70 among patients in the substudy was similar to the median PSI score of 76 among patients in the entire EPIC study (18). Since the decision to admit a patient to the ICU is closely tied to both age and PSI score, it may not have significantly differed between the two groups for similar reasons.

The risk ratio for history of liver disease was not able to be determined as all 7 patients with liver disease seroconverted to one or more influenza strain(s) after receiving the vaccine. While multiple studies have found seroprotection against influenza to be lower in patients with chronic liver disease, the same studies confirm that seroconversion to influenza vaccine is equal, if not better, in patients with chronic liver disease compared to healthy controls (23, 24, 25, 26).

Additionally, rates of seroconversion to H1N1, H3N2, and Victoria and Yamagata strains individually did not differ significantly between patients with liver disease and those without (H1N1 p=0.9, H3N2 p=0.8, Victoria p=0.4, Yamagata p=0.6). In this particular study, small study size is the mostly likely explanation for liver disease appearing protective against non-seroconversion although this finding could be confounded by other unmeasured variables.

This study has important limitations. It is a *post-hoc* analysis of data collected prospectively and subjects were not randomized to receiving influenza vaccine or not.

Since the EPIC study spanned from 2010 to 2012, the influenza vaccine differed slightly from season to season and different formulations were administered over time and across the centers. Unmeasured differences may have existed between the individuals who received the vaccine and returned for convalescent serology and those that did not return. Measurement bias should have been negligible as data collectors were not part of this future study. Additionally, individuals could have been subsequently infected with influenza between collection of acute and convalescent serology, causing HAI results to reflect actual infection and not vaccination. Use of hospital patients could have potentially caused a selection bias. Importantly, the estimates of this study lack precision and potential risk factors underappreciated due to the small sample size. Finally, the extent to which influenza vaccine works in preventing influenza-related illness in such patients (e.g., effectiveness) is much more complex and could be higher or lower than the observed rates of seroconversion.

This study of patients hospitalized with CAP suggests that individuals with diabetes, patients who received of the influenza vaccine in the previous season, patients with a bacterial pathogen, and those with an elevated influenza B (Yamagata) HAI titer are at risk for non-seroconversion after influenza vaccination. Although vaccinating individuals admitted to the hospital before discharge provides an opportunity to vaccinate vulnerable patients and to increase overall influenza vaccinations rates, the rates of seroconversion were lower in certain groups. Additional data are needed to better understand groups of hospitalized adults that have a suboptimal response to influenza vaccination. This could pave the way for using new strategies for influenza vaccination

(e.g., adjuvanted influenza vaccine, high dose influenza vaccine) in these groups ‘at risk’ for a suboptimal immune response.

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Tables

Table 1. Distribution of epidemiological and clinical factors among patients who did and did not seroconvert to influenza vaccination.

Variable		Seroconversion Group (n=66, 69%)	Non-Seroconversion Group (n=29, 31%)	P-value
Categorical Variables		n (%)	n (%)	
Gender	Female	31 (46.97)	9 (31.03)	0.1474*
	Male	35 (53.03)	20 (68.97)	
Race/Ethnicity	Hispanic	11 (16.67)	4 (13.79)	0.2135
	White	30 (45.45)	10 (34.48)	
	Black	24 (36.36)	12 (41.38)	
	Asian	1 (1.52)	3 (10.34)	
Smoking	Yes	20 (30.31)	10 (34.48)	0.8863
	No	46 (69.70)	19 (65.52)	
Study Hospital	Northwestern	27 (40.91)	9 (31.03)	0.7259
	Rush	15 (22.73)	7 (24.14)	
	Cook	11 (16.67)	5 (17.24)	
	Vanderbilt	9 (13.64)	7 (24.14)	
	Baptist	4 (6.06)	1 (3.45)	
Year of study enrollment	2010	21 (31.82)	11 (37.93)	0.3631
	2011	37 (56.06)	12 (41.38)	
	2012	8 (12.12)	6 (20.69)	
Self-reported receipt of influenza vaccine in prior season	Yes	11 (16.67)	10 (34.48)	0.0540*
	No	55 (83.33)	19 (65.52)	
COPD	Yes	9 (13.64)	5 (17.24)	0.6480
	No	57 (86.36)	24 (82.76)	
Asthma	Yes	19 (28.79)	10 (34.48)	0.5788
	No	47 (71.21)	19 (65.52)	
Chronic kidney disease	Yes	6 (9.09)	5 (17.24)	0.2529

	No	60 (90.61)	24 (82.76)	
Coronary artery disease or Heart failure	Yes	20 (30.30)	8 (27.59)	0.7891
	No	46 (69.70)	21 (72.41)	
Diabetes	Yes	10 (15.15)	10 (34.48)	0.0333*
	No	56 (84.85)	19 (65.52)	
Receipt of both influenza and PPV 23 vaccines	Yes	39 (59.09)	18 (62.07)	0.8496
	No	27 (40.91)	11 (37.93)	
Oral steroid use	Yes	6 (9.09)	3 (10.34)	0.8067
	No	60 (90.91)	26 (89.66)	
Liver disease	Yes	7 (10.61)	0 (0.00)	0.0684*
	No	59 (89.39)	29 (100.00)	
Stroke	Yes	4 (6.06)	3 (10.34)	0.4617
	No	62 (93.94)	26 (89.66)	
Previous pneumonia admission	Yes	18 (27.27)	9 (31.03)	0.7081
	No	48 (72.73)	20 (668.97)	
HIV+ (with CD4>200/14%)	Yes	1 (1.52)	1 (3.45)	0.5456
	No	65 (98.48)	28 (96.55)	
Non-skin cancer	Yes	6 (9.09)	3 (10.34)	0.8476
	No	60 (90.91)	26 (89.66)	
Immunosuppression	Yes	4 (6.06)	4 (13.79)	0.2114
	No	62 (93.94)	25 (86.21)	
Baseline HAI titer≥40: A(H1N1)	Yes	16 (24.24)	7 (24.14)	0.9913
	No	50 (75.76)	22(75.86)	
Baseline HAI	Yes	14 (21.21)	10 (34.48)	0.1704*

titer≥40: A(H3N2)				
	No	52 (78.79)	19 (65.52)	
Baseline HAI titer≥40: B (Victoria)	Yes	23 (34.85)	14 (48.28)	0.2165
	No	43 (65.15)	15 (51.72)	
Baseline HAI titer≥40: B (Yamagata)	Yes	34 (51.52)	22 (75.86)	0.0263*
	No	32 (48.48)	7 (24.14)	
Identification of bacterial pathogen	Yes	7 (10.61)	7 (24.14)	0.0866*
	No	59 (89.39)	22 (75.86)	
Identification of viral pathogen	Yes	24 (36.36)	8 (27.59)	0.4045
	No	42 (63.64)	21 (72.41)	
Identification of rhino virus	Yes	8 (12.12)	5 (17.24)	0.5037
	No	58 (87.88)	24 (82.76)	
ICU Admission	Yes	14 (21.21)	6(20.69)	0.9541
	No	52 (78.79)	23 (79.31)	

Continuous Variables	Median (IQR)	Median (IQR)	
Age	54.00 (42.00-65.00)	55.00 (49.00-71.00)	0.5048
BMI	26.87 (23.69-32.04)	28.58 (24.17-33.40)	0.6763
PSI score	68.00 (48.00-101.00)	71.00 (48.00-106.00)	0.5965
Duration of hospitalization	3.50 (2.00-5.00)	3.00 (2.00-5.00)	0.6739

*** P-value < 0.20**

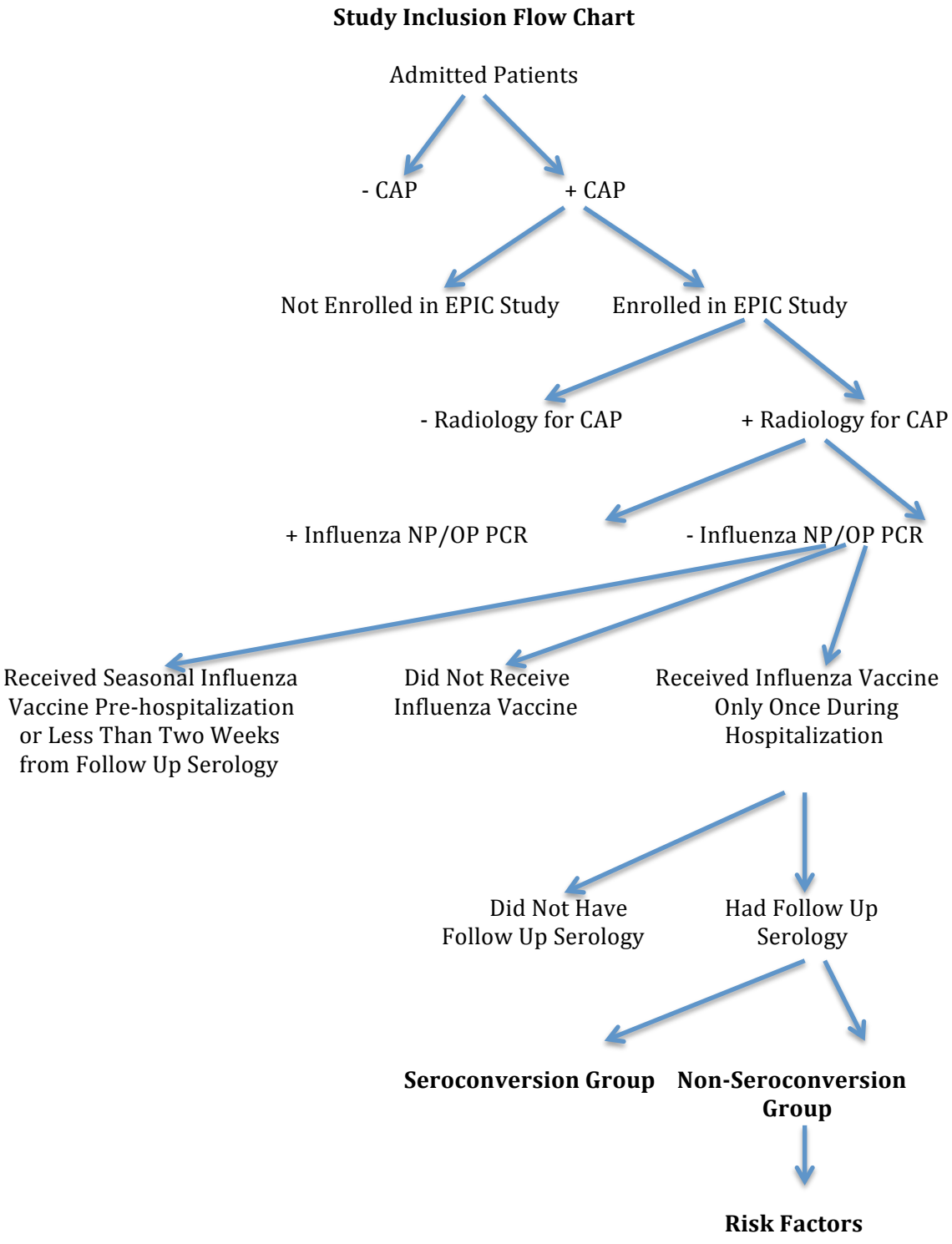
Table 2. Risk ratios (RR) with 95% confidence intervals (CI) for failure to seroconvert to any influenza strain after influenza vaccination, the adjusted risk ratios (aRR) fwith 95% CI for potential confounder of age of all variables found to be significant on univariate analysis, and percent change from RR to aRR (age groups 18-49 years old, 50-64 years old, and 65+ used).

Risk Factor	**RR**	**95% CI**	**aRR**	**95% CI**	**Change (%)**
History of liver disease	N/A*	N/A*	N/A*	N/A*	N/A
Elevated influenza B (Yamagata) baseline titer	2.19	(1.04, 4.61)	2.15	(1.02, 4.52)	-1.83
Bacterial infection identified	1.84	(0.98, 3.50)	1.95	(1.06, 3.58)	5.98
Received influenza vaccine in previous season	1.85	(1.03, 3.36)	1.76	(0.97, 3.21)	-4.86
History of diabetes	1.97	(1.10, 3.55)	1.86	(1.02, 3.39)	-5.58
Female gender	0.62	(0.32, 1.21)	0.61	(0.31, 1.19)	-1.61
Elevated influenza A (H3N2) baseline titer	1.56	(0.85, 2.87)	1.52	(0.83, 2.78)	-2.56

*Unable to calculate RR for patients with history of liver disease because zero patients with liver disease failed to seroconvert to at least one influenza strain.

Figures

Figure 1:



Chapter III: Summary

Further Discussion: Increased risk of non-seroconversion in individuals with an identified bacterial pathogen and pre-existing diabetes support the hypothesis that individuals with pre-existing conditions may not have the capacity to seroconvert (17) with administration of the non-adjuvanted vaccine.

Chronic Conditions Variable Analysis: When the variable "chronic conditions" (defined as individuals with two or more of the following conditions: asthma, chronic obstructive pulmonary disease, chronic kidney disease, coronary artery disease, diabetes mellitus, chronic liver disease, HIV, and any sort of chronic immunosuppression) was included in the multivariate model and stepwise selection was performed, the variables of chronic conditions (p=0.02), liver disease (p=0.004), sex (p=0.16), positive identification of bacterial infection (p=0.06), receipt of the influenza vaccine in the previous season (p=0.02), and elevated influenza B (Yamagata) HAI titer at baseline (p=0.02) were all found to be significant. Together, these six variables explain 27.72% of the result of seroconversion versus non-seroconversion, only a 5% higher percentage than without chronic conditions being included. Diabetes, therefore, was identified as the driving force of the variable "chronic conditions." Due to the determination, it was decided "chronic condtions" did not add significantly to the multivariate model independently from diabetes.

Throughout analysis, liver disease, elevated Influenza B (Yamagata) HAI antibody titer at baseline, receipt of influenza vaccine in previous season, and identification of a bacterial pathogen remained resilient in the multivariate model.

Potential Confounding: Risk ratios for failure to seroconvert were adjusted for age using several different categorizations in an attempt to identify potential confounding. After adjusting for age as a trichotomous variable (18-49 years old, 50-64 years old, and 65+) (**Table 2**), age was then broken into four categories (age groups 18-39, 40-54, 55-69, and 70+) (**Table 3**). Using four age categories, percent change of the risk ratios were greater but risk ratios for bacterial pathogen identified, receipt of influenza vaccine in previous season, history of diabetes, female gender, and multiple chronic conditions are all closer to the null when adjusted for age. To further assess age as a more-continuous variable, age was then broken into seven categories (age groups 18-29, 30-39, 40-49, 50-59, 60-69, 70-79, and 80+ used) in an attempt to more closely replicate controlling for age as a continuous variable (**Table 4**). The risk ratios for identification of a bacterial pathogen, receipt of influenza vaccine in previous season, and history of diabetes are all closer to the null when adjusted for age. The risk ratios for female gender and elevated influenza A (H3N2) baseline titer did not change.

What does all this mean for age as a confounder? While age is potentially a confounder, for the majority of variables it appears to move the risk ratio for each variable towards the null. This makes it less likely for age to be masking significant risk factors, but it could be amplifying risk factors to appear more significant than they actually are.

Stepwise selection was performed multiple times with various variable adjustments: with HAI titer as a dichotomous titer, with HAI titer as a continuous variable, with and without multiple chronic conditions, and with age and PSI each forced into the multivariate model. With every variation, however, liver disease, elevated

Influenza B (Yamagata) HAI antibody titer at baseline, receipt of influenza vaccine in previous season, and identification of a bacterial pathogen as remained significant in the model.

Chronic Liver Disease: All seven individuals with chronic liver disease meeting the study criteria seroconverted to one or more influenza strain after receiving the influenza vaccine. While multiple studies have found seroprotection against influenza to be lower in patients with chronic liver disease, the same studies confirm that seroconversion to influenza vaccine is equal, if not better, in patients with chronic liver disease compared to healthy controls (33, 34, 35, 36). One potential explanation for cirrhotic patients showing increased rates of seroconversion is that patients with cirrhosis tend to be older than healthy controls (36). Exposure in past years to previous influenza strains could potentially allow these individuals to create antibodies against these familiar strains (36). In this study, the mean age of subjects with chronic liver disease was 65 years, ten years older than the mean age of 55 years for all subjects. The median age of those with chronic liver disease (59 years) was also higher than the median age of all subjects (54 years, p= 0.10). It has been hypothesized that changes in liver and splenic pressure and functionality in cirrhotic patients could also explain changes in immune responses (37).

Multivariate analysis was also performed excluding the chronic liver disease variable. Without it, influenza vaccination in the previous season began to only approach significance (p=0.06) while the variables of diabetes (p=0.02), elevated baseline Yamagata titer (p=0.003), and bacterial infection p=0.03) all remained significant. It is

possible that liver disease is co-linear with another variable but no obvious physiological connection to one of the other four variables has been identified.

Summary: Vaccinating individuals admitted to the hospital before discharge is an excellent opportunity to reach more vulnerable patients and to increase overall influenza vaccinations rates. Increasing vaccination rates means little, however, if the individuals receiving the vaccine do not seroconvert after vaccination. Vaccinating without an immunological response is potentially a poor use of resources and could provide false reassurance regarding these individual's risk of subsequent influenza infection.

Overall, this study suggests that individuals with a bacterial pathogen detected, individuals with diabetes, patients who received of the influenza vaccine in the previous season, and those with an elevated influenza B (Yamagata) HAI titer are at risk for non-seroconversion. Future studies could examine whether an adjuvanted vaccine or a delay in influenza vaccination would increase rates of seroconversion. Future research could also compare outpatient versus inpatient seroconversion among adults with similar matched characteristics.

Additional Tables

Table 3. Examining age as a potential confounder using more narrow age groups, trying to more closely approximate a continuous variable (age groups 18-29, 30-39, 40-49, 50-59, 60-69, 70-79, and ≥80).

Risk Factor	RR	95% CI	aRR	adj. 95% CI
History of liver disease	N/A	N/A	N/A	N/A
Elevated influenza B (Yamagata) baseline titer	2.19	(1.04, 4.61)	2.17	(1.03, 4.58)
Bacterial infection identified	1.84	(0.98, 3.50)	1.93	(1.03, 3.61)
Received influenza vaccine in previous season	1.85	(1.03, 3.36)	1.80	(0.98, 3.29)
History of diabetes	1.97	(1.10, 3.55)	1.91	(1.05, 3.48)
Female gender	0.62	(0.32, 1.21)	0.60	(0.31, 1.18)
Elevated influenza A (H3N2) baseline titer	1.56	(0.85, 2.87)	1.55	(0.84, 2.84)
Multiple chronic conditions	2.03	(1.10, 3.77)	1.98	(1.06, 3.70)

Table 4. Examining age as a potential confounder using four more evenly distributed age groups (age groups 18-39, 40-54, 55-69, and ≥70).

Risk Factor	RR	95% CI	aRR	adj. 95% CI
History of liver disease	N/A	N/A	N/A	N/A
Elevated influenza B (Yamagata) baseline titer	2.19	(1.04, 4.61)	2.16	(1.02, 4.56)
Bacterial infection identified	1.84	(0.98, 3.50)	1.88	(1.01, 3.53)
Received influenza vaccine in previous season	1.85	(1.03, 3.36)	1.81	(0.99, 3.31)
History of diabetes	1.97	(1.10, 3.55)	1.93	(1.07, 3.50)
Female gender	0.62	(0.32, 1.21)	0.61	(0.31, 1.20)
Elevated influenza A (H3N2) baseline titer	1.56	(0.85, 2.87)	1.52	(0.82, 2.81)
Multiple chronic conditions	2.03	(1.10, 3.77)	2.00	(1.07, 3.76)

Table 5. ≥2 Chronic conditions variable (individuals with two or more of the following conditions: asthma, chronic obstructive pulmonary disease, chronic kidney disease, coronary artery disease, diabetes mellitus, chronic liver disease, HIV, and any sort of chronic immunosuppression).

Variable		Seroconversion Group (n=66, 69%)	Non-Seroconversion Group (n=29, 31%)	P-value
Categorical Variables		n (%)	n (%)	
≥2 Chronic conditions (including diabetes)	Yes	22 (33.33)	17 (56.62)	0.0210*
	No	44 (66.67)	12 (41.38)	
≥2 Chronic conditions (excluding diabetes)	Yes	19 (28.79)	8 (27.59)	0.9048
	No	47 (71.21)	21 (72.41)	

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