Acute Respiratory Infection Among Hospitalized Individuals: Prediction and Prevention of Severe Influenza

by

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Dedication

To my family and my friends, and most of all, to my husband. I couldn't have done this without all of you.

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List of Abbreviations

ILI	Influenza-Like Illness
RSV	Respiratory Syncytial Virus
CV	Coronavirus
PIV	Parainfluenza Virus
AV	Adenovirus
HMPV	Human Metapneumovirus
RV	Rhinovirus
VE	Vaccine Effectiveness
TND	Test Negative Design
ARI	Acute Respiratory Infection
PCR	Polymerase Chain Reaction
OR	Odds Ratio
aOR	Adjusted Odds Ratio
ICU	Intensive Care Unit
CHF	Congestive Heart Failure
COPD	Chronic Obstructive Pulmonary Disease
HAIVEN	Hospitalized Adult Influenza Vaccine Effectiveness Network
CHS	Clalit Health Services

CI	Confidence Interval
MoH	Ministry of Health
TIV	Trivalent Inactivated Influenza Vaccine
QIV	Quadrivalent Inactivated Influenza Vaccine
LAIV	Live Attenuated Influenza Vaccine
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
EHR	Electronic Health Record
EMR	Electronic Medical Record
BMI	Body Mass Index
LOS	Length of Stay
CCI	Charlson Comorbidity Index
UMH	University of Michigan Hospital
HFH	Henry Ford Hospital

Abstract

Influenza is a serious respiratory virus in terms of global morbidity and mortality. Patients hospitalized with influenza generally have comorbidities contributing to their disease severity and are most at risk for further severe influenza-related outcomes. Despite the importance of this group, there are few studies investigating interventions in populations of patients hospitalized due to influenza. Specifically, robust evaluation is needed of the two most used interventions against severe influenza, vaccination and neuraminidase inhibitors. The best available protection against influenza illness is vaccination, which is recommended annually in the United States and in many other countries worldwide. Treatment with neuraminidase inhibitors has been shown to prevent severe influenza outcomes and reduce symptomatic illness; antiviral treatment is recommended for all hospitalized patients with suspected or confirmed influenza in the United States.

This dissertation examines two components of prevention of severe influenza: vaccine effectiveness against hospitalization, and the prevention of severity in individuals at high risk for severe influenza outcomes. Influenza vaccine effectiveness against hospitalization of Israeli children who are fully or partially vaccinated was determined through use of medical record data over three influenza seasons in chapter 2. Vaccination was found to be effective for fully, but not partially, vaccinated children over all three seasons. This result supports guidelines by the Advisory Committee on Immunization Practices in the United States and the Israeli Ministry of Health, which recommend two inoculations in the first season of vaccination for children under nine years of age.

In chapter 3 we focused on the methodological validity of the test negative design in the inpatient setting. We tested specimens previously collected for vaccine effectiveness estimation against hospitalization in adults participating in the HAIVEN study for a variety of respiratory viruses. We calculated VE in three ways: using the traditional influenza-negative control group, using an influenza negative but other virus positive control group, and using a pan-negative control group, in order to evaluate whether inclusion of individuals without a true ARI in the influenza-negative control group biases VE estimates in the hospital. We did not find consistent differences in VE by control group, suggesting that this bias is not a persistent problem when estimating vaccine effectiveness against hospitalization.

In the next two chapters, we focused on characterization of influenza severity and risk factors for severe influenza. In chapter 4 we studied adults hospitalized with influenza over two seasons. Using inverse probability weighted logistic and linear models, we found that rapid antiviral treatment was associated with reduced odds of lower pulmonary disease and that obese patients were treated more rapidly with antiviral medication than non-obese patients, making antiviral treatment timing a potential confounder of the relationship between obesity and severe influenza. In chapter 5, we evaluated predictors of ICU admission, 30-day readmission, and extended length of stay among hospitalized adults over two seasons of the HAIVEN study. Frailty and lack of prior year health care visits were associated with reduced influenza and acute respiratory infection severity. Through linear models stratified by vaccination status, we found that antiviral treatment was associated with reduced hospital length of stay in vaccinated, but not unvaccinated patients. Ongoing research to measure the underlying disease severity in these groups at presentation to the hospital will aid in further interpretation of this result.

Chapter 1 Introduction

In this dissertation, I will explore predictors and prevention of severe respiratory infection and influenza virus infection specifically among hospitalized individuals. In chapter 1, I discuss the impact of influenza and other respiratory viruses on human health, and the importance and difficulties of studying acute respiratory infection in hospitalized individuals. Throughout the dissertation, I examine the effectiveness of the influenza vaccine in preventing severe influenza infection, focusing on the effectiveness of influenza vaccine at preventing disease in understudied groups as well as the validity of using common methods for calculating vaccine effectiveness among hospitalized individuals. I also discuss which hospitalized individuals are most at risk for severe influenza outcomes and what interventions can help prevent these disease manifestations. Further, I suggest new methods to more accurately conduct these studies to be used in the future.

1.1 Specific Aims and Hypothesis

The specific aims and hypotheses addressed by this dissertation are:

Aim 1. Estimate influenza vaccine effectiveness among hospitalized Israeli children who are fully or partially vaccinated according to the Advisory Committee on Immunization Practices (ACIP) and Israeli Ministry of Health.

Hypothesis 1. Influenza vaccination according to the ACIP and Ministry of Health guidelines will be significantly protective against influenza. Vaccine effectiveness of partial vaccination will be lower than that of full vaccination.

Aim 2. Estimate influenza vaccine effectiveness using alternate control groups (all influenza negative, influenza negative and other respiratory virus positive, negative for all viruses) to determine if an over inclusion of individuals with a non-infectious illness is biasing influenza vaccine effectiveness estimates among hospitalized adults.

Hypothesis 2. Influenza vaccine effectiveness using alternate control groups will be significantly lower than vaccine effectiveness estimates using pan-negative or the traditional influenza-negative control groups, indicating bias due to inclusion of individuals in the study without an infectious illness.

Aim 3. Determine whether obesity, previously identified as a predictor of severe influenza, was associated with severe outcomes among hospitalized adults in Detroit, Michigan. Also evaluate the impact of neuraminidase-inhibitor administration on severe influenza outcomes.

Hypothesis 3. Obese individuals will have increased odds of severe disease manifestations, including increased hospital length of stay, increased odds of lower pulmonary disease, and increased odds of intensive care unit admission.

Aim 4. Determine predictors of severe influenza and acute respiratory infection related outcome among hospitalized adults, such as increased hospital length of stay, ICU admission, and mechanical ventilation. Estimate the impact of influenza vaccination and neuraminidase inhibitor administration on severe influenza outcomes.

Hypothesis 4. Older individuals, individuals with more comorbid conditions, and individuals who are more frail will have increased severe manifestations of influenza. Influenza vaccination and early administration of neuraminidase inhibitors will be associated with a reduction in severe outcomes.

1.2 Background and Significance

Viral respiratory infections are an important cause of morbidity and mortality globally among individuals of all ages[1,2]. Influenza, influenza like illness (ILI), and pneumonia together comprise the eighth most common cause of death in the United States[3]. While influenza is a major cause of morbidity and mortality during the Winter, a variety of respiratory viruses circulate during the influenza season and cause illness that is clinically indistinguishable from

influenza, including: respiratory syncytial virus (RSV), human coronaviruses (hCVs), parainfluenzaviruses (PIV), adenovirus (AV), human metapneumovirus (HMPV), and rhinovirus (RV)[4–6]. In addition to the morbidity burden caused by these viruses, the economic burden of these infections is also not trivial. It has been estimated that the direct and indirect costs of non-influenza viral respiratory infection approach $40 billion in a single year in the United States[7].

Despite the importance of non-influenza viruses, particular attention is devoted to studying influenza viruses due to the impact that these viruses have on morbidity and mortality; influenza viruses were estimated to cause 48.8 million illnesses, 959,000 hospitalizations, and 79,400 deaths in the United States during the 2017-2018 season[8]. In addition, influenza viruses are characterized by frequent genetic changes that are reflected in antigenic changes, meaning that antigenically distinct influenza viruses circulate each season and that individuals may be susceptible to influenza infection from the same influenza subtype in consecutive seasons. Periodically, novel influenza A viruses can emerge, leading to influenza pandemics[9,10]. The most recent influenza pandemic in 2009 was caused by the emergence of the influenza A(H1N1)pdm09 subtype which now circulates seasonally. In comparison with previous pandemics, the 2009 influenza pandemic was mild, but still there were an estimated 12,000 deaths and 300,000 hospitalizations associated with this emergence of a new influenza strain, indicating the huge impact that even a "mild" influenza pandemic can have on human health[11]. Influenza vaccination is the most effective way to prevent influenza; treatment with neuraminidase inhibitors is also recommended to reduce adverse outcomes of influenza infection and to shorten illness duration in cases of severe influenza or cases of influenza among individuals at high risk for adverse outcomes[12]. While these methods are routinely used to

prevent and treat seasonal influenza, it is also key to understand their effectiveness in order to quickly develop potent vaccines and antiviral drugs in the case of an influenza pandemic.

As mentioned previously, the most effective way to prevent influenza infection is through vaccination. The influenza vaccine is recommended for all individuals 6 months and older in the United States and in many other countries around the world, including Israel[13,14]. Due to seasonal variation in circulating influenza strains, new vaccines are made each season and vaccination is recommended each season. Children from age 6 months through 8 years are recommended to receive two influenza vaccines the first time that they are vaccinated[13]. The universal influenza vaccination recommendation means that it is not ethical to conduct randomized control trials to estimate vaccine effectiveness (VE), as one cannot ethically allocate a study participant to a "no vaccination" group. For this reason, numerous observational studies across the world are designed to estimate influenza vaccine effectiveness at preventing medically attended illness annually[15–19].

Throughout this dissertation, we examine two components of prevention of severe influenza: vaccine effectiveness against hospitalization, and the prevention of severity in individuals at high risk for severe influenza outcomes.

1.2.1 Test Negative Design

The observational study design most commonly used to estimate influenza VE is the test negative design (TND)[20,21]. Generally, studies that use this design are embedded in an influenza surveillance system, and prospectively identify patients with acute respiratory infection (ARI) symptoms or influenza like illness (ILI) when they present for medical care due to their illness. In most cases, information is collected from the medical record and from a brief enrollment interview about the participant's vaccination status, comorbid conditions, and illness course. In

addition, a respiratory swab is collected either by the research team or clinical team and tested for influenza by reverse-transcriptase polymerase chain reaction (RT-PCR). Individuals who test positive for influenza are considered cases and individuals who test negative are controls. As the main exposure of this study is vaccination, confirmation of accurate vaccination status is a key part of the study protocol. In countries without centralized medical record data, such as the United States, numerous efforts are made to confirm vaccination status, which can involve contacting outside medical systems, pharmacies, and a variety of vaccination records. VE is calculated by comparing odds of vaccination between influenza positive and influenza negative participants, and it is expressed as $(1- OR_{vaccination}) *100$.

The theoretical underpinnings and assumptions in the test negative design have been described in a variety of manuscripts[22–26]. While traditional cohort studies can be used in lieu of TND studies to follow individuals throughout the entire influenza season, catching all influenza positive participants upon illness onset, this is not an efficient way to calculate influenza VE as most people will not be infected with influenza, and many will not experience any type of respiratory illness. The TND avoids this inefficiency by detecting influenza positives upon presentation for medical care due to a respiratory illness.

Jackson et al. published a formal methodological description of the TND in 2013[27]. In it, they divide the population into two groups of individuals, those who would seek care if they had an ARI and those who would not. They explain that because the test negative design enrolls participants who have presented to a doctor for medical care, a traditional cohort study, in which a cohort of individuals in a certain catchment area are the study subjects, cannot be used. Bias due to health care seeking behavior would be a major problem in this scenario, as health care seeking behavior is related to both vaccination status and detection of influenza if only

individuals who seek care can be cases. The TND solves this problem by restricting enrollment to individuals who seek care; effectively ensuring that care seeking behavior is the same in case and control groups. As Jackson et al. explain, individuals who would seek care if they had an ARI fall into three categories: those who have influenza, those who have a non-influenza respiratory infection, and those who do no have a respiratory infection and are therefore not currently seeking care for an ARI (Figure 1.1). In this scenario, n_1 represents the total number of vaccinated individuals who seek care for ARI and n_3 represents the number of unvaccinated individuals who seek care. Vaccinated individuals who would seek care if they had an ARI who are infected with influenza, infected with a non-influenza virus, or not infected with a viruse are represented by a, b, and c, respectively. Those who are unvaccinated are represented by d if they are infection with influenza, e if they are infected with a non-influenza virus, or c if they do not have an infection. Ideally, vaccine effectiveness could be calculated using a risk ratio comparing the risk of influenza among vaccinated and unvaccinated individuals. In this case, VE = $\left(1 - \frac{a/n_1}{g/n_3}\right) * 100$. However, n_1 and n_3 cannot be calculated in this scenario; the number of individuals who fall into groups c and i is unknown, as these individuals are not seeking care because they do not have a respiratory infection currently (Figure 1.1). Instead, the assumption is made that the incidence of non-influenza ARI is not different between the vaccinated and unvaccinated population, that $b/n_1 = h/n_3$, indicating that $h/b = n_3/n_1$. Following this assumption, VE = $\left(1 - \frac{a*h}{g*b}\right) * 100$, this is equivalent to (1- $OR_{vaccination}$) * 100.

In addition to the assumption that the influenza vaccine does not impact the incidence of non-influenza ARI, the validity of TND also depends on various other assumptions. One key assumption is that the rate of influenza ARI must vary proportionally with the rate of non-

influenza ARI across health care seeking thresholds[27]. If this condition is not met, the study will be biased by health care seeking behavior, this bias will only be eliminated by adjustment for health care seeking behavior[26]. In addition, it is important to note that the influenza vaccine is hypothesized to reduce influenza severity as well as incidence[28,29]. If this is the case, vaccine effectiveness against medically attended influenza is not a direct measurement of protection from infection. Furthermore, if disease severity also differs by influenza status, in addition to being related to health care seeking behavior and vaccination, then this may lead to confounding, necessitating adjustment for illness severity[20].

1.2.2 Influenza Vaccine Effectiveness against Hospitalization

The test negative design was first implemented in studies measuring VE against medically attended influenza in outpatient clinics. Most of the validation of this study design has occurred in this setting. However, hospitalized individuals, who have already experienced an adverse outcome due to their influenza infection, are especially vulnerable to further complication including necessity for mechanical ventilation, admission to an intensive care unit (ICU), or even death. For this reason, understanding the efficacy of vaccination in this population is particularly important. Certain subsets of hospitalized patients are particularly understudied. The majority of all TND studies occur in countries or regions with well-established networks, such as Canada, Australia, the United States, and many countries across Europe, and most other countries either have very few or no understanding of their local and regional VE[18,30–33]. Hospitalized children are also understudied, with few networks producing VE estimates against hospitalization in children annually[34–38]. In addition, while there is some evidence to suggest that two influenza vaccines provide increased protection compared to one vaccine in vaccine naïve children, the impact of receiving full influenza vaccination on prevention of

hospitalization in children is not fully understood[39–42]. In chapter two, I address this limitation by estimating influenza VE against hospitalization in Israeli children, a previously unstudied population.

Hospitalized populations vary quite significantly from populations of outpatients: hospitalized patients are older and have more comorbid conditions than outpatients. Due to the differences between these populations, it is possible that some of assumptions made ensuring the validity of the TND in the outpatient setting may not follow in the hospital[43,44]. For example, due to the high prevalence of comorbid conditions among hospitalized patients, some of the patients in the influenza negative group may not have a true ARI, but a non-infectious exacerbation of a chronic condition such as congestive heart failure (CHF) or chronic obstructive pulmonary disease (COPD). If these individuals with more chronic conditions seek medical care at a lower threshold than others and are also more likely to be vaccinated, this would bias the VE estimates. Despite this potential bias, due to the importance of understanding VE against hospitalization, numerous studies produce annual influenza VE estimates against vaccination in this setting[19,45,46]. In chapter three, I use alternate control groups, including one group of patients with a PCR confirmed non-influenza infection, to evaluate whether inclusion of patients without an ARI is biasing our study, the data used to evaluate this aim come from the HAIVEN study.

1.2.3 The HAIVEN Study

The Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN) study is a CDC-funded study conducted at University of Pittsburgh, Vanderbilt University, Baylor Scott and White, and the University of Michigan[19]. In this study, adults hospitalized with an ARI are enrolled and a TND protocol is used to calculate influenza VE against hospitalization. Before the official creation of HAIVEN during the 2015-16 season, the same protocol was used only at the

University of Michigan as an optional component of the similar outpatient network study. Chapters three and five both use data from the optional 2014-15 season and the preliminary year of HAIVEN, the 2015-16 season. The protocols were the same for both seasons; adults hospitalized within the previous three days were enrolled if they had evidence of a respiratory symptom (such as cough, sore throat, wheezing etc.) and a constitutional symptom (such as fever, myalgia, etc.) with onset in the last ten days. Participants were consented, interviewed, and swabbed, and the swabs were tested for influenza. Enrollment interview questions for each season are available as Figure 1.2 for the 2014-15 season and Figure 1.3 for the 2015-16 season.

1.2.4 Influenza Surveillance and Hospital Records in Israel

As mentioned, many countries are not members of annual VE networks, and therefore do not produce VE estimates routinely. Israel is one such country, though they do conduct robust sentinel surveillance in which patients with ILI are swabbed at certain primary care offices. Previously, these data were used to produce VE estimates against outpatient visits published for three seasons; one manuscript covered the 2014-2015 and 2015-2016 seasons, and one covered the 2016-2017 season[47,48]. There are no manuscripts in Israel or the Middle East estimating VE against hospitalization. There is no robust sentinel influenza surveillance in the hospital that could be used to calculate VE, however, Israel has a detailed medical record system that can be utilized to get accurate vaccination information from birth in addition to data on laboratory confirmed influenza outcomes.

In Israel, there are four major state-mandated health service organizations, the largest of which is Clalit Health Services (CHS). CHS provides healthcare for over half of Israelis at hospitals and clinics geographically distributed throughout Israel, covering Jewish Israelis, Arab Israelis and the Ultra Orthodox. The majority of Israelis stay insured by the same insurer

provider throughout their entire lives, meaning that detailed influenza vaccine information can be collected through the medical record from birth. In addition, vaccines are not offered at pharmacies or other third party locations, save certain school vaccine campaigns which are now being reported to insurer/providers after the fact. For this reason the medical record contains a complete reports of vaccination history. The availability of a detailed and accurate exposure measurement in addition to the lack of data on influenza VE in this region makes Israel an ideal location to study VE. In chapter 2, I use data from CHS to estimate influenza VE in hospitalized children who are fully vaccinated and 'partially' vaccinated (i.e. have only received one influenza vaccine).

1.2.5 Predictors and Prevention of Severe Outcomes During Acute Respiratory Infection Related Hospitalization

During seasonal influenza epidemics, young children are most at risk for influenza infection and the elderly (adults 65 years of age and older) are most at risk for severe influenza related outcomes. From 1977 until 2009, seasonal influenza epidemics were characterized by circulation of seasonal influenza A viruses A(H3N2) and A(H1N1) in addition to influenza B viruses. In April 2009, the rapid emergence of a new influenza A(H1N1) virus was characterized by a rise in hospitalizations among previously healthy young adults. This increase in severity associated with the A(H1N1)pdm09 strain lead to the declaration of an influenza pandemic. Since 2009, the influenza A(H1N1)pdm09 strain has replaced the former seasonal A(H1N1) in co-circulation with influenza A(H3N2).

During the 2009 influenza A(H1N1) pandemic individuals thought to be at low risk for severe influenza, such as those under the age of 65 without recognized underlying conditions, were hospitalized at a higher than expected rate[49]. There were also new predictors of severe

influenza identified during this pandemic, with morbid obesity being the most consistently identified factor[50–52]. As population level immunity for the A(H1N1)pdm09 increases with increased exposure through seasonal infection and vaccination, the epidemiology of this virus must be monitored. Since the 2009 pandemic, the age of those hospitalized for influenza A(H1N1)pdm09 infection has increased [53–55]. Understanding who is most at risk for severe influenza is critical, so that they can be targeted for antiviral treatment and vaccination in the event of a vaccine-shortage or influenza pandemic.

There are two main ways to mediate the burden of severe influenza infection; to vaccinate to prevent influenza onset among individuals at high risk for severe outcomes, and to give antiviral drugs, specifically neuraminidase inhibitors, to individuals who already have influenza to reduce severe outcomes. There is some evidence that the influenza vaccine may reduce severity as well as incidence of influenza, though the results evaluating this association are mixed[28,29,56,57]. In the past five years there have been two influenza A(H3N2) seasons that have been particularly severe; in part due to very low vaccine effectiveness against influenza A(H3N2).[58,59]. Understanding the role of the influenza vaccine in reducing severity in these seasons when the vaccine did not succeed in reducing influenza A(H3N2) incidence is critical, both for informing messaging in the context of poor VE and for improving current vaccines.

There is more extensive evidence linking neuraminidase inhibitors and influenza severity reduction. The most commonly used neuraminidase inhibitor is oseltamivir. The CDC currently recommends that hospitalized patients be treated with antivirals upon clinical suspicion of influenza and that clinicians should not wait for influenza test results before treatment[60]. Despite this recommendation and the relative severity of the 2009 influenza season, treatment rates fell in the immediate post-pandemic years, though levels have been increasing since then[61].

Many studies have indicated that antiviral drugs can reduce influenza symptom length, especially when used within two-days of symptom onset[62,63]. While most studies of antiviral effectiveness have focused on the ability of antivirals to shorten and lessen the symptoms of relatively mild illness, recently, the ability of antiviral drugs to prevent death among individuals hospitalized with influenza has been shown[60,64–67]. Additionally, numerous studies have reported that oseltamivir is most effective when prescribed within two-days of symptom onset which has affected prescribing behavior, leading some physicians to not treat patients whose illness onset is greater than 48 hours from hospital admission[68,69]. There is evidence that while antivirals are more effective the closer they are used to illness onset, they are effective at reducing inpatient mortality up to five days from symptom onset[49,66]. Treatment is recommended in individuals at high risk of severe influenza irrespective of time from illness onset[70].

Numerous studies annually evaluate influenza VE against hospitalization; harnessing these data to understand the effectiveness of antivirals and vaccination at preventing severe influenza outcomes is critical. However, using TND data to understand influenza severity in the hospital is challenging. While the strength of the TND is its control for health care seeking behavior, when these data are used to evaluate severity, this control is no longer present. This is particularly a problem if individuals who frequently seek care are older and/or have numerous chronic conditions, as both are more likely to present and to be admitted to the hospital with a less severe disease. There is no direct way to adjust for this admission bias; variables such as age and comorbid conditions can be adjusted for, but it is not possible to directly measure the impetus for a physician to admit a patient to the hospital.

This problem persists when evaluating the impact of vaccination and antivirals on influenza severity. The same factors that are related to vaccine receipt are also likely related the

threshold of illness severity that causes a patient to present and be admitted to the hospital, as well as some factors that may be related to their likelihood of further deteriorating and having a severe outcome. The differential administration of antivirals should not, in theory, be as problematic; all individuals hospitalized with suspected or confirmed influenza are recommended to be treated as close to illness onset as possible. However, in practice, these guidelines may not be followed completely, with patients who present earlier in their illness being more likely to be treated or with physician driven testing practices also influencing treatment. In addition, antivirals that are given to patients with very severe disease who are far along in their disease course may not be effective, therefore, both timing of hospital presentation as well as antiviral administration needs to be taken into account.

Figure 1.1 Calculation of Influenza Vaccine Effectiveness using the Test Negative Design

	Do seek care for ARI			
	Infected with influenza	Infected with other pathogen	Not infected	Total
Vaccinated	a	b	c	n_1
Unvaccinated	g	h	i	n_3

$$\widehat{VE} = 1 - \frac{\left(\frac{a}{n_1}\right)}{\left(\frac{g}{n_3}\right)} = 1 - \frac{a}{g} * \frac{n_3}{n_1} \cong \widehat{VE}_{test-negative} = 1 - \frac{ah}{gb}$$

Figure 1.2 Eligibility Scheme for Enrollment in 2014-2015 US Flu VE Option A Study

2014-15 Patient Eligibility Scheme

Influenza-like Illness
Influenza-like illness (ILI)
Influenza-like disease (ILD)
Influenza
Upper Respiratory Infection (URI)
Viral URI
Cough
Bronchitis
Pneumonia
Pneumonia (PNA)
Bacterial Pneumonia
Community Acquired Pneumonia (CAP)
Healthcare-Acquired Pneumonia (HAP)
Rule out Pneumonia
Evaluate Pneumonia
Bibasilar Pneumonia
Asthma and COPD
COPD Exacerbation
Asthma Exacerbation
Status Asthmaticus
Asthmatic Bronchitis
Other Respiratory Conditions (require evidence an acute infectious respiratory illness in admission note*)
Fever
Acute Respiratory Distress Syndrome (ARDS)
Respiratory Distress
Shortness of Breath (SOB)
Difficulty in Breathing (DIB)
Dyspnea
Cystic Fibrosis Exacerbation (CF)
Respiratory Medical Other
Congestive Heart Failure (CHF)
Idiopathic Pulmonary Fibrosis (IPF)
Altered Mental Status

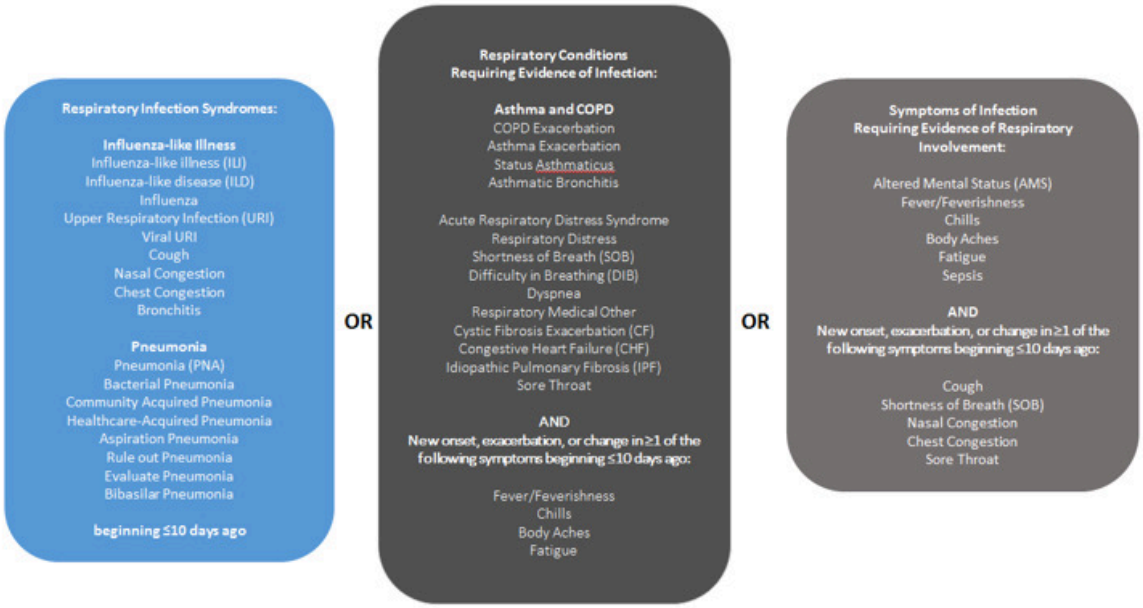
* Note of: a new or worsening cough, cold, flu, or acute respiratory infection symptoms, fever, or sick contacts

Patient eligibility is determined by examining the chief complaint, admission diagnosis, or hospital problem for a diagnosis of interest (listed above). The admission note examined for evidence of onset in the previous 10 days.

Figure 1.3 Eligibility Scheme for Enrollment in the 2015-2016 Season of the HAIVEN Study

2015-16 Patient Eligibility Scheme

Patients require a respiratory infection syndrome (left box) with onset in previous 10 days or respiratory condition with new onset of a symptom of infection (middle box), or a symptom of infection with new onset of a respiratory condition symptom (right box)



Chapter 2 Influenza Vaccine Effectiveness Against Hospitalization in Fully and Partially Vaccinated Children in Israel; 2015-16, 2016-17, and 2017-18[1]

2.1 Author Summary

There are no estimates of influenza VE against hospitalization in Israel, and very few analyses from any country comparing VE in fully and partially vaccinated hospitalized children. In this analysis we found that influenza vaccines were effective at preventing influenza A and B related hospitalization in fully vaccinated children. Our results support recommendations that Israeli children receive two influenza vaccines in their first season of vaccination.

2.2 Abstract

Influenza vaccine effectiveness (VE) varies by season, circulating influenza strain, age, and geographic location. There have been few studies of influenza VE among hospitalized children, particularly in Europe and the Middle East. We estimated VE against influenza hospitalization among children six months to eight years at Clalit Health Services hospitals in Israel in the 2015-16, 2016-17, and 2017-18 influenza seasons using the test-negative design. Estimates were computed for full and partial vaccination. We included 326 influenza-positive cases and 2821 influenza-negative controls (140 cases and 971 controls from 2015-16, 36 cases and 1069 controls from 2016-17, and 150 cases and 781 controls from 2017-18). Over all seasons, VE was 53.9% for full vaccination (95% CI:(38.6,68.3)), and 25.6% for partial vaccination (95% CI:(-3,

[1] Chapter 2 has been published as: Segaloff H.E., Leventer-Roberts M., Riesel D., Malosh R.E., Feldman B. S., Shemer-Avni Y., Key C., Monto A.S., Martin E.T., Katz M.A. Influenza Vaccine Effectiveness Against Hospitalization in Fully and Partially Vaccinated Children; 2015-16, 2016-17 and 2017-18. *Clin Infect Dis*. (2019).

47)). In 2015-16, most viruses were influenza A(H1N1) and vaccine lineage-mismatched influenza B/Victoria; VE for fully vaccinated children was statistically significant for influenza A (80.7%, 95% CI:(40.3,96.1)) but not B (23.0%, 95% CI:(-38.5, 59.4)). During 2016-17, influenza A(H3N2) predominated, and VE was (70.8%, 95% CI:(17.4, 92.4)). In 2017-18, influenza A(H3N2), H1N1 and lineage-mismatched influenza B/Yamagata co-circulated; VE was statistically significant for influenza B (63.0% 95% CI: (24.2,83.7)), but not A (46.3%, 95% CI:(-7.2, 75.3)). Influenza vaccine was effective in preventing hospitalizations among fully vaccinated Israeli children over three influenza seasons, but not among partially vaccinated children. There was cross-lineage protection in a season where the vaccine contained B/Victoria and the circulating strain was B/Yamagata, but not in a season with the opposite vaccine-circulating strain distribution.

2.3 Introduction

Influenza viruses circulate globally each year and cause substantial morbidity and mortality. Young children are at high risk for severe influenza-related outcomes including hospitalization and death [1,71–74] Vaccination is the most effective strategy for prevention of influenza-related illness and is recommended annually by the Israeli Ministry of Health (MoH) and the Advisory Committee on Immunization Practices in the United States for individuals six months and older [13,14]. Both recommendations specify that children aged six months through eight years receive two doses of influenza vaccine if they have not received more than one influenza vaccine previously [14].

Due to frequent genetic changes among circulating influenza viruses that require regular updates to vaccine composition, influenza vaccine effectiveness (VE) is evaluated each year. Annual network studies in the United States and Europe have shown substantial variation in VE

by circulating influenza virus, season, age, and geographic location [75–77]. However, few studies have evaluated influenza VE in preventing hospitalization in children spanning multiple years, and evidence is particularly sparse from Europe and the Middle East [34–37].

In Israel, influenza circulation is seasonal and peaks in the winter. Administration of inactivated influenza vaccines is part of covered services in outpatient clinics run by four large, national healthcare funds. Two recent studies evaluated influenza VE in preventing medically-attended influenza in outpatient clinics in Israel [47,48], but VE against hospitalization has not been described.

In this study, we evaluated the VE of trivalent inactivated influenza vaccines (TIV) among hospitalized Israeli children insured by Clalit Health Services (CHS), the largest Israeli healthcare organization, during the 2015-16, 2016-17, and 2017-18 influenza seasons. For each season, we estimated VE for fully and partially vaccinated children using complete vaccine receipt history from birth recorded by CHS.

2.4 Methods

2.4.1 Study Population and Data Source

CHS is the largest integrated payer-provider healthcare organization in Israel. It provides care to 4.5 million people, over 50% of Israel's population, and CHS hospitals are located throughout the country.

We included hospitalizations of children aged six months through eight years who were tested for influenza as part of clinical care by reverse-transcriptase polymerase chain reaction (RT-PCR) during hospitalization at any CHS hospital during the 2015-16, 2016-17 and 2017-18 influenza seasons. Individuals were excluded if they were not lifetime CHS members, received their vaccine <14 days before hospitalization**,** received the live attenuated influenza vaccine

(LAIV) or quadrivalent inactivated vaccine (QIV) in the current influenza season, were hospitalized outside of the influenza season, or were tested for influenza >10 days post-hospital admission.

2.4.2 Study Design

This was a test-negative design study that examined data retrospectively over three influenza seasons. We considered the beginning of the season to be the week when the first influenza-positive sample was reported, and the end of the season to be the week when sentinel surveillance ended, based on Israeli MoH Surveillance [78]. The 2015-16 season occurred between October 11, 2015 and April 16, 2016, the 2016-17 season between October 8, 2016 and April 16, 2017. For the 2017-18 season we included hospitalizations from October 21, 2017 through March 18 2018, the latest date that data were available at the time of analysis, and three weeks before the end of the influenza season.

2.4.3 Outcome and Vaccination Status

Influenza cases were individuals who tested positive for influenza by RT-PCR during a hospital admission**.** Vaccinated individuals were those who had received TIV from September 1st of the influenza season of interest ≥14 days before hospital admission.

Fully vaccinated individuals were those who had received a current season vaccine (TIV only) and had received ≥2 influenza vaccines previously, or had received two current season vaccines ≥14 days apart. Partially vaccinated individuals were those who had received a current season vaccine and did not meet the criteria for full vaccination [14,79]. Children with no electronic health record (EHR) evidence of receiving the current season vaccine were considered unvaccinated.

2.4.4 Covariates

Demographic covariates included age at hospitalization, sex, and ethnicity. Ethnicity was defined at the clinic catchment level as predominantly Jewish or Arab. Clinical covariates included comorbid conditions, number of hospitalizations in the prior year, time from hospital admission to RT-PCR test, and number of weeks from hospital admission to the peak of the influenza season. Comorbid conditions were defined for the two years before hospitalization using ICD-9 discharge codes previously described [80,81] (Table 2.1). Time from hospital admission to season peak, determined from Israeli MoH surveillance, was divided into two-week intervals and included a maximum of ten two-week intervals per season because data were sparse beyond this point [78]. All variables were extracted from CHS's integrated clinical and administrative EHR.

2.4.5 Statistical Analysis

We compared demographic and clinical characteristics between influenza-positive and influenza-negative children, using chi-square or Fisher's exact tests for categorical variables and Wilcoxon Rank-Sum test for continuous variables.

We used Firth's corrected logistic regression to estimate the adjusted odds ratio (aOR) for vaccination, comparing those who tested positive for influenza to those who tested negative [82]. VE was calculated separately for fully and partially vaccinated children as 1-(aOR) x 100. VE was computed for each season separately and for all seasons combined, for two age groups (children <2 and children aged ≥2) and for children with at least one comorbidity. Adjusted models included admission hospital (hospital A vs. others), log-transformed age at hospitalization in months, presence of any comorbidity, number of hospitalizations in the past year, days from hospital admission to RT-PCR test. In order to adjust for confounding caused by

variations in influenza positivity and cumulative vaccination rates throughout each season across the three seasons, number of weeks between hospital admission and peak of the influenza season were also included in adjusted models, as described in previous VE studies [[83]]. For VE estimates pooled across seasons, influenza season of hospitalization was a covariate. VE estimates were calculated for influenza A and B separately and were considered statistically significant if the 95% confidence interval did not include zero. As influenza B did not circulate in 2016-17, pooled season estimates of influenza B did not include data from the 2016-17 season. All analysis was completed using R Studio. The logistf package was used to compute Firth's corrected models.

2.4.6 Sensitivity Analysis

We included all qualifying hospitalizations in our primary analysis. We also performed a sensitivity analysis including only the first hospitalization for each individual in each season. During 2016-17, children in second grade received influenza vaccine in school, but records of these vaccinations were not routinely entered into the CHS EMR. To evaluate the impact of these potentially incomplete vaccination records, we conducted two additional analyses; we removed 2016-17 data from the overall VE estimate, and we estimated influenza VE in the 2016-17 excluding individuals >5 years old as of September 1, 2016.

2.4.7 Ethics Approval

The CHS research ethics committee approved this de-identified, medical-record based study.

2.5 Results

2.5.1 Participant Characteristics

We identified 3,746 hospitalizations of children who were tested for influenza by RT-PCR in six CHS hospitals over three influenza seasons. After exclusions, 3,147 hospitalizations remained in the sample (Figure 2.1). The majority of these hospitalizations were in children who were male (55.2%), six months to < 2 years old (60.7%), and had at least one comorbid condition (54.3%).

Over all three seasons, 326 of 3,147 hospitalizations (10.4%) included a positive influenza test (Table 1); In the 2015-16 season there were 140 influenza-positive specimens; 45 (32%) influenza A and 95 (68%) influenza B (Figure 2.2 A). The 2016-17 season had few positives (N=36), all of which were influenza A (Figure 2.2 B). In 2017-18 there were 150 positive specimens; 71 (47%) influenza A and 79 (53%) influenza B (Figure 2.2 C). The mean time from admission to RT-PCR test was 1.5 days, 99% of children were tested within 7 days of admission and 96% were tested within 4 days (data not shown).

Only 7.7% of hospitalized children under 2 years old tested positive for influenza compared to 13.3% of children aged 2-4, and 16.7% of children aged 5-8 ($p<.0001$) . Children with at least one comorbid condition, and with more than one hospitalization in the year prior to admission were less likely to be influenza-positive (Table 2.2).

Overall, 504 (16.0%) hospitalizations were among fully vaccinated children, 575 (18.3%) were among partially vaccinated children, and 2068 (65.7%) were among unvaccinated children. Frequency of partial vaccination was highest in children under 2 years old (22.8%). (Table 2.3). Children with at least one comorbid condition and children with more than one hospitalization in

the prior year were more likely to be vaccinated compared to those with no comorbid conditions and those who had one or zero hospitalizations in the previous years, respectively (Table 2.3).

2.5.2 Vaccine Effectiveness Estimates

Influenza VE pooled over the three seasons was 53.9% (95% CI: 38.6%, 68.3%) for fully vaccinated children; TIV was effective against influenza A (63.9%, 95% CI: 38.7%, 80.1%) and influenza B (42.3%, 95% CI: 8.6%, 64.9%) in fully vaccinated children. Pooled VE for partial vaccination was 25.6% (95% CI: -3.0%, 47.0%). Partial vaccination was effective against influenza A (45.1%, 95% CI: 12.3%, 67.1%), but not against influenza B (4.1%, 95% CI: -45.4%, 38.1%) (Table 2.4).

Influenza vaccine was effective in preventing hospitalizations in each of the three seasons among fully vaccinated children; VE was 45.8% (95%CI: 7.2%, 69.9%) in 2015-16, 70.8% in 2016-17 (95%CI: 17.4%, 92.4%) and 56.5% (95% CI: 25.5%, 75.7%) in 2017-18 (Table 2.4). VE was consistently lower for partial vaccination compared to full vaccination, and none of the season-specific estimates were statistically significant.

VE against influenza A was 80.7% in the 2015-16 season (95% CI: 40.3%, 96.1%) but was lower and not significant in the 2017-18 season (46.3%, 95% CI: -7.2%, 75.3%). Conversely, VE against influenza B was only 23.0% (95% CI: -38.5%, 59.4%) in 2015-16 but was 63.0% in 2017-18 (95% CI: 24.2%, 83.7%).

Overall VE did not vary by age; VE was 48.1% among individuals under 2 years old, and 49.6% among children 2 -8 years old. Compared to children 2-8 years old, VE point estimates for children under 2 were higher against influenza A and lower against influenza B, but CIs overlapped for both comparisons. Adjusted Influenza VE for fully vaccinated children with at

least one comorbidity was 35.8%, (95% CIs 0.8, 59.5), which did not vary significantly from the overall VE estimate (Table 2.5).

When analyses were restricted to the first hospitalization per individual in each influenza season, VE did not significantly change (Table 2.6). Results were similar in additional analyses removing individuals affected by the in-school vaccination program in 2016-17 (Table 2.7).

2.6 Discussion

Our study, the first to evaluate influenza VE against hospitalization in Israel, found that influenza vaccine was effective in preventing hospitalization associated with laboratory-confirmed influenza in fully vaccinated children aged six months to eight years across three influenza seasons. Influenza vaccine was effective in each season, although the degree of effectiveness varied. Among fully vaccinated children, the vaccine was effective against influenza A in two of three seasons, and influenza B in 2017-18. Estimated VE for partial vaccination was consistently lower than VE for full vaccination. These findings reinforce current recommendations in Israel and many other countries to immunize young children with influenza vaccine annually and to give vaccine-naïve children two influenza vaccines [13,14]. They also demonstrate that the vaccine is effective at reducing the risk for the most severe complications of influenza.

Due to a mismatch in circulating and vaccine-contained influenza B lineage in 2015-16 and 2017-18, we were able to approximate the degree of cross-lineage protection conferred by TIV in both seasons. In 2017-18, according to surveillance data from the Israeli MoH, over 95% of influenza B viruses were of the Yamagata lineage [unpublished data, Israel Centers for Disease Control]. We found that TIV, which contained influenza B/Victoria viruses in 2017-18, was 63% effective at reducing influenza-related hospitalizations despite this mismatch.

Interestingly, we did not find evidence for cross-lineage protection in the 2015-16 season, when the vaccine contained the B/Phuket-like (Yamagata lineage) virus but 95% of the circulating influenza B was of the Victoria lineage [84], and VE against influenza B was low (23%).

Our finding of significant cross-lineage protection against influenza B in 2017-18 was also seen in Canada, where circulating influenza B was nearly exclusively B/Yamagata and the vaccine contained B/Victoria. In 2017-2018, approximately 70% of vaccine used in Canada was TIV, and VE against outpatient influenza B was 55% [85]. In Australia, where QIV was used exclusively and B/Yamagata also predominated, VE against influenza B was 57% [86]. The similar VE estimates against influenza B in our study in Israel, along with estimates from Canada and Australia, suggest that QIV did not confer additional protection beyond that provided by TIV in the 2017-2018 season, despite the lineage mismatch.

The lack of influenza B cross-lineage protection that we observed in the 2015-16 season is consistent with VE findings among children from outpatient studies in Finland, in a multisite study in Europe and an outpatient study in Israel in the same year [47,87,88]. In all three settings, the same mismatch occurred between the vaccine (Yamagata) and circulating (Victoria) influenza B lineages, and TIV was not effective against influenza B. In contrast, in Canada, where approximately 85% of the vaccines distributed were TIV, VE was 54% against influenza B/Victoria [89].

Our high VE estimates for influenza A in 2015-16 and 2016-17 were similar to results described in other populations. We found a VE of 81% against influenza A in 2015-16,when virtually all circulating influenza in Israel was influenza A(H1N1)pdm [90]. This was similar to estimates of TIV effectiveness against influenza A among young Finnish children (78%) in the same year [88]. Studies in Canada and the US also demonstrated significant VE against influenza

A(H1N1)pdm in children in 2015-2016 [59,89,91]. In contrast, a study in outpatient clinics in Israel estimated a VE of -8% against influenza A among children <17 years old [48]. Notably, the low VE in the Israeli study occurred in a broader age group and at a different level of care (outpatient setting) compared to our study. In 2016-17, our VE estimate against influenza A (71%) was consistent with estimates against influenza A in an Israeli outpatient study among children aged 5-17 (69%), but much higher than estimates for children aged 6 months to 4 years in the same study (39%) [48]. The US Flu VE network also found significant VE against influenza A in 2016-2017 in children <8 years old [32]. In 2017-18, we estimated a VE of 46% against influenza A, which was consistent with interim estimates of VE against influenza A/H3N2 in children <8 years old in the US but higher than interim estimates from Australia in children <17 years old [86,92].

While the vaccine was effective among fully vaccinated children, estimated VE was consistently lower among partially vaccinated children, though confidence intervals overlapped. This discrepancy between full and partial vaccination has been demonstrated in previous studies [39–42]. In our study, children aged 6 months to 2 years were much more likely than older children to be partially vaccinated. Concerns exist about lower influenza VE in young children [93]. In our study, however, VE among children < 2 years old was very similar to VE in children 2-8 years old.

Our study has limitations. We relied on a retrospective analysis based on clinical testing, rather than an acute respiratory infection (ARI) inclusion criterion. This approach could have led to rates of influenza positivity that are different compared to other influenza VE studies that used specific case definitions such as ARI, and could have introduced potential bias to our influenza VE estimates. In addition, we could not confirm the date of illness onset prior to admission. We

may have included individuals who had been ill for long periods of time before hospital admission. Delayed hospital presentation could increase the likelihood of false-negative RT-PCR results if the infection has cleared before testing [94]. We would expect this bias to be non-differentially associated with vaccination status, likely biasing our VE results to the null and leading to an underestimation of VE. In order to reduce inclusion of false-negatives, we excluded individuals who were tested more than ten days after hospital admission. We included individuals tested up to 10 days before admission because previous studies suggest that children may shed influenza for a long period of time, with a substantial decrease in shedding after 10 days [94–96]. Although this strategy could still allow inclusion of initially infected individuals who had ceased shedding virus, nearly all children were tested within 4 days, suggesting that the inclusion of individuals far from illness onset was likely minimal. Clinical test results did not include subtype or lineage information, leaving us unable to make subtype or lineage specific estimates. However, the subtyping results from annual surveillance from the Israeli MoH allowed us to make inferences about the relative frequency of subtype and lineage specific influenza infections in our study. In addition, while CHS serves over 50% of the Israeli population, representing a geographically and socioeconomically diverse group, the exact representativeness of CHS members to the population of Israel is not known, and our results may not be generalizable to the entire Israeli population. Finally, recent interest in the impact of previous year vaccination on current season VE has grown, and many studies now consider repeat vaccination when reporting VE [97,98]. Unfortunately, our small sample size prevented us from evaluating the impact of sequential vaccination on VE. A future, larger study in this population addressing the impact of repeat vaccination would benefit from the detailed life course vaccination history available for CHS members.

In conclusion, we found influenza vaccine to be effective against hospitalization for laboratory-confirmed influenza in fully vaccinated children aged six months to eight years in Israel from 2015-2018. The VE for partial vaccination was consistently lower than for full vaccination. There was also high VE against influenza A in 2015-16 and 2016-17, and influenza B in 2017-18. We found evidence for cross-lineage protection from vaccination when TIV included B/Victoria but not when TIV included B/Yamagata, though these estimates rely on small sample sizes and have wide confidence intervals. Our findings suggesting that influenza VE reduces the risk for severe disease in children further strengthen current recommendations for annual influenza vaccine in children. The higher VE for children who were fully vaccinated is consistent with current guidelines that recommend two vaccines for vaccine -naïve children through age eight.

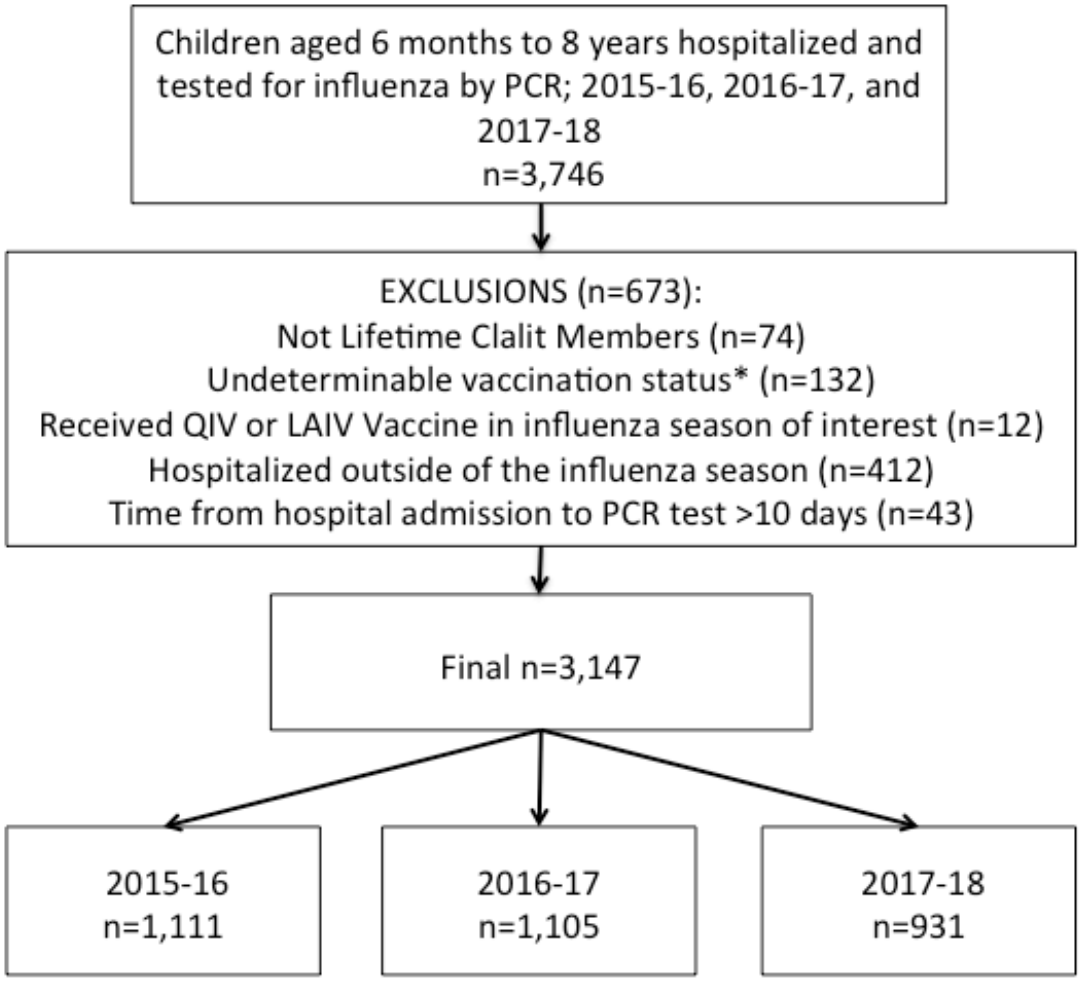
Table 2.1 ICD9 Codes Used to Define Comorbidity Categories

Categories	Subcategories	ICD 9 Codes
Neuromuscular	Brain and spinal cord malformations	740.0-742.9
	Mental retardation	318.0-318.2
	Central nervous system degeneration and disease	330.0-330.9, 334.0-334.2, 335.0-335.9
	Infantile cerebral palsy	343.0-343.9
	Muscular dystrophies and myopathies	359.0-359.3
Cardiovascular	Heart and great vessel malformations	745.0-747.4
	Cardiomyopathies	425.0-425.4, 429.1
	Conduction disorders	426.0-427.4
	Dysrhythmias	427.6-427.9
Respiratory	Respiratory malformations	748.0-748.9
	Chronic respiratory disease	770.7
	Cystic fibrosis	277.0
Renal	Congenital anomalies	753.0-753.9
	Chronic renal failure	585
Gastrointestinal	Congenital anomalies	750.3, 751.1-751.3, 751.6-751.9
	Chronic liver disease and cirrhosis	571.4-571.9
	Inflammatory bowel disease	555.0-556.9
Hematologic or Immunologic	Sickle cell disease	282.5-282.6
	Hereditary anemias	282.0-282.4
	Hereditary immunodeficiency	279.0-279.9, 288.1-288.2, 446.1
	Acquired immunodeficiency	0420-0421
Metabolic	Amino acid metabolism	270.0-270.9
	Carbohydrate metabolism	271.0-271.9
	Lipid metabolism	272.0-272.9
	Storage disorders	277.3, 277.5

	Other metabolic disorders	275.0-275.3, 277.2, 277.4, 277.6, 277.8-277.9
Other congenital or genetic defect	Chromosomal anomalies	758.0-758.9
	Bone and joint anomalies	259.4, 737.3, 756.0-756.5
	Diaphragm and abdominal wall	553.3, 756.6-756.7
	Other congenital anomalies	759.7-759.9
Malignancy	Malignant Neoplasms	140.0-208.9, 235.0-239.9
Asthma		519.1, 493.0-493.9

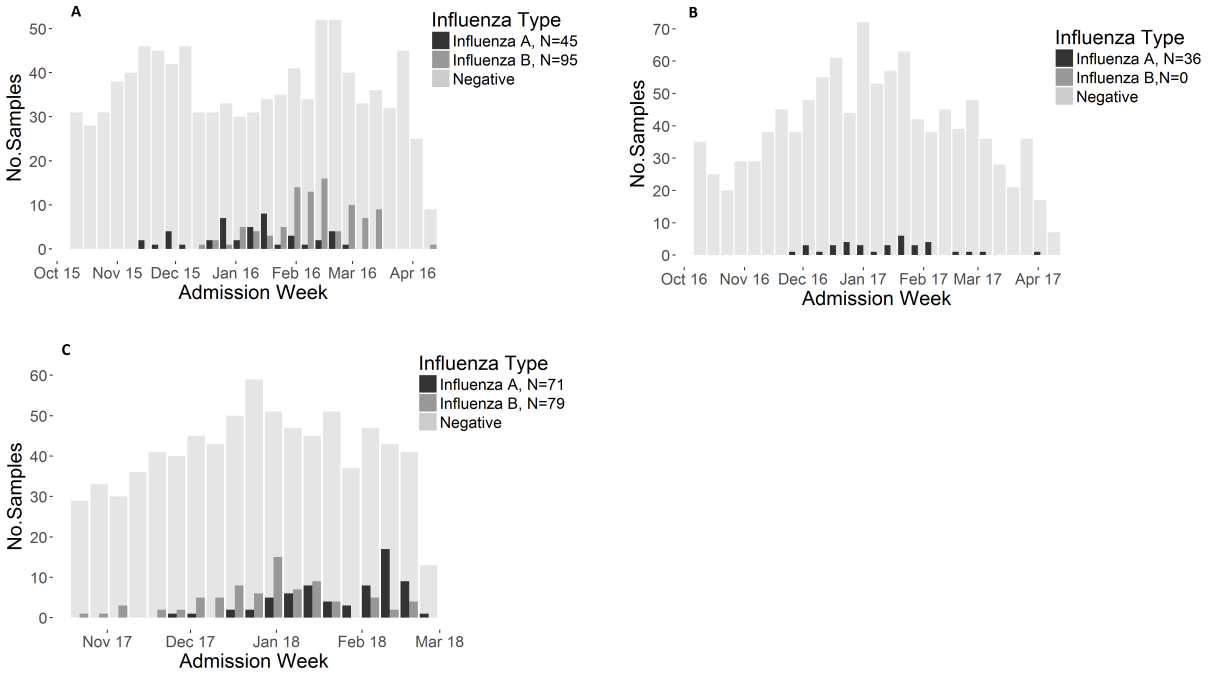
Codes and categories taken from Feudtner et al [79]. and Martin et al [80].

Figure 2.1 Flow Chart of Inclusion of Hospitalizations of Patients at Clalit Health Services Hospitals Tested for Influenza by RT-PCR During the 2015-16, 2016-17, and 2017-18 Influenza Seasons.



Indeterminate vacation status refers to a vaccination received 1 to 13 days before hospital admission, or two vaccinations received less than 14 days apart in the same season for an individual who was previously vaccine naïve.

Figure 2.2 Number of Influenza-Positive Samples from Children Hospitalized at Clalit Health Services Hospitals and Tested for Influenza by RT-PCR by Week of Hopsitalization



Each panel represents a different influenza season, A is the 2015-16 season, B is the 2016-17 season and C is the 2017-18 season. Shades of gray indicate PCR results; the darkest gray indicates the number of Influenza-A positive specimens, the medium gray indicates influenza B, and the lightest gray indicates the number of influenza-negative specimens.

Table 2.2 Descriptive Characteristics of Hospitalizations of Children Aged Six Months Through Eight Years Tested for Influenza by RT-PCR, by Influenza Status

Characteristics, n (Column %)	Total (n=3147)	Influenza Positive (n=326)	Influenza Negative (n=2821)	P[a]
Season				<.0001
2015-2016	1111 (35.3%)	140 (42.9%)	971 (34.4%)	
2016-2017	1105 (35.1%)	36 (11.0%)	1069(37.9%)	
2017-2018	931 (29.6%)	150 (46.0%)	781 (27.7%)	
Male	1736 (55.2%)	185 (56.7%)	1551 (55.0%)	0.54
Admission Hospital				<.0001
Hospital A	1407 (44.7%)	123 (37.7%)	1284 (45.5%)	
Hospital B	133 (4.2%)	25 (7.7%)	108 (3.8%)	
Hospital C	90 (2.9%)	15 (4.6%)	75 (2.7%)	
Hospital D	856 (27.2%)	94 (28.8%)	762 (27.0%)	
Hospital E	369 (11.7%)	50 (15.3%)	319 (11.3%)	
Hospital F	292 (9.3%)	19 (5.8%)	273 (9.7%)	
Age on September 1st				<.0001
6 months to <2 years	1910 (60.7%)	147 (45.1%)	1763 (62.5%)	
2 years to < 5 years	805 (25.6%)	107 (32.8%)	698 (24.7%)	
5 years to <9 years	432 (13.7%)	72 (22.1%)	360 (12.8%)	
Ethnicity				0.72
Arab	1158 (36.8%)	117 (35.9%)	1041 (36.9%)	
Jewish	1989 (63.2%)	209 (64.1%)	1780 (63.1%)	
Underlying Chronic Condition				
Any	1709 (54.3%)	158 (48.5%)	1551 (55.0%)	0.02
Asthma	563 (17.9%)	60 (18.4%)	503 (17.8%)	0.8
Neuromuscular	335 (10.6%)	32 (9.8%)	303 (10.7%)	0.61
Cardiac	417 (13.3%)	34 (10.4%)	383 (13.6%)	0.11
Respiratory	142 (4.5%)	6 (1.8%)	136 (4.8%)	0.01
Renal	59 (1.9%)	3 (0.9%)	56 (2.0%)	0.18
Gastrointestinal	84 (2.7%)	5 (1.5%)	79 (2.8%)	0.18
Hematologic	195 (6.2%)	22 (6.7%)	173 (6.1%)	0.66
Metabolic	183 (5.8%)	19 (5.8%)	164 (5.8%)	0.99
Genetic	254 (8.1%)	22 (6.7%)	232 (8.2%)	0.35
Malignancies	474 (15.1%)	34 (10.4%)	440 (15.6%)	0.01
Vaccination Status				0.04
Unvaccinated	2068 (65.7%)	234 (71.8%)	1834 (65.0%)	
Partially Vaccinated	575 (18.3%)	53 (16.3%)	522 (18.5%)	
Fully Vaccinated	504 (16.0%)	39(12.0%)	465(16.5%)	
Number of Hospitalizations in Prior Year				<.01
0	1934 (61.5%)	224 (68.7%)	1710 (60.6%)	

1	458 (14.6%)	45 (13.8%)	413 (14.6%)	
>1	755 (24.0%)	57 (17.5%)	698 (24.7%)	
Length of Stay (mean, std)	5.1 days (6.4)	4.8 days (7.4)	5.1 days (6.3)	0.15
Days from Admission to PCR testing (mean, std)	1.53 days (1.3)	1.48 days (1.26)	1.53 days (1.3)	0.59

a. P Values are calculated byMcNemar's Chi Square tests or Fisher's exact tests for categorical variables and Wilcoxon's Rank Sum tests for continuous variables

Abbreviations: RT-PCR: Reverse Transcriptase PCR
std: standard deviation

Table 2.3 Descriptive Characteristics of Hospitalizations of Children Aged Six Months Through Eight Years Tested for Influenza by RT-PCR by Vaccination Status

Characteristics, N (Column%)	Total (N=3147)	Fully Vaccinated[a] (N= 504)	Partially Vaccinated (N=575)	Not Vaccinated (N=2068)	P[b]
Season					<.01
2015-2016	1111 (35.3%)	173 (34.3%)	209 (36.3%)	729 (35.2%)	
2016-2017	1105 (35.1%)	171 (33.9%)	169 (29.4%)	765 (37.0%)	
2017-2018	931 (29.6%)	160 (31.7%)	197 (34.3%)	574 (27.8%)	
Male	1736 (55.2%)	288 (16.6%)	329 (18.9%)	1119 (64.5%)	0.26
Admission Hospital					<.0001
Hospital A	1407 (44.7%)	158 (31.3%)	238 (41.4%)	1011 (48.9%)	
Hospital B	133 (4.2%)	27 (5.4%)	10 (1.7%)	96 (4.6%)	
Hospital C	90 (2.9%)	21 (4.2%)	20 (3.5%)	49 (2.4%)	
Hospital D	856 (27.2%)	170 (33.7%)	164 (28.5%)	522 (25.2%)	
Hospital E	369 (11.7%)	58 (11.5%)	80 (13.9%)	231 (11.2%)	
Hospital F	292 (9.3%)	70 (13.9%)	63 (11.0%)	159 (7.7%)	
Age at September 1st					<.0001
6 months to <2 years	1910 (60.7%)	246 (48.8%)	436 (75.8%)	1228 (59.4%)	
2 years to <5 years	805 (25.6%)	140 (27.8%)	103 (17.9%)	562 (27.2%)	
5 years to <9 years	432 (13.7%)	118 (23.4%)	36 (6.3%)	278 (13.4%)	
Ethnicity					0.07
Arab	1158 (36.8%)	199 (39.5%)	228 (39.7%)	731 (35.3%)	
Jewish	1989 (63.2%)	305 (60.5%)	347 (60.3%)	1337(64.7%)	
Underlying Chronic Condition					
Any	1709 (54.3%)	349 (69.2%)	328 (57.0%)	1032 (49.9%)	<.0001
Asthma	563 (17.9%)	129 (25.6%)	108 (18.8%)	326 (15.8%)	<.0001
Neuromuscular	335 (10.6%)	91 (18.1%)	62 (10.8%)	182 (8.8%)	<.0001
Cardiac	417 (13.2%)	92 (18.2%)	95 (16.5%)	230 (11.1%)	<.0001
Respiratory	142 (4.5%)	39 (7.7%)	40 (7.0%)	63 (3.0%)	<.0001
Renal	59 (1.9%)	19 (3.8%)	13 (2.3%)	27 (1.3%)	<0.001
Gastrointestinal	84 (2.7%)	13 (2.6%)	18 (3.1%)	53 (2.6%)	0.75
Hematologic	195 (6.2%)	32 (6.3%)	33 (5.7%)	130 (6.3%)	0.88
Metabolic	183 (5.8%)	24 (4.8%)	37 (6.4%)	122 (5.9%)	0.48
Genetic	254 (8.1%)	78 (15.5%)	56 (9.7%)	120 (5.8%)	<.0001
Malignancies	474 (15.1%)	94 (18.7%)	67 (11.6%)	313 (15.1%)	<.01
Influenza Status					0.06
Influenza A	152 (4.8%)	15 (3.0%)	21 (3.6%)	116 (5.6%)	
Influenza B	174 (5.5%)	24 (4.8%)	32 (5.6%)	118 (5.7%)	

Influenza Negative	2821 (89.6%)	465 (92.3%)	522 (90.8%)	1834 (88.7%)	
Number of Hospitalizations in Prior Year					<.0001
0	1934 (61.5%)	257 (51.0%)	331 (57.6%)	1346 (65.1%)	
1	458 (14.5%)	84 (16.7%)	85 (14.8%)	289 (14.0%)	
>1	755 (24.0%)	163 (32.3%)	159 (27.6%)	433 (20.9%)	
Length of Stay (mean, std)	5.1 days (6.4)	5.3 days (6.7)	5.1 days (6.1)	5.0 days (6.4)	.36
Admission to PCR testing (mean, std)	1.5 days (1.3)	1.5 days (1.3)	1.6 days (1.4)	1.5 days (1.2)	0.53

a. Individuals were considered fully vaccinated if they received two current season vaccinations at least 14 days apart at least 28 days before their hospital admission or if they received >1 vaccine before the current season and a current season vaccine 28 days before hospitalization. Individuals were considered partially vaccinated if they did not meet the above categories but did receive a current season vaccine 28 days before admission.

b. P Values were calculated using McNemar's Chi Square tests or Fisher's exact tests for categorical variables and Wilcoxon's Rank Sum tests for continuous variables

Abbreviations: RT-PCR: Reverse Transcriptase PCR

std: standard deviation

Table 2.4 Percentage of Fully and Partially Vaccinated Children and Adjusted Influenza Vaccine Effectiveness Estimates by Season and Influenza Type

	No. Positive/ No. Evaluated (%)			Adjusted VE (95% CI)	
	Fully Vaccinated	**Partially Vaccinated**	**Unvaccinated**	**Fully Vaccinated**	**Partially Vaccinated**
All Seasons					
All Influenza	39/504 (7.7%)	53/575 (9.2%)	234/2016 (11.3%)	53.9 (38.6, 68.3)	25.6 (-3.0, 47.0)
Influenza A	15/480 (3.1%)	21/543 (3.9%)	116/1950 (5.9%)	63.9 (38.7, 80.1)	45.1 (12.3, 67.1)
Influenza B*	24/321 (7.5%)	32/389 (8.2%)	118/1216 (9.7%)	42.3 (8.6, 64.9)	4.1 (-45.4, 38.1)
2015-2016					
All Influenza	17/173 (9.8%)	24/209 (11.5%)	99/729 (13.6%)	45.8 (7.2, 69.9)	14.1 (-38.9, 48.5)
Influenza A	2/158 (1.3%)	6/191 (3.1%)	37/667 (5.5%)	80.7 (40.3, 96.1)	47.7 (-18.3, 80.1)
Influenza B	15/171 (8.8%)	18/203 (8.9%)	62/692 (9.0%)	23.0 (-38.5, 59.4)	-13.6 (-97.4, 37.2)
2016-2017					
All Influenza	3/171 (1.8%)	4/169 (2.4%)	29/765 (3.8%)	70.8 (17.4, 92.4)	30.1 (-79.6, 78.3)
Influenza A	3/171 (1.8%)	4/169 (2.4%)	29/765 (3.8%)	70.8 (17.4, 92.4)	30.1 (-79.6, 78.3)
Influenza B	-	-	-	-	-
2017-2018					
All Influenza	19/160(11.9%)	25/197 (12.7%)	106/574 (18.5%)	56.5 (25.5, 75.7)	25.9 (-21.1, 55.8)
Influenza A	10/151 (6.6%)	11/183 (6.0%)	50/518 (9.7%)	46.3 (-7.2, 75.3)	28.9 (-39.4, 66.2)
Influenza B	9/150 (6.0%)	14/186 (7.5%)	56/524 (10.7%)	63.0 (24.2, 83.7)	23.2 (-43.1, 60.8)

Models were adjusted for log-adjusted age in months, hospital (Hospital A vs others), presence of any comorbidity, prior year hospital admission (yes/no), distance of hospital admission from the influenza season peak (in two-week intervals), days from admission date to influenza PCR. Season is included in pooled models.
Individuals were considered fully vaccinated if they received two current season vaccinations at least 14 days apart at least 28 days before their hospital admission or if they received >1 vaccine before the current season and a current season vaccine 28 days before hospitalization. Individuals were considered partially vaccinated if they did not meet the above categories but did receive a current season vaccine 28 days before admission.
Influenza B estimates do not include information from 2016-17 when influenza B was not circulating

Table 2.5 Adjusted Influenza Vaccine Effectiveness Estimates by Season and Influenza Type Stratified by Age (<2 years old vs. 2-8 years old) and for Children with at Least One Comorbidity, Israel, 2015-2018

	No. Positive/ No. Evaluated (%)			Adjusted VE (95% CI)	
	Fully Vaccinated	Partially Vaccinated	Unvaccinated	Fully Vaccinated	Partially Vaccinated
All Seasons- Any Influenza					
Age <2	13/246 (5.3%)	34/436 (7.8%)	100/1228 (8.1%)	48.1 (8.3, 72.6)	9.3 (-27.1, 40.9)
Age 2-8	26/258 (10.1%)	19/139 (13.7%)	134/840 (16.0%)	49.6 (20.2, 69.2)	15.8 (-40.4, 51.7)
Any Comorbidity	30/349 (8.6%)	30/328 (9.1%)	98/1032 (9.5%)	35.8 (0.8, 59.5)	-2.4 (-58.0, 35.1)
All Seasons- Flu A					
Age <2	4/237 (1.7%)	15/417 (3.6%)	63/1191 (5.3%)	73.1 (35.4, 91.3)	31.9 (-18.7, 63.1)
Age 2-8	11/243 (4.5%)	6/126 (4.8%)	53/759 (7.0%)	49.7 (1.1, 76.3)	32.5 (-117.2, 74.0)
Any Comorbidity	13/332 (3.9%)	13/311 (4.2%)	50/984 (5.1%)	43.3 (-4.5, 71.2)	14.1 (-57.5, 56.0)
All Seasons- Flu B*					
Age <2	9/242 (3.7%)	19/421 (4.5%)	37/1165 (3.2%)	10.4 (-100.0, 55.4)	-32.2 (-131.2, 26.6)
Age 2-8	15/247 (6.1%)	13/133 (9.8%)	81/787 (10.3%)	50.4 (12.2, 73.5)	-.05 (-81.4, 48.4)
Any Comorbidity	17/336 (5.1%)	17/315 (5.4%)	48/982 (4.9%)	26.8 (-28.8, 60.2)	-27.9 (-124.7, 30.1)
2015-2016					
Age <2	8/93 (8.6%)	11/139 (7.9%)	47/409 (11.5%)	46.4 (-14.6, 77.4)	32.6 (-34.6, 68.5)
Age 2-8	9/80 (11.3%)	13/70 (18.6%)	52/320 (16.3%)	44.2 (-17.6, 75.8)	-28.2 (-152.4, 37.9)
Any Comorbidity	11/114 (9.6%)	14/120 (11.7%)	42/381 (11.0%)	39.4 (-21.9, 72.1)	-11.6 (-112.8, 44.1)
2016-2017					
Age <2	1/75 (1.3%)	3/129 (2.3%)	14/468 (3.0%)	47.1 (-127.7, 94.3)	-7.1 (-234.7, 73.3)
Age 2-8	2/96 (2.1%)	1/40 (2.5%)	15/297 (5.1%)	71.7 (-5.6, 94.9)	39.6 (-189.4, 93.8)
Any Comorbidity	3/125 (2.4%)	3/106 (2.8%)	12/388 (3.4%)	46.5 (-74.2, 87.2)	-20.6 (-302.5, 71.1)
2017-2018					
Age <2	4/78 (5.1%)	20/168 (11.9%)	39/351 (11.1%)	64.5 (5.3, 89.3)	4.5 (-74.7, 49.2)
Age 2-8	15/82 (18.3%)	5/29 (17.2%)	67/223 (30.0%)	51.3 (4.5, 76.5)	63.7 (1.8, 89.1)
Any Comorbidity	16/110 (14.5%)	13/102 (12.7%)	43/263 (16.3%)	35.1 (-24.9, 67.7)	21.2 (-58.4, 62.6)

Models were adjusted for log-adjusted age in months, hospital (Hospital A vs others), presence of any comorbidity, prior year hospital admission (yes/no), distance of hospital admission from the influenza season peak (in two-week intervals), days from admission date to influenza PCR. Season is included in pooled models.
Individuals were considered fully vaccinated if they received two current season vaccinations at least 14 days apart at least 28 days before their hospital admission or if they received >1 vaccine before the current season and a current season vaccine 28 days before hospitalization. Individuals were considered partially vaccinated if they did not meet the above categories but did receive a current season vaccine 28 days before admission.
Influenza B estimates do not include information from 2016-17 when influenza B was not circulating

Table 2.6 Percentage of Fully and Partially Vaccinated and Adjusted Influenza Vaccine Effectiveness Estimates by Season and Influenza Type Restricting to First Hospitalization for Each Patient

	No. Positive/ No. Evaluated (%)			Adjusted VE (95% CI)	
	Fully Vaccinated	**Partially Vaccinated**	**Unvaccinated**	**Fully Vaccinated**	**Partially Vaccinated**
All Seasons					
All Influenza	32/432 (7.4%)	51/511 (10.0%)	218/1834 (11.9%)	58.8 (38.9, 73.1)	26.0 (-3.5,47.9)
Influenza A	12/412 (2.9%)	20/480 (4.2%)	103/1719 (6.0%)	65.9 (39.2, 82.4)	42.7 (7.1, 66.2)
Influenza B*	20/278 (7.2%)	31/351 (8.8%)	115/1088 (10.6%)	50.4 (18.8, 71.0)	0.9 (-39.4, 41.9)
2015-2016					
All Influenza	14/152 (9.2%)	23/183 (12.6%)	94/645 (14.6%)	52.9 (16.1, 75.2)	16.3 (-37.5, 50.6)
Influenza A	2/140 (1.4%)	6/166 (3.6%)	33/584 (5.7%)	78.4 (32.5, 95.7)	43.1 (-30.2, 78.5)
Influenza B	12/150 (8.0%)	17/177 (9.6%)	61/612 (9.3%)	36.9 (-18.8, 68.8)	-5.2 (-86.0, 42.9)
2016-2017					
All Influenza	1/142 (0.7%)	3/143 (2.1%)	27/670 (4.0%)	85.1 (40.2, 98.4)	36.9 (-76.8, 83.4)
Influenza A	1/142 (0.7%)	3/143 (2.1%)	27/670 (4.0%)	85.1 (40.2, 98.4)	36.9 (-76.8, 83.4)
Influenza B	-	-	-	-	-
2017-2018					
All Influenza	17/137 (12.4%)	25/160 (13.5%)	97/519 (18.7%)	57.2 (24.2, 76.9)	23.4 (-26.6, 54.7)
Influenza A	9/129 (7.0%)	11/171 (6.4%)	43/465 (9.2%)	44.0 (-16.5, 75.4)	21.6 (-56.5, 63.2)
Influenza B	8/128 (6.2%)	14/174 (8.0%)	54/476 (11.3%)	64.4 (24.7, 84.9)	24.9 (-40.5, 61.8)

Models were adjusted for hospital (Hospital A vs others), log-adjusted age in months, presence of any comorbidity, prior year hospital admission (yes/no), distance of hospital admission from the influenza season peak (in two-week intervals), days from admission date to influenza PCR. Season is included in pooled models.
Individuals were considered fully vaccinated if they received two current season vaccinations at least 14 days apart at least 28 days before their hospital admission or if they received >1 vaccine before the current season and a current season vaccine 28 days before hospitalization. Individuals were considered partially vaccinated if they did not meet the above categories but did receive a current season vaccine 28 days before admission.
*Influenza B results do not include contribution from 2016-17 when influenza B was not circulating
Abbreviations: Vaccine effectiveness (VE), confidence interval (CI)

Table 2.7 Percentage of Fully and Partially Vaccinated and Adjusted Influenza Vaccine Effectiveness Estimates by Season and Influenza Type Restricting to Individuals Unaffected by In School Influenza Vaccination Strategy

	No. Positive/ No. Evaluated (%)			Adjusted VE (95% CI)	
	Fully Vaccinated	**Partially Vaccinated**	**Unvaccinated**	**Fully Vaccinated**	**Partially Vaccinated**
All Seasons excluding 2016-2017					
All Influenza	36/333 (10.8%)	49/406 (12.1%)	205/1303 (15.7%)	50.8 (27.7, 67.2)	22.4 (-9.5, 45.8)
All Influenza A	12/309 (3.9%)	17/374 (4.5%)	87 /1185(7.3%)	61.2 (29.9, 80.1)	44.4 (6.2, 68.7)
All Influenza B	24/321 (7.5%)	32/389 (8.2%)	118/1216 (9.7%)	42.3 (8.6, 64.9)	4.1 (-45.4, 38.1)
2016-2017 excluding individuals aged 6 years and older					
All Influenza	1/138 (0.7%)	4/155 (2.5%)	27/675 (3.8%)	83.3 (32.9, 98.2)	28.2 (-86.8, 77.9)
Influenza A	1/138 (0.7%)	4/155 (2.5%)	27/675 (3.8%)	83.3 (32.9, 98.2)	28.2 (-86.8, 77.9)
Influenza B	-	-	-	-	-

Models adjusted for hospital (Hospital A vs others), log-adjusted age in months, presence of any comorbidity, prior year hospital admission (yes/no), distance of hospital admission from the influenza season peak (in two-week intervals), days from admission date to influenza PCR. Season is included in pooled models.
Individuals were considered fully vaccinated if they received two current season vaccinations at least 14 days apart at least 28 days before their hospital admission or if they received >1 vaccine before the current season and a current season vaccine 28 days before hospitalization. Individuals were considered partially vaccinated if they did not meet the above categories but did receive a current season vaccine 28 days before admission.
Abbreviations: Vaccine effectiveness (VE), confidence interval (CI)

Chapter 3 Influenza Vaccine Effectiveness in the Inpatient Setting; Evaluation of Potential Bias in the Test Negative Design by Use of Alternate Control Groups[2]

3.1 Author Summary

The test negative design is commonly used to estimate influenza vaccine effectiveness. This study design was validated in the outpatient setting, but certain biases may persist among hospitalized patients. We found that VE estimates did not change consistently over two seasons when using alternate control groups, suggesting that the population differences in the inpatient setting do not lead to bias of VE estimates from the test negative design. We also found that the influenza vaccine did not alter the risk of non-influenza infections, an important assumption of the test negative design.

3.2 Abstract

The test negative design (TND) is used to estimate influenza vaccine effectiveness (VE) and is well validated in outpatient but not inpatient settings, where specific biases may differ. For example, the high prevalence of chronic pulmonary disease among enrollees of inpatient studies may lead to a non-representative control group. TND estimates are biased if influenza vaccine administration is associated with incidence of non-influenza viruses. We evaluated potential biases correlated with inpatient control group selection and effects of influenza vaccination on the incidence of other respiratory viruses. Patients with acute respiratory infection were enrolled

[2] Chapter 3 is in preparation for submission to the International Journal of Epidemiology. The full author list is: H.E. Segaloff, B. Cheng, A.V. Miller, J.G. Petrie, R.E. Malosh, C.K. Cheng, A.S. Lauring, L. Lamerato, J.M. Ferdinands, A.S. Monto, E.T. Martin.

from two hospitals during the 2014-15 and 2015-16 influenza seasons and tested for respiratory viruses. VE against influenza was estimated using three control groups: influenza negative, other respiratory virus positive, and pan-negative individuals. VE was also estimated for other common respiratory viruses. In 2014-15, VE was 41.1% (95% CI: 1.7%, 64.7%) using the influenza negative control group, 24.5% (95% CI: -42.6%, 60.1%) using the other-virus positive group, and 45.8% (95% CI: 5.7%, 68.9%) using the pan-negative group. In 2015-16, VE was 68.7% (95% CI: 44.6%, 82.5%) using the influenza negative control group, 63.1% (95% CI: 25.0%, 82.2%) using the other-virus positive group, and 71.1% (46.2%, 84.8%) using the pan-negative group. Influenza vaccination did not alter the odds of any other respiratory virus. We did not find evidence of substantial bias related to control group selection or vaccine effects on the incidence of non-influenza viruses, supporting the use of the TND in inpatient studies.

3.3 Introduction

In the United States, between 140,000 and 960,000 individuals are hospitalized due to influenza each year, and those hospitalized are most at risk for severe influenza-related outcomes, including death[99]. Hospitalized individuals often have chronic diseases that impact their ability to recover from influenza, making effective vaccines particularly important in this population[100–102]. However, the study design most commonly used to evaluate influenza vaccine effectiveness (VE), the test negative design (TND), has primarily been validated in outpatient settings. Its validity in inpatient settings is largely unknown[20,26,27,43]. Hospital-based studies add to knowledge of the full spectrum of protection afforded by the influenza vaccine. When VE estimates from hospital-based studies differ from estimates from ambulatory-based studies, an understanding of the validity of hospital-based estimates is necessary to fully interpret these results, and to evaluate the ability of the vaccine to prevent the most severe infections[103].

The validity of the TND relies on various assumptions, most relevantly that influenza vaccination is not associated with incidence of non-influenza respiratory infections, and that the influenza-negative control group is drawn from the same population that gave rise to the cases[23,24]. In order to assure this comparability between cases and controls, a symptom-based case definition is used to restrict participants to those experiencing an acute respiratory infection (ARI), presumably caused by a virus or bacterium that elicits similar symptoms to influenza[26,27]. Outpatient and inpatient populations vary substantially; beyond obvious differences in illness severity, hospitalized patients tend to be older, frailer, and more chronically ill than patients in outpatient settings. There is concern that features that differentiate the inpatient population from the well-validated outpatient population may lead to biased VE estimates. Specifically, the increased prevalence of chronic respiratory and related conditions among hospitalized adults may lead to biased influenza VE estimates using the TND[43,19]. Bias in hospital-based TND estimates would occur if individuals with chronic respiratory conditions such as chronic obstructive pulmonary disease (COPD) or congestive heart failure (CHF) have symptoms that mimic ARI symptoms even when they are not experiencing a true ARI. These same individuals may more frequently present to seek medical care. If these patients who are at an increased probability to test negative for influenza and are more likely to present to the hospital are also more likely to be vaccinated, this could bias VE estimates upwards in TND studies. Because influenza vaccine may provide greater protection against severe illness compared to mild-moderate illness, differentiating bias from a true increase in VE can be challenging.

To understand the potential role of this selection bias in TND studies, we calculated VE against influenza hospitalization using three control groups that were laboratory confirmed to be negative for influenza infection in two influenza seasons. The 2014-15 season that was

dominated by a mismatched influenza A(H3N2) virus, and the 2015-16 season with a well-matched influenza A(H1N1) virus. The control groups included the traditional "influenza-negative" control group, an alternative “other virus positive” control group that included patients testing negative for influenza but positive for another respiratory virus, and a "pan-negative" control group including patients who were negative for all viruses. We also tested the assumption that influenza vaccination does not affect incidence of other respiratory viruses by calculating VE against pooled non-influenza respiratory viruses and specifically against respiratory syncytial virus (RSV) and human rhinovirus (hRV).

3.4 Methods

3.4.1 Participant enrollment and interview

Participants were adults (age ≥18) hospitalized for ARI (respiratory and constitutional symptoms with onset in the past 10 days) at the University of Michigan Hospital (hospital A) in Ann Arbor, Michigan or Henry Ford Hospital (hospital B) in Detroit during the 2014-15 and 2015-16 influenza seasons[19]. Enrollment occurred from November 5th 2014 to March 6th 2015 in 2014-15 and from January 11th 2016 to April 15th 2016 in 2015-16. Eligibility criteria, enrollment procedures, and ARI definitions have been described previously[19,104]. Briefly, adults admitted to the hospital in the previous 72 hours with an ARI of ≤10 days duration were identified by daily review of electronic medical records (EMRs) by study staff. Eligible participants or their representatives provided written informed consent. The Institutional Review Boards at the University of Michigan Medical School and Henry Ford Health System approved all study procedures.

Participants were interviewed at enrollment to collect information about demographics, influenza vaccination, illness characteristics and subjective assessment of frailty (unexplained

>10 pounds weight loss [yes/no], little energy for desired activities [yes/no], difficulty walking 100 yards [no difficulty…unable to do], difficulty carrying 10 pounds [no difficulty…unable to do] and frequency of low/moderate activity [more than once/week…hardly ever/never]) [103].

Evidence for comorbid health conditions was extracted from the EMR and used to calculate the Charlson Comorbidity Index (CCI) score for each patient as previously described[103,105]. Intensive care unit (ICU) admission and hospital length of stay were also extracted from the EMR. Influenza vaccine receipt was documented using the EMR, the state immunizations registry, or plausible patient self-report on the enrollment interview. Self- report was considered plausible if timing and location of immunization could be provided.

3.4.2 Specimen collection and laboratory methods

Nasal and throat specimens were acquired from enrolled patients, combined, and tested for influenza by reverse transcription polymerase chain reaction (RT-PCR). All primers, probes and protocols were developed and provided by the Influenza Division of the CDC. At a later date, RNA from specimens from the 2014-15 season, stored in viral transport media at -80° C, were re-extracted. For specimens collected during the 2015-16 season, RNA extracted for influenza testing during the season was stored at -80° C and thawed for additional multiplex testing. All extracted RNA was tested for viral pathogens using the FTD Respiratory Pathogen 33 multiplex PCR kit (Fast Track Diagnostics). Results for hRV, coronavirus HKU1 (hCoV-HKU1), coronavirus OC43 (hCoV-OC43), coronavirus NL63 (hCoV-NL63), coronavirus 229E (hCoV-229E), parainfluenza viruses 1-4, human metapneumovirus (HMPV), human bocavirus, paraechovirus, enterovirus, adenovirus, and RSV were recorded.

3.4.3 Statistical methods

Patients who tested positive for influenza by RT-PCR were cases. We defined three control groups by multiplex testing: "influenza negative", "other virus positive", and "pan-negative". Individuals with an indeterminate positive by multiplex testing (unable to determine presence of exponential curve) were excluded from the other virus positive and pan-negative control groups but were still included in the influenza positive or negative groups depending on their influenza status.

Patients were considered vaccinated if they had documented or plausible self-report of vaccination ≥14 days before illness onset; if patients self-reported no vaccination and had no documentation of vaccine receipt they were considered unvaccinated. Individuals who received the vaccine 1 to 13 days before illness onset or who could not report either location or timing of vaccination and had no documentation were excluded, and individuals who received the vaccine after onset were considered unvaccinated.

CCI score were categorized as 0,1,2 or ≥3. Our frailty score was defined as the sum of the dichotomized subjected assessments of frailty. Characteristics of cases were compared to all three control groups. Characteristics of vaccinated and unvaccinated enrollees were compared using χ^2 tests or Fisher's exact tests for categorical variables and Wilcoxon rank-sum test for continuous variables.

VE was estimated separately for each influenza season and control group, and was calculated as 100*(1-Odds Ratio) comparing case and control patients in Firth's corrected logistic regression models. Adjusted models contained sex, age group (18-49, 50-64, 65+), Hospital (A vs. B), frailty score, CCI, days between illness onset and specimen collection, and calendar time of illness onset measured in two-week windows. In addition VE using hRV

positive individuals and RSV positive individuals as case groups compared to hRV negative and RSV negative individuals, respectively, as controls was calculated. Influenza-positive individuals were excluded from these analyses. These models were used to test the assumption that influenza does not impact incidence of other viruses, any difference from the null would be evidence for this bias.

3.5 Results

3.5.1 Participant inclusion and viral testing

In the 2014-15 season, we enrolled 756 participants; after exclusions 624 were included (Figure 3.1). Ninety-eight (15.7%) individuals tested positive for influenza A(H3N2) and 526 tested negative (Figure 3.1). Of the influenza-negative individuals, 181 (34.4%) tested positive for a respiratory virus, and 338 (64.2%) tested negative for all viruses (Figure 3.2); 7 (1.3%) individuals who had indeterminate status for a non-influenza virus were excluded from analyses using the other virus positive or pan-negative control groups. In the 2015-2016 season, 482 individuals were enrolled. After exclusions, 441 individuals remained in the final sample (Figure 3.1). Eighty-seven (19.7%) individuals tested positive for influenza A(H1N1), and 354 tested negative . Of those 354, 107 (30.2%) tested positive for a non-influenza respiratory virus and 247 (69.8%) tested negative for all viruses (Figure 3.2).

3.5.2 Participant characteristics by case status and control group

Participant demographics and clinical characteristics did not vary by control group in either season, with a few exceptions (Tables 3.1 and 3.2). In 2014-15, influenza positive individuals had significantly lower frequency of CHF and significantly lower mean frailty score than controls. The other virus positive group had the lowest frequency of individuals aged ≥65

(29.8% of the other virus group, 34.4% of the influenza negative group); opposite to the relationship seen in 2015-16, when participants who tested positive for a non-influenza virus tended to be older than influenza negative participants. In both 2014-15 and 2015-16, the other virus positive group had the highest frequency of individuals enrolled >4 days post illness onset. The other virus positive group had the lowest mean frailty compared to other control groups in 2014-15; this trend was not seen in 2015-16 (3.1 and 3.2). In 2015-16, influenza positive individual had significantly less frequency of a CCI>3 and less CHF than controls. Among controls, a higher proportion of individuals in the other virus positive group (61.7%) had a CCI >3 compared to those in the influenza-negative (55.9%) control group; this was not seen in 2014-15.

3.5.3 Chronic Respiratory Illness by Case Status and Control Group

Due to concerns regarding over-representation of individuals with chronic respiratory illness in the influenza negative control group, we examined the frequency of COPD and CHF by control group. In 2014-15, prevalence of COPD was higher in the other virus positive group (69.1%) than in the all influenza negative group (62.4%); there was a similarly higher prevalence in the influenza positive group (67.3%). CHF was similarly prevalent in all control groups. In 2015-16, COPD and CHF were distributed similarly across all control groups (Table 3.2).

3.5.4 Participant characteristics by vaccination status

In both seasons, roughly two-thirds of participants were vaccinated against influenza (Tables 3.1 and 3.2) and vaccination varied by enrollment hospital, age, race, CCI, CHF, and whether participants received primary care from the enrollment hospital (Tables 3.3 and 3.4). COPD was more prevalent among vaccinated participants in 2015-16 but not 2014-15. Vaccination was less frequent among cases compared to influenza-negative and pan-negative

controls in the 2014-15 season, but this difference was not significant between case and other virus positive controls (Table 3.5). In 2015-16, vaccination was significantly less frequent among cases compared to all control groups (Table 3.6).

3.5.5 Influenza VE estimates using alternate control groups

In the 2014-15 influenza season, the vaccine was 41.1% (95% CI: 1.7%, 64.7%) effective at preventing influenza A(H3N2)-related hospitalizations using the influenza negative control group, but VE was lower (24.5%, 95% CI: -42.6%, 60.1%) when other virus positive controls were used, though confidence intervals were wide and overlapped substantially (Figure 3.3). VE was highest (45.8%, 95% CI: 5.7%, 68.9%) when using pan-negative controls. In 2015-2016, using the influenza negative control group, the vaccine was 68.7% (95% CI: 44.6%, 82.5%) effective at preventing influenza A(H1N1) associated hospitalizations. VE was consistent when using the other virus positive group (63.1%, 95% CI: 25.0%, 82.2%) or the pan-negative group (71.1%, 95% CI: 46.2%, 84.8%) as controls.

3.5.6 VE estimates against non-influenza viruses

In order to evaluate the assumption of the TND that the influenza vaccine is not associated with the incidence of non-influenza respiratory infections, we tested VE against RSV, hRV, and all pooled non-influenza respiratory viruses. The vaccine was not protective against any non-influenza virus in either season. In 2014-15, VE against hRV was -34.6% (95% CI: -176.8%, 22.8%) and VE against all non-influenza viruses was 23.3% (-18.4%, 48.1%) (Figure 3.4). In the 2015-16 season all VE estimates against these non-influenza viruses were near 0% (Figure 3.4).

3.6 Discussion

Many large network studies evaluate annual VE in various clinical settings globally[30,106–108]. However, there are few direct comparisons of VE estimates generated in hospital and ambulatory settings from the same source population[44–46,109,110] With a lack of comparative data from TND in outpatient and inpatient studies, differences in VE estimates from these settings are challenging to interpret. For example, a moderate and significant VE found in Michigan inpatient adults in 2014-15 was in stark contrast to VE estimated in outpatient settings that was near 0% both among Michigan adults and across the US[58,103,111].

In order to evaluate the hypothesis that over-inclusion of individuals with chronic respiratory illnesses among influenza-negative controls biases inpatient VE estimates, we compared influenza negative and other virus positive controls. In both seasons examined, the assumption that influenza-negative controls had higher frequency of chronic diseases and increased age was not supported. Controls did not differ consistently by age, CCI, CHF, or COPD. These data suggest that the hypothesis that influenza negative control group contains an inappropriate frequency of patients with chronic respiratory conditions was unfounded. This is a particularly important conclusion for TND studies, as exacerbations of chronic respiratory conditions such as COPD are important complications of influenza infection and frequent causes of hospitalization, meaning that exclusion of these individuals to reduce bias would not be a viable solution.

Our results did not indicate consistent over-estimation of VE using an influenza negative control group. In both 2014-15 and 2015-16, VE calculated using other virus positive controls had 95% CIs that overlapped those of VE calculated using influenza negative controls. In 2015-16, VE was consistent regardless of the control group used. We did observe some variation

between VE estimates in the 2014-15 season, when the VE estimate using the traditional control group (41.1%) was higher than VE calculated using the other virus positive group (24.5%). However, this difference does not fully explain the discrepancy found between the outpatient and inpatient VE estimates that we measured in this season[112]. Our results suggest that while it is possible that selection bias accounted for a portion of the high VE in preventing hospitalization in 2014-15, this bias probably did not fully account for this discrepancy. It is possible that while a mismatched vaccine was unable to prevent mild influenza illness, it was able to prevent severe illness, possible supported by the lower rates of ICU admission among influenza positive individuals in 2014-15, a year with low VE[28,113]

Other studies, mostly conducted in outpatient settings, have also shown that VE is similar using influenza-negative and other virus positive controls[109,114,115]. A recent meta-analysis evaluating VE using alternate control groups found no difference in VE when using influenza-negative, other virus positive, or pan-negative control groups among 12 studies[25]. Similar to our study, more variation between estimates was observed when VE was low than when it was high. However, only three of the studies evaluated included inpatients, and only two included inpatient adults[38,109,116]. Both studies found no difference between VE when using alternate control groups [109,116].Notably, like these two studies, our study used research case definitions to define eligibility for study enrollment. The potential for selection bias due to inclusion of patients without ARI is more likely when studies rely on physician directed clinical testing rather than rigorous case definitions as inclusion criteria. While our results are suggestive, they may not be generalizable to TND studies that use clinical testing or other methods to determine inclusion with no case definition.

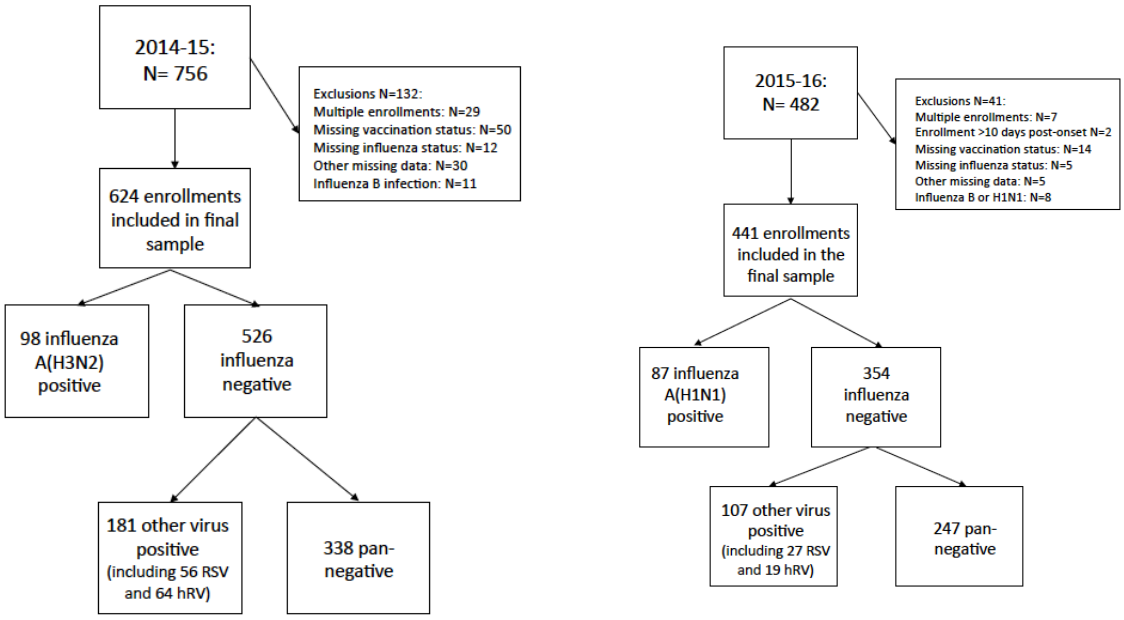
We also tested the assumption that the influenza vaccine does not affect incidence of other respiratory viruses by calculating VE against these viruses. While it has been suggested that the influenza vaccine may increase incidence of non-influenza respiratory viruses in children[117], most studies have found no association between influenza vaccine receipt and incidence of non-influenza respiratory viruses[118,119]. Our results confirm these null findings in an inpatient setting. In the 2014-15 season there was some variation in VE estimates against non-influenza viruses likely due to small numbers, but all point estimates were not statistically different than zero.

It is possible that the true discrepancy between VE calculated using different control groups is larger in certain age groups, especially considering that older adults tend to be more frail and have more chronic illnesses than younger adults. However, our small sample size prevented us from stratifying by age. Another limitation to our analysis was our inability to differentiate between molecular detection of virus and determination of the causal agent of illness, a common dilemma with PCR-based testing panels. Certain respiratory viruses can be shed and detected via PCR for long periods of time post-infection (e.g. adenovirus) or can be frequently detected among asymptomatic individuals (e.g. hRV). If individuals were enrolled into our study with symptoms unrelated to the respiratory virus that they were shedding, we may have misclassified their exposure, as they would truly belong in the pan-negative control group. However, hRV has been shown to cause severe clinical illness in some cases, so exclusion of these individuals or classification of these individuals as pan-negative is also likely to cause bias[120–122]. In addition, it is possible that certain individuals with a respiratory virus were allocated to the pan-negative control group because they had ceased shedding their respiratory virus, as we enrolled individuals with onset as long as ten days before enrollment. However, time

from onset to enrollment was shorter in the pan-negatives compared to the other virus positive and influenza-positive groups.

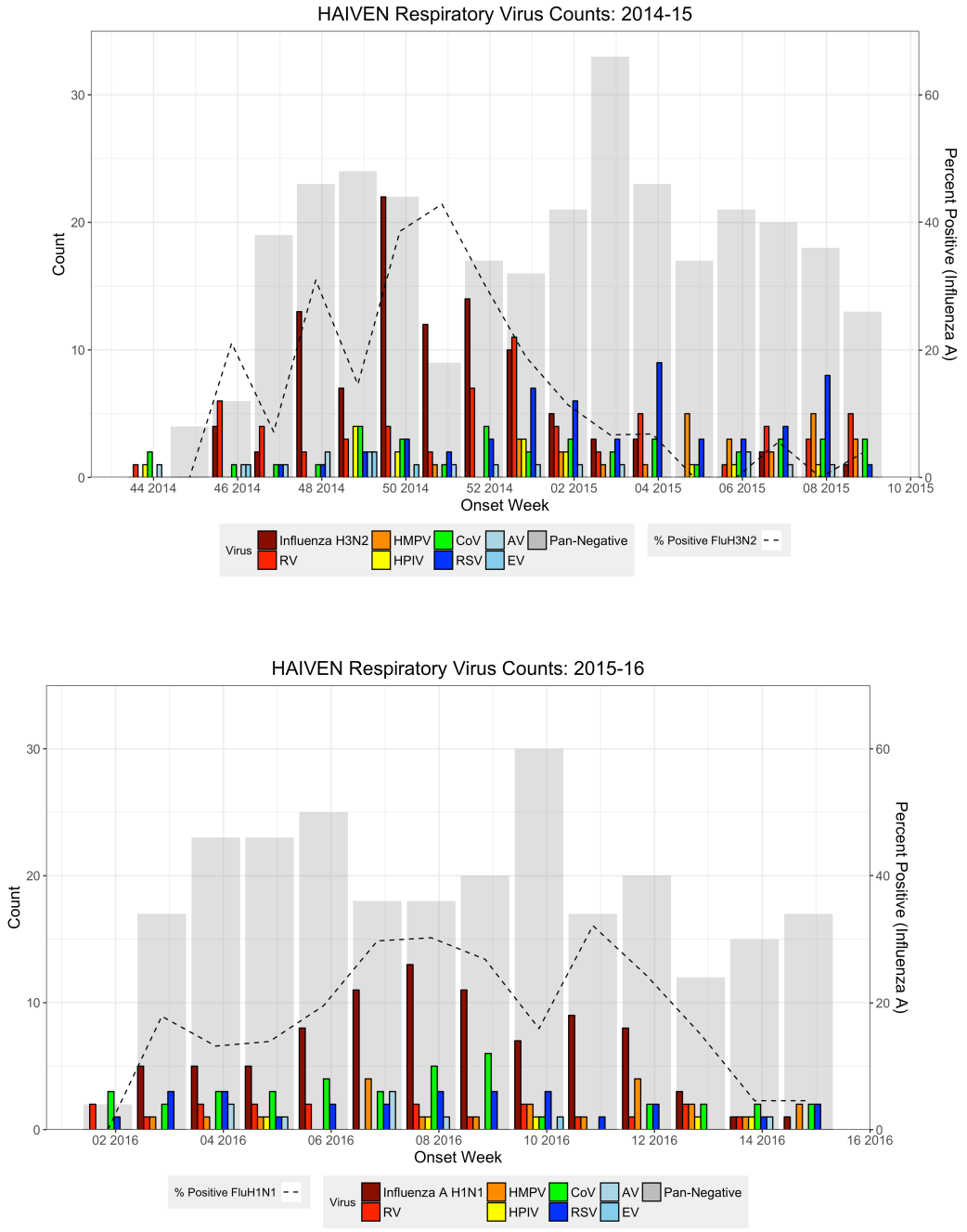
We did not find evidence of systematic bias due to an association between the influenza vaccine and the incidence of non-influenza respiratory infections, or a non-representative influenza-negative control group in our study. Our results indicate that non-representative controls and non-specific vaccine effects are not consistent sources of bias in inpatient VE estimates calculated in studies with a systematic case definition. These data suggest that the use of TNDs in VE studies enrolling influenza-infected inpatients does not introduce systematic bias.

Figure 3.1 Flow Chart of Exclusions for Determination of the Final Sample for the 2014-15 and 2015-16 Influenza Seasons.



Other virus positive refers to participants who tested negative for influenza but positive for a different respiratory virus. Pan-negative refers to participants who tested negative for all respiratory viruses. In the 2014-15 season 7 participants who had an undeterminable PCR result on one or more respiratory virus targets and did not test positive for any viruses were classified as influenza negative but not as either other virus positive or pan-negative.

Figure 3.2 Respiratory Virus Counts by Two Week Period in the 2014-15 and 2015-16 Influenza Seasons



The percent influenza A positive (H3N2 in 2014-15, and H1N1 in 2015-16) is represented by the dashed line. Each color bars represent counts of the frequency that each virus was detected in a two-week period. The translucent gray bars indicate counts of individuals who test negative for all viruses.

Table 3.1 Characteristics of Influenza A(H3N2) Outcome Status Using Alternate Control Groups; 2014-1

			Control Groups[a]		
	Total (n=624)	Influenza Positive (n=98)	All Influenza Negative (n=526)	Other Virus Positive (n=181)	Pan-Negative (n=338)
Enrolled at Hospital A	341 (54.6%)	53 (54.1%)	288 (54.8%)	98 (54.1%)	186 (55.0%)
Female Sex	337 (54.0%)	60 (61.2%)	277 (52.7%)	93 (51.4%)	181 (53.6%)
Age Group					
18-49	165 (26.4%)	27 (27.6%)	138 (26.2%)	48 (26.5%)	90 (26.6%)
50-64	239 (38.3%)	32 (32.7%)	207 (39.5%)	79 (43.6%)	126 (37.3%)
65+	220 (35.3%)	39 (39.8%)	181 (34.4%)	54 (29.8%)	122 (36.1%)
Race[b]					
White	329 (53.1%)	52 (53.6%)	277 (53.0%)	87 (48.1%)	185 (55.2%)
Black	217 (35.0%)	33 (34.0%)	184 (35.2%)	67 (37.0%)	116 (34.6%)
Other	74 (11.9%)	12 (12.4%)	62 (11.9%)	27 (14.9%)	34 (10.1%)
Vaccinated[c]	421 (67.5%)	57 (58.2%)	364 (69.2%)	119 (65.7%)	240 (71.0%)
Charlson Score					
0	49 (7.9%)	8 (8.2%)	41 (7.8%)	15 (8.3%)	26 (7.7%)
1	180 (28.8%)	36 (36.7%)	144 (27.4%)	49 (27.1%)	93 (27.5%)
2	97 (15.5%)	12 (12.2%)	85 (16.2%)	32 (17.7%)	51 (15.1%)
3+	198 (47.8%)	42 (42.9%)	256 (48.7%)	85 (47.0%)	168 (49.7%)
Congestive Heart Failure	189 (30.3%)	22 (22.4%)	167 (31.7%)	57 (31.5%)	109 (32.2%)
Chronic Obstructive Pulmonary Disease	394 (63.1%)	66 (67.3%)	328 (62.4%)	125 (69.1%)	200 (59.2%)
Frailty Score[d]					
Mean ± SD	1.83 ± 1.42	1.52 ± 1.33	1.88 ± 1.43	1.71 ± 1.45	1.98 ± 1.42
Enrolled ≤4 days from Illness Onset	382 (61.2%)	66 (67.3%)	316 (60.1%)	93 (51.4%)	218 (64.5%)
Receiving Primary Care from enrollment hospital health system[e]	463 (74.9%)	74 (76.3%)	389 (74.7%)	134 (74.9%)	249 (74.3%)
Length of Hospital Stay					
Mean ± SD	4.36 ± 4.35	3.25 ± 2.28	4.56 ± 4.61	4.46 ±4.17	4.65 ± 4.86
Intensive Care Unit Admission	69 (11.1%)	6 (6.1%)	63 (12.0%)	17 (9.4%)	46 (13.6%)

Abbreviation: SD, standard deviation

[a.] Control groups used include all influenza negative enrollees and two subsets of this group. Other virus positive includes enrollees who tested positive for one or more respiratory viruses but negative for influenza. Pan-negative includes enrollees who tested negative for all viruses. Seven individuals who had unknown status for one or more viruses and did not test positive for any virus were excluded from the other virus positive and pan-negative groups.

[b]. Four participants refused to report their race[c.] Participants were considered vaccinated if documented or plausible self-reported influenza vaccine receipt was ≥14 days before illness onset; participants were considered unvaccinated if there was no evidence of documented vaccination and they self-reported no vaccination.[d]. Subjective assessments of frailty across 5 items were dichotomized (present/absent, difficult/not difficult, frequent/not frequent) and summed across items to create a frailty score (0 = not frail … 5 = very frail).

[e]. One participant had missing location of primary care physician

Table 3.2 Characteristics of Enrolled Patients by Influenza A(H1N1) Outcome Status Using Alternate Control Groups; 2015-16

			Control Groups[a]		
	Total (n=441)	Influenza Positive (n=87)	All Influenza Negative (n=354)	Other Virus Positive (n=107)	Pan-Negative (n=247)
Enrolled at Hospital A	259 (58.7%)	45 (51.7%)	214 (60.5%)	67 (62.6%)	147 (59.5%)
Female Sex	250 (56.7%)	44 (50.6%)	206 (58.2%)	69 (64.5%)	137 (55.5%)
Age Group					
18-49	146 (33.1%)	33 (37.9%)	113 (31.9%)	28 (26.2%)	85 (34.4%)
50-64	159 (36.1%)	31 (35.6%)	128 (36.2%)	42 (39.3%)	86 (34.8%)
65+	136 (30.8%)	23 (26.4%)	113 (31.9%)	37 (34.6%)	76 (30.8%)
Race[b]					
White	221 (50.8%)	43 (50.6%)	178 (50.9%)	53 (49.5%)	125 (51.4%)
Black	161 (37.0%)	35 (41.2%)	126 (36.0%)	35 (32.7%)	91 (37.4%)
Other	53 (12.2%)	7 (8.2%)	46 (13.1%)	19 (17.8%)	27 (11.1%)
Vaccinated[c]	293 (66.4%)	38 (43.7%)	255 (72.0%)	80 (75.5%)	174 (70.4%)
Charlson Score					
0	66 (15.0%)	21 (24.1%)	45 (12.7%)	10 (9.3%)	35 (14.2%)
1	91 (20.6%)	19 (21.8%)	72 (20.3%)	17 (15.9%)	55 (22.3%)
2	53 (12.0%)	14 (16.1%)	39 (11.0%)	14 (13.1%)	25 (10.1%)
3+	231 (52.4%)	33(37.9%)	198 (55.9%)	66 (61.7%)	132 (53.4%)
Congestive Heart Failure	148 (33.6%)	19 (21.8%)	129 (36.4%)	41 (38.3%)	88 (35.6%)
Chronic Obstructive Pulmonary Disease	261 (59.2%)	38 (43.7%)	223 (63.0%)	67 (62.6%)	195 (66.6%)
Frailty Score[d]					
Mean ± SD	1.77 ± 1.43	1.62 ± 1.37	1.81 ± 1.45	1.80 ± 1.44	1.81 ±1.45
Enrolled ≤4 days from Illness Onset	280 (63.5%)	67 (77.0%)	213 (60.2%)	58 (54.2%)	155 (62.8%)
Receiving Primary Care from enrollment hospital health system[e]	295 (66.9%)	61 (70.1%)	234 (66.1%)	81 (75.7%)	153 (61.9%)
Length of Hospital Stay					
Mean ± SD	4.47 ± 4.82	5.23 ± 7.50	4.28 ± 3.88	4.20 ± 3.65	4.31 ± 3.98
Intensive Care Unit Admission	50 (11.3%)	12 (13.8%)	38 (10.7%)	10 (9.3%)	28 (11.3%)

Abbreviation: SD, standard deviation

[a.] Control groups used include all influenza negative enrollees and two subsets of this group. Other virus positive includes enrollees who tested positive for one or more respiratory viruses but negative for influenza. Pan-negative includes enrollees who tested negative for all viruses. Seven individuals who had unknown status for one or more viruses and did not test positive for any virus were excluded from the other virus positive and pan-negative groups.

[b]. Six participants refused to report their race[c.] Participants were considered vaccinated if documented or plausible self-reported influenza vaccine receipt was ≥14 days before illness onset; participants were considered unvaccinated if there was no evidence of documented vaccination and they self-reported no vaccination.[d]. Subjective assessments of frailty across 5 items were dichotomized (present/absent, difficult/not difficult, frequent/not frequent) and summed across items to create a frailty score (0 = not frail … 5 = very frail).

[e]. One participant had missing location of primary care physician

Table 3.3 Characteristics of Enrolled Patients by Vaccination Status; 2014-15

	Total (n= 624)	Vaccinated (n=421)[a]	Unvaccinated (n=203)	P-value
Enrolled at Hospital A	341 (54.6%)	253 (60.1%)	88 (43.3%)	<.0001
Female Sex	337 (54.0%)	226 (53.7%)	111 (54.7%)	0.81
Age Group				<.0001
18-49	165 (26.4%)	77 (18.3%)	88 (43.3%)	
50-64	239 (38.3%)	169 (40.1%)	70 (34.5%)	
60+	220 (35.3%)	175 (41.6%)	45 (22.2%)	
Race[b]				0.0001
White	329 (53.1%)	246 (58.7%)	83 (41.3%)	
Black	217 (35.0%)	125 (29.8%)	92 (45.8%)	
Other	74 (11.9%)	48 (11.5%)	26 (12.9%)	
Charlson Score				<.0001
0	49 (7.9%)	25 (5.9%)	24 (11.8%)	
1	180 (28.8%)	103 (24.5%)	77 (37.9%)	
2	97 (15.5%)	63 (15.0%)	34 (16.7%)	
3+	198 (47.8%)	230 (54.6%)	68 (33.5%)	
Congestive Heart Failure	189 (30.3%)	145 (34.4%)	44 (21.7%)	0.001
Chronic Obstructive Pulmonary Disease	394 (63.1%)	267 (63.4%)	127 (62.6%)	0.83
Frailty Score[c]				
Mean ± SD	1.83 ± 1.42	1.89 ± 1.42	1.70 ± 1.42	0.10
Enrolled ≤4 days from Illness Onset	382 (61.2%)	257 (61.0%)	125 (61.6%)	0.90
Receiving Primary Care from enrollment hospital health system[d]	463 (74.9%)	337 (80.8%)	126 (62.7%)	<.0001
Length of Hospital Stay				
Mean ± SD	4.36 ± 4.35	4.43 ± 4.12	4.21 ± 4.80	0.03
Intensive Care Unit Admission	69 (11.1%)	44 (10.5%)	25 (12.3%)	0.49

Abbreviation: SD, standard deviation

[a.] Participants were considered vaccinated if documented or plausible self-reported influenza vaccine receipt was ≥14 days before illness onset; participants were considered unvaccinated if there was no evidence of documented vaccination and they self-reported no vaccination.

[b.] Four participants refused to report their race.[c.] Subjective assessments of frailty across 5 items were dichotomized (present/absent, difficult/not difficult, frequent/not frequent) and summed across items to create a frailty score (0 = not frail … 5 = very frail).

[d.] One participant had missing location of primary care physician

Table 3.4 Characteristics of Enrolled Patients by Vaccination Status; 2015-16

	Total (n=441)	Vaccinated (n=293)[a]	Unvaccinated (n=148)	P-value
Enrollment at Hospital A	259 (58.7%)	201 (68.6%)	58 (39.2%)	<.0001
Female Sex	250 (56.7%)	173 (59.0%)	77 (52.0%)	0.16
Age Group				<.0001
18-49	146 (33.1%)	76 (25.9%)	70 (47.3%)	
50-64	159 (36.1%)	105 (35.8%)	54 (36.5%)	
60+	136 (30.8%)	112 (38.2%)	24 (16.2%)	
Race[b]				<.0001
White	221 (50.8%)	173 (59.2%)	48 (33.6%)	
Black	161 (37.0%)	87 (29.8%)	74 (51.7%)	
Other	53 (12.2%)	32 (11.0%)	21 (14.7%)	
Charlson Score				<.0001
0	66 (15.0%)	20 (6.8%)	46 (31.1%)	
1	91 (20.6%)	50 (17.1%)	41 (27.7%)	
2	53 (12.0%)	40 (13.7%)	13 (8.8%)	
3+	231 (52.4%)	183 (62.5%)	48 (32.4%)	
Congestive Heart Failure	148 (33.6%)	121 (41.3%)	27 (18.2%)	<.0001
Chronic Obstructive Pulmonary Disease	261 (59.2%)	195 (66.6%)	66 (44.6%)	<.0001
Frailty Score[c]				
Mean ± SD	1.77 ± 1.43	1.92 ± 1.45	1.47 ± 1.35	0.002
Enrolled ≤4 days from Illness Onset	280 (63.5%)	189 (64.5%)	91 (61.5%)	0.53
Receiving Primary Care from enrollment hospital health system[d]	295 (67.0%)	211 (72.0%)	84 (57.1%)	0.002
Length of Hospital Stay				
Mean ± SD	4.47 ± 4.82	4.20 ± 3.81	4.99 ± 6.34	0.84
Intensive Care Unit Admission	50 (11.3%)	29 (9.9%)	21 (14.2%)	0.18

Abbreviation: SD, standard deviation

[a.] Participants were considered vaccinated if documented or plausible self-reported influenza vaccine receipt was ≥14 days before illness onset; participants were considered unvaccinated if there was no evidence of documented vaccination and they self-reported no vaccination.

[b.] Seven participants refused to report their race[c]. Subjective assessments of frailty across 5 items were dichotomized (present/absent, difficult/not difficult, frequent/not frequent) and summed across items to create a frailty score (0 = not frail … 5 = very frail).

[d]. One participant had missing location of primary care physician

Table 3.5 Influenza Status by Vaccination Status, 2014-15

	Total	Vaccinated [a]	Unvaccinated	P Value[b]
Influenza H3N2 Positive Cases	98	57 (58.2%)	41 (41.8%)	
Control Groups				
All Influenza Negative	526	364 (69.2%)	162 (30.8%)	0.03
Other Virus Positive	181	119 (65.7%)	62 (34.3%)	0.21
Pan-Negative	338	240 (71.0%)	98 (29.0%)	0.02

[a] Participants were considered vaccinated if documented or plausible self-reported influenza vaccine receipt was ≥14 days before illness onset; participants were considered unvaccinated if there was no evidence of documented vaccination and they self-reported no vaccination.

[b.] P values are comparing the percent vaccinated of each control group compared to the influenza positive group.

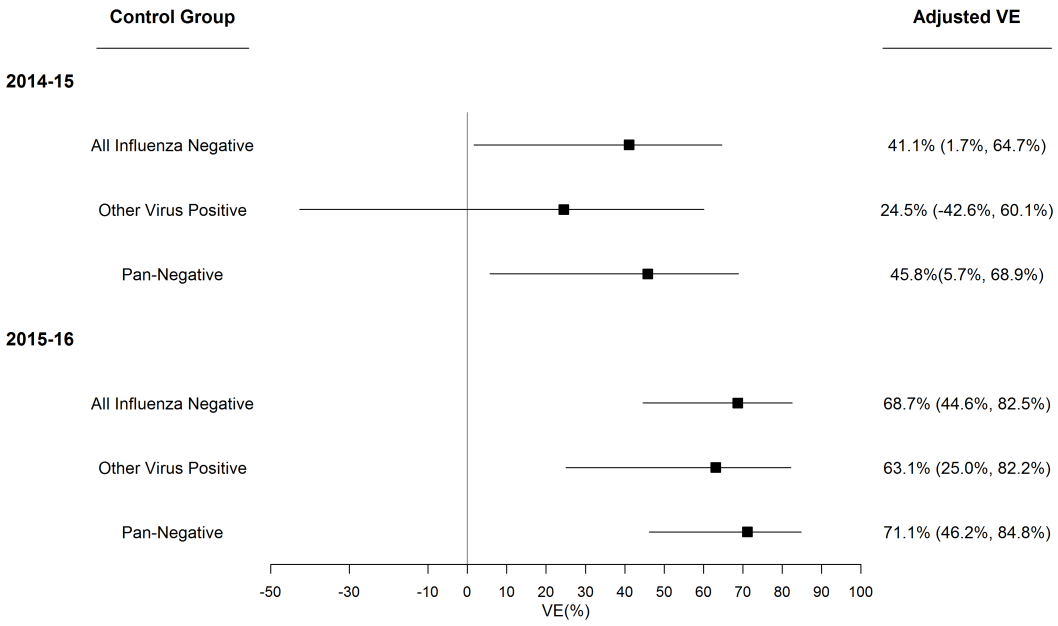
Table 3.6 Influenza Status by Vaccination Status, 2015-16

	Total	Vaccinated[a]	Unvaccinated	P Value[b]
Influenza H1N1 Positive Cases	87	38 (43.7%)	49 (56.3%)	
Control Groups				
All Influenza Negative	354	255 (72.0%)	99 (28.0%)	<.0001
Other Virus Positive	107	81 (75.7%)	26 (24.3%)	<.0001
Pan Negative	247	174 (70.4%)	73 (29.6%)	<.0001

[a] Participants were considered vaccinated if documented or plausible self-reported influenza vaccine receipt was ≥14 days before illness onset; participants were considered unvaccinated if there was no evidence of documented vaccination and they self-reported no vaccination.

[b.] P values are comparing the percent vaccinated of each control group compared to the influenza positive group.

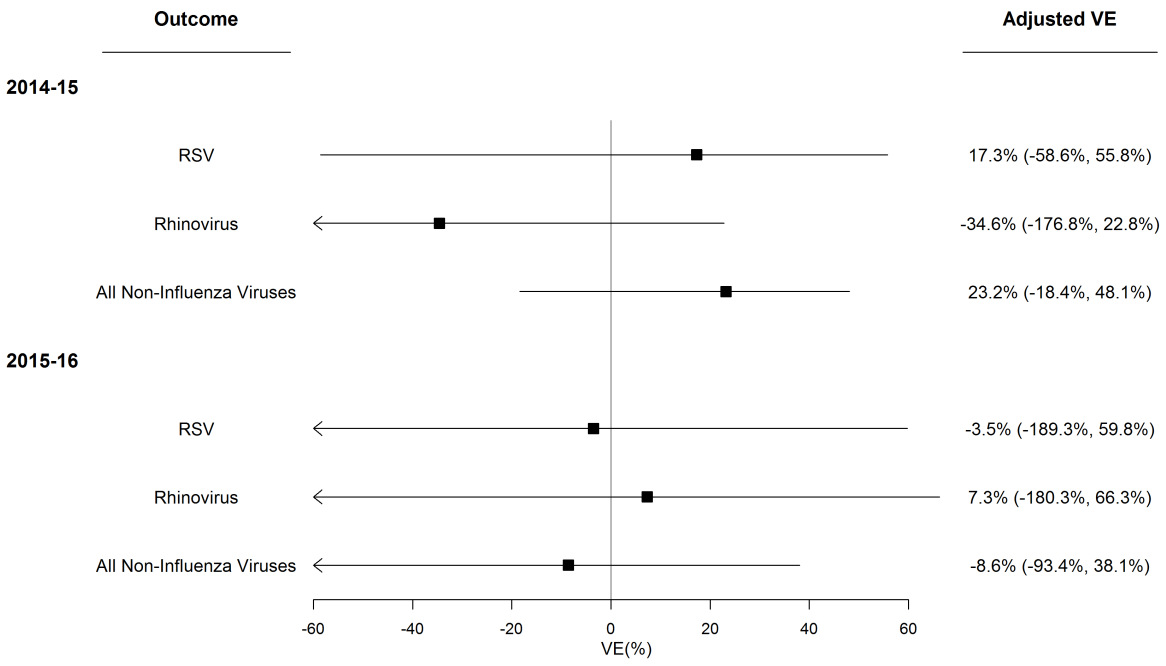
Figure 3.3 Influenza vaccine effectiveness estimates using three different control groups in the 2014-15 and 2015-16 influenza seasons.



VE was estimated separately for each influenza season using each control group, and was calculated as 100*(1-Odds Ratio) comparing case and control patients in Firth's corrected logistic regression models. Adjusted models contained sex, age group (18-49, 50-64, 65+), Hospital (A vs. B), frailty score, CCI, days between illness onset and specimen collection, and calendar time of illness onset measured in two-week windows

Other virus positive refers to participants who tested negative for influenza but positive for a different respiratory virus. Pan-negative refers to participants who tested negative for all respiratory viruses. In the 2014-15 season 7 participants who had an undeterminable PCR result on one or more respiratory virus targets and did not test positive for any viruses were classified as influenza negative but not as either other virus positive or pan-negative.

Figure 3.4 Vaccine effectiveness against hRV, RSV, and pooled non-influenza viruses in the 2014-15 and 2015-16 seasons.



Abbreviations: hRV human Rhinovirus, RSV Respiratory Syncytial Virus

VE was estimated separately for each influenza season using each virus separately, and was calculated as 100*(1-Odds Ratio) comparing case and control patients in Firth's corrected logistic regression models. Adjusted models contained sex, age group (18-49, 50-64, 65+), Hospital (A vs. B), frailty score, CCI, days between illness onset and specimen collection, and calendar time of illness onset measured in two-week windows

Chapter 4 The Impact of Obesity and Timely Antiviral Administration on Severe Influenza Outcomes Among Hospitalized Adults[3]

4.1 Author Summary

During the 2009 influenza A(H1N1)pdm09, increased influenza severity was observed among morbidly obese patients. Consequently, obesity was added to the list of high-risk conditions for severe influenza outcomes and it was recommended that obese patients be treated with neuraminidase inhibitors even in the outpatient setting. We found that in the hospital, where neuraminidase inhibitor administration is recommended for all patients upon suspicion of influenza infection, obese patients were treated earlier in their disease course than other patients. When evaluating the impact of obesity on influenza severity, it is important to take into account neuraminidase inhibitor treatment and treatment timing.

4.2 Abstract

Obesity was identified as a risk factor for severe influenza during the 2009 influenza A(H1N1) pandemic, but evidence of this association has been mixed since. Post-pandemic antiviral treatment guidelines may have increased antiviral treatment among obese individuals.

A prospective study of adults hospitalized with laboratory-confirmed influenza in Detroit, Michigan in 2011-2012 and 2012-2013 was conducted. Patient information was collected from interviews and medical chart abstraction. Obese (BMI≥30) and non-obese (BMI<30) participants

[3] Chapter 4 has been published as: Segaloff, H. E., Evans, R., Arshad, S., Zervos, M. J., Archer, C., Kaye, K. S., & Martin, E. T. (2018). The impact of obesity and timely antiviral administration on severe influenza outcomes among hospitalized adults. *Journal of medical virology*, *90*(2), 212-218.

were compared. Late antiviral treatment (>2 days from symptom onset), obesity (30≤BMI<40), and morbid obesity (BMI≥40) were evaluated as predictors of lower respiratory tract disease (LRD), ICU admission, and length of stay (LOS) using logistic regression and inverse probability weighted models.

Forty-eight participants were included in the study after exclusions and all patients received antiviral treatment. Participants who were obese were significantly more likely to have a cough and to take steroids than non-obese participants, and had a shorter time from hospital admission to antiviral treatment (median time from admission to treatment of 0 days for obese patients and 1 day for non-obese patients (p=0.001)). In all models, late antiviral treatment was associated with increased odds of LRD (OR: 3.9(1.1,15.9) in fully adjusted model). After adjustment for treatment timing, the odds of ICU admission (OR: 6.4(0.8,58.2) to 7.9(0.9, 87.1) and LRD (OR: 3.3 (0.5, 23.5) to 4.0 (0.6, 35.0) associated with morbid obesity increased.

Obese individuals were treated with antivirals earlier than others. Late antiviral treatment was associated with severe influenza in the hospital.

4.3 Introduction

Influenza virus, though usually a self-limiting infection, is associated with increased morbidity and mortality during annual outbreaks[123]. In general, older adults and individuals with underlying comorbid conditions are at high risk for adverse events after influenza infection[124]. In 2009, the emergence of the 2009 pandemic influenza A (H1N1) virus led to an increased prevalence of severe outcomes among populations that had not previously been considered at high risk for these consequences of disease. Specifically, children and young adults were more likely to be hospitalized for influenza; and morbid obesity (body mass index of 40 or greater) was identified as a predictor of hospitalization, ICU admission and of death[125,51,126].

Subsequent analyses using data from non-pandemic influenza seasons have left an unresolved picture of the relationship between obesity and influenza severity and whether this relationship persists beyond the influenza A (H1N1)pdm09 subtype. A study by Cocoros et al. regarding seasonal and pandemic influenza A(H1N1) found that obesity had a small association with severe influenza like illness (ILI) in seasons dominated by H1N1 before and after the 2009 pandemic, leading to hospitalization[127]. However, these results were only seen in certain age groups in the study population and these results have not been consistently validated in other populations[128,129].

Differences in antiviral treatment timing add further complexity to this issue in the post-pandemic period. A meta-analysis analyzing the relationship between obesity and influenza A (H1N1)pdm09 severity in 2009-2011 influenza seasons globally found that the significant relationship between obesity and influenza complications was attenuated and non-significant after adjustment for antiviral prescription timing; the authors found that obese individuals were less likely to receive antivirals in a timely fashion and that this treatment timing was an important confounder of the relationship between obesity and influenza severity[130]. However, in the United States, a recent change to antiviral treatment recommendations may have altered this relationship between treatment timing and weight status. After review of data from the 2009 pandemic, the Center for Disease Control and Prevention (CDC) added morbid obesity to their list of indicators for high influenza severity risk and recommended that these individuals be prescribed antiviral treatment empirically in the outpatient setting[60]. These recommendations may lead physicians to treat hospitalized obese patients earlier than patients without a high-risk condition, either through empiric treatment in outpatient settings before presentation to the hospital, or through immediate treatment upon hospital admission. Previous studies have

demonstrated that treatment with oseltamivir within 48 hours of symptom onset reduces severe complications of influenza and that oseltamivir is most effective when given within 48 hours[131,132]. However, oseltamivir is also associated with reduced duration of shedding when it is given within 72 hours of symptom onset, and increased survival and decreased severity for up to five days after illness onset[133–135]. If obese individuals are treated earlier than others, antiviral receipt timing may complicate the ability to detect an association between obesity and influenza severity.

In order to evaluate the relationship between antiviral timing, obesity, and influenza severity, a prospective study of hospitalized influenza-positive adults in Detroit, Michigan was conducted. A previous study in this region found that nearly 50% of the study population had a body mass index (BMI) classified as obese, well over the state average of 30.7%[136]. The results of this study indicated that obese individuals were more likely than non-obese individuals to be admitted to inpatient care, to have hospital stays of greater than 7 days, and to have lower respiratory tract disease manifestations, following influenza infection, predominately with influenza A (H1N1)pdm09[137]. The aim of this current study is to use a prospective design and data from the 2011-2012 and 2012-13 influenza seasons to expand on the previous data linking obesity to influenza severity, and to evaluate the role of the timing of antiviral administration in this association.

4.4 Methods

4.4.1 Study Population

Hospitalized adults admitted to one of five Detroit, Michigan area hospitals with laboratory confirmed influenza from February through April 2012 and November 2012 through March 2013 were prospectively identified. Participants were enrolled from Detroit Receiving

Hospital, Harper Hospital, Hutzel Women's Hospital, Sinai-Grace Hospital, and Henry Ford Hospital. Patients were identified from a clinical microbiology laboratory results using a clinical alert system (Theradoc) and their eligibility status was confirmed with their treating physician. Patients admitted to the hospital were eligible if they were 18 years old or greater, and if they tested positive for influenza A (H1N1)pdm09, influenza A (H3N2), or influenza B, and if they had any symptom compatible with influenza like illness (ILI) including cough, chills, rhinorrhea, myalgia, dyspnea, diarrhea, vomiting, and/or subjective fever. Patients were excluded from the analysis if they had symptom duration longer than 10 days before admission and if they had a BMI less than 18.5. Eligible patients were approached for informed consent.

4.4.2 Patient Survey and Data Abstraction

After affirmative consent, patients were surveyed to determine their illness onset date, physical characteristics, alcohol and smoking histories, living situation, and influenza and pneumococcal vaccine information. There were also asked about any physical, mental, or emotional limitations and if they routinely used special equipment due to a health problem (i.e. wheelchair, cane, special bed, special telephone). All day 1 interview questions were adapted from the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System Survey Questionnaire[138]. Additional information regarding demographics, insurance information, and medical information pertaining to symptoms, admission timing, antiviral and antibiotic therapy, vaccination, readmission, ICU admission, laboratory results, virus testing results, diagnoses and comorbidities was collected from the electronic medical record (EMR). Specific conditions evaluated were a history of cancer, lung disease (bronchiectasis, COPD/emphysema, asthma, restrictive disease, interstitial lung disease), history of heart conditions (myocardial infarction, coronary artery disease, coronary artery bypass grafting),

diabetes, renal disease, and HIV. Steroid use, including use of glucocorticoid steroids, prednisone or methylprednisolone in the last month, was also abstracted from the EMR. The patients were interviewed again by phone 30 days after enrollment to collect information on any new hospital admissions and the reason for these visits as well as any visit to a doctor in a doctor's office and the reason for these visits. Information on medications prescribed since discharge was also collected and patients were asked whether they had made diet or exercise changes since their discharge date.

4.4.3 Statistical Analysis

Severe outcomes in this study were defined as ICU admission, lower respiratory tract disease (intubation, hypoxia, lung infiltrates or consolidation) and increased length of stay. For the determination of lower respiratory tract disease, hypoxia was defined as oxygen saturation percentage marked as abnormal (below 94%), and lung infiltrates as well as consolidation were defined by a description of infiltrates or consolidation on chest x-ray impressions. In frequency models, obese (BMI of 30 or greater) and non-obese (BMI less than 30) participants were compared; p-values were determined using Fisher's exact tests. Differences in distributions of continuous variables were tested using the Wilcoxon rank-sum test. Unadjusted and adjusted Firth-penalized logistic models were run to predict odds of severity; unadjusted models contain either categorical BMI (BMI less than 30, BMI of 30 to less than 40, BMI of 40 or greater) or dichotomized antiviral treatment timing (late treatment defined as >2 days from symptom onset), adjusted models contained both variables. Inverse probability weighting (IPW) of propensity scores was used to efficiently adjust for age, diabetes, and poor/fair self rated health despite the small sample size. Diabetes was deemed the most important clinical confounder due to the impact of immune system disruption on influenza outcomes and to the recommendations that

antivirals be used promptly in these patients[139,140]. Asthma was not included in the model because it did not improve model fit. IPW of propensity scores was used to reduce bias in effect estimates by balancing the baseline characteristics between those with and without the outcome of interest. Due to collinearity between steroid use and other elements in the model it was not used as an adjustment factor. For the outcome of increased length of stay, length of stay was log-transformed and linear regression was used to predict percentage change in length of stay. All statistics were run on SAS 9.4 (Statistical Analysis System).

4.5 Results

There were 55 individuals enrolled in this study with laboratory-confirmed influenza. Individuals were excluded if they had missing data on BMI, lower respiratory tract disease, or ICU admission. Additionally, individuals who reported symptom onset of greater than 10 days before admission were excluded. The final analysis was performed on the 48 individuals with complete data. All (N=48) participants were treated with oseltamivir, 3 of these patients were given oseltamivir one day before their study admission. Of these 48 participants, 34 (70.8%) completed the 30-day follow up survey.

Twenty-four (50%) participants were obese and 5 (10.4%) were morbidly obese. The median age was 54.5 years of age in obese individuals and 60.5 years of age in non-obese individuals. Obese individuals had significantly shorter duration from admission to antiviral therapy and significantly greater frequency of steroid prescription in the prior three months (Table 4.1). At study enrollment, approximately 50% (n=23) of participants self-reported the need for special equipment due to a health problem, almost (n=28) 60% reported having physical, mental, or emotional limitations, over 50% (n=25) reported poor/fair health, and nearly 80% (n=37) reported having shortness of breath that affects their quality of life. All of these self-

reported health conditions were more common among obese patients, though these differences were not statistically significant (Table 4.2). Fifty percent (n=24) of participants reported receiving an influenza vaccine in the last year and 62.5% (n=30) reported receiving a pneumococcal vaccine during their lifetime (Table 4.2).

Thirty-four individuals participated in the survey given approximately one month post-discharge. 79.4% (n=26) of individuals reported having an appointment with their primary care physician, 15 (57.7%) of these appointments were follow up appointments and 20.5% (n=7) reported being readmitted to the hospital within 30-days of hospital discharge. Reasons for readmission included deep vein thrombosis, chest pain and coronary artery disease, pneumonia, ischemic cardiopathy, chronic obstructive pulmonary disease exacerbation, and congestive heart failure exacerbation. Almost 70% (n=26) of participants reported receiving a new prescription at discharge and nearly 70% (n=17) of those who received a prescription completed the full dose of the new medication. A variety of medications were prescribed in the 30 days post-discharge including antibiotics, antivirals, steroids (inhaled and ingested), blood pressure medication, pain medication, and blood thinners, among others. Over half (52.9%, n=18) of participants reported making a positive change to their diet or exercise habits 30-days after hospital discharge (Table 4.3). There were no deaths within 30 days of discharge.

In univariate models, late antiviral treatment was significantly associated with increased odds of lower respiratory tract disease and increased length of hospitalization (OR: 3.6 (1.1, 14.2), Percent change: 40.8 (2.6, 93.2)) (Table 4.4). After adjustment for BMI group, the odds of lower respiratory tract disease and ICU admission associated with late antiviral treatment increased in magnitude. In the IPW models the association between late antiviral treatment and lower respiratory tract disease remained significant (OR: 3.9 (1.1, 15.9)).

In the univariate model, obese individuals (BMI from 30 to less than 40) had significantly shorter length of hospitalization than non-obese individuals (Percent change: -29.2 (-49.6, -0.6))(Table 4.4). Obesity and morbid obesity were also associated with increased odds of ICU admission and lower respiratory tract disease, and these relationships were monotonic, though not significant (Table 4.4). After adjustment for late-antiviral treatment the odds of lower respiratory tract disease and ICU admission increased in the obese group, though they did not reach statistical significance.

4.6 Discussion

This study demonstrated that antiviral treatment within two days of symptom onset was associated with reduced odds of lower respiratory tract disease among adults hospitalized with laboratory-confirmed influenza. In 2011, the Advisory Committee on Immunization Practices (ACIP) released their updated recommendations for antiviral treatment. They recommended that all hospitalized patients with suspected influenza be treated empirically, even before confirmation of influenza status[60].The results of this study add to the existing literature that support this treatment recommendation change by emphasizing the importance of early antiviral treatment on improving patient outcomes[141,142]. Late antiviral treatment also appears to be an important confounder between obesity and influenza severity, as the likelihood of severe disease increased among obese individuals after adjustment for treatment in all models. These findings add to previous observations of the connection between antiviral treatment and obesity; a 2016 paper examined antiviral treatment among hospitalized patients from 2010-2015 and found that individuals with high risk conditions, including morbid obesity, were significantly more likely than non-obese individuals to receive antivirals; however the 2016 study did not evaluate timing of therapy[61].

This study took place at hospitals in Detroit, Michigan, a city with high levels of obesity and poverty[143]. As expected in hospitals in an underserved area, many participants had self-reported poor health in addition to the need for special medical equipment and physical, mental, or emotional limitations. Forty six percent of patients received health insurance from Medicaid or a Medicare/Medicaid combination and an additional 4% had no insurance. Individuals with public insurance are more likely to visit the emergency department over their primary care physician. It is unclear whether or not this is due to decreased access to their primary care physician, more complex conditions that require emergency facilities, or a preference for the hospital over outpatient clinics[144,145]. In the current study, the increased use of the emergency room among those with public insurance is reflected in the high readmission rate (20.6%) reported from respondents to the 30-day survey. Encouragingly, 52.9% of participants who completed the 30-day survey reported efforts to improve their diet or exercise routine post-hospitalization, indicating that the hospital discharge may have the potential to be an effective time to counsel the patient on modifiable health behaviors.

There are limitations to this analysis. The in-hospital observational nature of the study complicated our ability to study some commonly used severity endpoints. Reverse causation could have masked associations between timely antiviral treatment and severe outcomes if individuals were treated when admitted to the ICU or if lower respiratory tract disease was already present at the time of treatment. Lower than expected enrollment, particularly during the mild 2011-12 season, presented numerous difficulties. Though steroid use was likely an important confounder of obesity and severe influenza, steroid use could not be adjusted for due to collinearity between steroids and other adjustment factors. A variety of other confounders were able to be adjusted for using inverse-probability weighting despite the small sample size;

however, it is possible that residual confounding remains. Future studies should be conducted to re-evaluate the associations studied in this analysis, as the small sample size led to a reduction in statistical power.

The findings of this prospective study highlight the need to evaluate confounding from antiviral treatment timing when studying the relationship between obesity and influenza severity. Additionally, the association between late antiviral treatment and increased likelihood of serious disease highlights the importance of timely antiviral treatment. This reinforces the treatment recommendations from the ACIP and emphasizes the need for continued evaluation of antiviral prescription rates among hospitalized adults.

Table 4.1 Patient Characteristics by Obesity Status

Characteristics	Total (N=48)	BMI≥30 (N=24)	BMI<30 (N=24)	P Value[1]
Age (median, range)	59.0 (21-91)	54.5 (21-83)	60.5 (21-91)	0.30
BMI (mean, 95% CI)	30.3 (27.6, 33.0)	-	-	-
Days from Admission to Antiviral Treatment (median, range)	0.0 (0.0-6.0)	0.0 (0.0-4.0)	1.0 (0.0-6.0)	0.001
	N (% of Total)	**N (% of Obese)**	**N (% of Not Obese)**	**P Value[3]**
Female Sex	23 (47.9%)	11 (45.8%)	12 (50.0%)	0.77
Race[4]				0.45
White	5 (10.6%)	2 (8.7%)	3 (12.5%)	
Black	39 (83.0%)	21 (91.3%)	18 (75.0%)	
Other	3 (6.4%)	0 (0.0%)	2 (8.3%)	
Symptoms				
GI Symptoms	20 (41.7%)	7 (29.2%)	13 (54.2%)	0.08
Cough	37 (77.1%)	22 (92.1%)	15 (62.5%)	0.02
Fever	26 (54.2%)	15 (62.5%)	11 (45.8%)	0.25
Chills	22 (45.8%)	13 (54.2%)	9 (37.5%)	0.25
Shortness of Breath	29 (60.4%)	17 (70.8%)	12 (50.0%)	0.14
Fatigue	12 (25.0%)	4 (16.7%)	8 (33.3%)	0.18
Clinical Factors				
Diabetes	16 (31.2%)	8 (33.3%)	7 (29.2%)	0.76
Chronic Obstructive Pulmonary Disease	11 (22.9%)	7 (29.2%)	4 (16.7%)	0.30
Asthma	8 (16.7%)	5 (20.8%)	3 (12.5%)	0.44
Congestive Heart Failure	11 (22.9%)	5 (20.8%)	6 (25.0%)	0.73
Sepsis	20 (41.7%)	11 (45.8%)	9 (37.5%)	0.56
Steroid Use[5]	13 (27.7%)	10 (43.5%)	3 (12.5%)	0.02

[1]P values are the result of Wilcoxon tests
[2]3 obese patients were given oseltamivir one day before admission, these individuals were classified as treated 0 days from hospital admission
[3]P values are the result of Fisher's exact tests.
[4]One individual with obesity is missing race status
[5]One individual with obesity is missing information on steroid medication

Table 4.2 Survey Results by Obesity Status

Characteristics	Total (N=48)	BMI≥30 (N=24)	BMI<30 (N=24)	P Value[1]
	N (Column %)	N (Column %)	N (Column %)	
Self-Reported Limitations	28 (58.3%)	16 (66.7%)	12 (50.0%)	0.24
Self-Reported Poor/Fair Health	25 (52.1%)	15 (62.5%)	10 (41.7%)	0.24
Self-Reported Need for Equipment Due to Medical Condition	23 (47.9%)	13 (54.2%)	10 (41.7%)	0.39
Report that Shortness of Breath Affects Quality of Life	37 (77.0%)	20 (83.3%)	17 (70.8%)	0.30
100 Cigarette Smoking History	24 (50.0%)	12 (50.0%)	12 (50.0%)	1.0
Influenza Vaccine Receipt	24 (50.0%)	13 (54.2%)	11 (45.8%)	0.56
Pneumonia Vaccine Receipt	30 (62.5%)	14 (58.3%)	16 (66.7%)	0.55
	Median (Range)	Median (Range)	Median (Range)	P Value[2]
Alcoholic Drinks per Day in Past Month	0 (0-5)	0 (0-4)	0 (0-5)	0.22
Number of Children in the Household	0 (0-5)	0 (0-2)	0 (0-5)	0.30

[1]P values are the result of Fisher's exact tests
[2]P values are the result of Wilcoxon tests

Table 4.3 Day 30 Survey Results by Obesity Status

Characteristics	**Total (N=34)**	**BMI≥30 (N=20)**	**BMI<30 (N=14)**	**P Value[1]**
Hospital Readmission	7 (20.6%)	3 (15.0%)	4 (28.6%)	0.41
Visit to PCP	26 (79.4%)	16 (80.0%)	10 (71.4%)	0.69
Positive Diet Change	18 (52.9%)	11 (55.0%)	7 (50.0%)	0.77
New Medication Prescribed[2]	26 (67.6%)	14 (73.7%)	12 (85.7%)	0.67
Completed Dose of New Medication[3]	17 (68.0%)	11 (84.6%)	6 (50.0%)	0.10

[1] P Values are calculated from Fisher's exact tests
[2] One individual with obesity is missing information on new medication prescription
[3]. Percentages are calculated out of the number of individuals who reported having a new medication prescribed. One obese individual who reported receiving a new prescription had missing information on dose, so this percentage was calculated out of 13.

Table 4.4 Predictive Models of Influenza Severity among Hospitalized Adults Treated with Antivirals

ICU Admission (OR, 95% CI)		**Univariate Associations**	**Multivariable Model**	**IPW Model**
	Admitted to ICU (N, %)(N=7)			
BMI<30 (N=24)	2 (8.3%)	1.0	1.0	1.0
30≤BMI<40 (N=19)	3 (13.8%)	1.9 (0.3, 12.6)	2.9 (0.5, 20.9)	2.5 (0.4, 17.0)
BMI≥40 (N=5)	2 (11.6%)	6.4 (0.8, 58.2)	7.9 (0.9, 87.1)	7.8 (0.8, 88.9)
Late Antiviral Treatment (N=27)	6 (22.2%)	4.13 (0.8, 42.2)	5.1 (0.9, 53.4)	5.2 (0.95, 54.3)
Lower Respiratory Tract Disease (OR, 95% CI)				
	Lower Respiratory Tract Disease (N, %) (N=17)			
BMI<30 (N=24)	7 (41.7%)	1.0	1.0	1.0
30≤BMI<40 (N=19)	7 (36.8%)	1.4 (0.4, 5.0)	2.1 (0.5, 8.6)	3.0 (0.8, 12.3)
BMI≥40 (N=5)	3 (60.0%)	3.3 (0.5, 23.5)	4.0 (0.6, 35.0)	4.0 (0.5, 37.5)
Late Antiviral Treatment (N=27)	13 (48.1%)	3.6 (1.1, 14.2)	4.2 (1.2, 17.7)	3.9 (1.1, 15.9)
Length of Stay (Percent Change, 95% CI)	**Length of Stay (Median, Range) (5, 1-17)**			
BMI<30 (N=24)	5 (1-17)	0.0	0.0	0.0
30≤BMI<40 (N=19)	4 (1-8)	-29.2 (-49.6, -0.6)	-24.1 (-46.0, 6.7)	-26.8 (-47.5, 2.1)
BMI≥40 (N=5)	5 (2-8)	-13.6(-49.8, 48.7)	-12.0 (-48.3, 49.3)	-11.3 (-49.3, 55.3)
Late Antiviral Treatment (N=27)	5 (1-17)	40.8 (2.6, 93.2)	32.5 (-4.1, 83.2)	37.2 (-.3, 88.9)

Univariate associations model displays Firth corrected univariate associations
Multivariable model displays Firth corrected associations adjusted for BMI and late antiviral treatment (treatment >2 days from symptom onset compared to treatment ≤2 days from onset)
IPW model displays inverse probability weighted models adjusted for age, diabetes and poor/fair self-rated health
Length of stay models were modeled as log(length of stay); results were transformed to display percent changes.

Chapter 5 Severe Morbidity Among Hospitalized Adults with Acute Influenza and Other Respiratory Infections; 2014-15 and 2015-16[4]

5.1 Author Summary

During the 2009 influenza A(H1N1)pdm09, young adults and morbidly obese patients were at increased risk for severe influenza. During the post-pandemic period, it is important to understand whether these risk factors persist, and whether they are only associated with influenza A(H1N1)pdm09 infection, or also influenza A(H3N2) infection. Furthermore, in order to support recommendation for universal vaccination as well as neuraminidase inhibitor administration for hospitalized patients with suspected influenza, it is important to establish the effectiveness of these interventions at prevention of influenza severity. We found that frail patients and patients who did not visit the doctor in the past year were most at risk for severe influenza outcomes. We also found that neuraminidase inhibitor administration reduced hospital length of stay only among vaccinated patients, suggesting either that vaccination and antiviral treatment interact in their reduction of influenza severity, or that there are unexplained differences in vaccinated and unvaccinated patients in our study.

5.2 Abstract

Our objective was to identify predictors of severe acute respiratory infection in hospitalized patients and understand the impact of vaccination and neuraminidase inhibitor

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administration on severe influenza. We analyzed data from a study evaluating influenza vaccine effectiveness in two Michigan hospitals during the 2014-2015 and 2015-2016 influenza seasons. Adults admitted to the hospital with an acute respiratory infection were eligible. Through patient interview and medical record review, we evaluated potential risk factors for severe disease, defined as ICU admission, 30-day readmission, and hospital length of stay. Two hundred sixteen of 1119 participants had PCR-confirmed influenza. Frailty score, Charlson score, and tertile of prior-year health care visits were associated with length of stay. Charlson score >2 (OR:1.5[1.0, 2.3]) was associated with ICU admission. Highest tertile of prior-year visits (OR:0.3[0.2, 0.7]) was associated with decreased ICU admission. Increasing tertile of visits (OR: 1.5[1.2, 1.8]) was associated with 30-day readmission. Frailty and prior-year health care visits were associated with 30-day readmission among influenza-positive participants. Neuraminidase inhibitors were associated with decreased length of stay among vaccinated participants with influenzaA (HR:1.6 [1.0, 2.4]). Overall, frailty and lack of prior-year health care visits were predictors of disease severity. Neuraminidase inhibitors were associated with reduced severity among vaccine recipients.

5.3 Introduction

It is widely recognized that seasonal respiratory illness, which peaks in fall and winter in temperate regions, is associated with corresponding peaks in doctor's office visits and hospital admissions [146,147]. Numerous respiratory pathogens are associated with hospitalization; notably, influenza, human metapneumovirus, respiratory syncytial virus, rhinovirus, and parainfluenza virus; all of which cause similar symptoms [6]. However, influenza-associated illness accounts for a substantial proportion of these medical events [147,148]. Influenza is a viral pathogen that causes an estimated 12,000 to 56,000 deaths in the United States annually [149]. Influenza-

related severe outcomes, such as death, ICU admission, or the need for invasive mechanical ventilation, generally occur in elderly individuals or individuals with numerous comorbidities; however, previously healthy adults are also at risk for serious illness [150,151].

During the 2009 influenza A(H1N1) pandemic, individuals thought to be at low risk for severe influenza, such as those under the age of 65 and without recognized underlying conditions, were hospitalized at a higher than expected rate [49]. During the pandemic, previously unknown risk factors for influenza severity were identified with morbid obesity being one of the most consistently identified factors [50,51]. In post-pandemic seasons the age of those hospitalized for influenza A(H1N1)pdm09 infection increased along with an increase in the severity of influenza-related pneumonia [53–55]. There was, paradoxically, a corresponding decrease in the use of antiviral treatment initially, though rates of treatment have since risen [55,61]. With the continued circulation of the A(H1N1) pandemic strain along with A(H3N2) and B viruses it is critical to identify and monitor groups at risk for severe disease in order to optimize strategies, including use of neuraminidase inhibitors and vaccine prioritization when the vaccine supply is limited, to prevent adverse outcomes.

In order to identify predictors of influenza and acute respiratory illness (ARI) severity and, specifically, to understand the impact of vaccination and neuraminidase inhibitor administration on illness severity, we present data from adults hospitalized with ARI from two hospitals in Southeast Michigan over the 2014-2015 and 2015-2016 influenza seasons. Severe outcomes evaluated include ICU admission, length of stay (LOS), and 30-day readmission.

5.4 Methods

5.4.1 Participant Enrollment, Interview and Specimen Collection

Participants were adults hospitalized for ARI at University of Michigan Hospital (UMH, Hospital A) in Ann Arbor, Michigan and Henry Ford Hospital (HFH, Hospital B) in Detroit. Enrollment occurred from November 5th 2014 to March 6th 2015, and from January 11th 2016 to April 15th 2016. Staff reviewed electronic medical records (EMRs) daily to identify newly admitted patients (≤72 hours) with ARI as previously described [[152]]. Eligible participants were approached, and they or their proxy provided written consent for participation in the study. All study procedures were approved by the Institutional Review Boards of the University of Michigan Medical School and the Henry Ford Health System.

Patients were interviewed at enrollment to collect information about demographics, influenza vaccination status, general health status, illness characteristics, and subjective assessment of frailty (unexplained >10 pounds weight loss [yes/no], little energy for desired activities [yes/no], difficulty walking 100 yards [no difficulty…unable to do], difficulty carrying 10 pounds [no difficulty…unable to do] and frequency of low/moderate activity [more than once/week…hardly ever/never]). Number of health care encounters in the past year and evidence of neuraminidase inhibitor prescription from the study hospital admission were extracted from EMRs. Information about comorbid health conditions were also extracted to calculate the Charlson Comorbidity Index (CCI) for each patient. The following outcome variables were collected from the EMR: death, ICU admission, ventilator use, length of stay, and 30-day readmission. Outcomes that were experienced by more than 10 influenza-positive participants, including ICU admission, length of stay, and 30-day readmission, were used in models.

5.4.2 Laboratory Methods

Nasal and throat swabs collected at enrollment were combined and tested for influenza viruses using reverse transcriptase polymerase chain reaction (RT-PCR). All primers, probes and protocols were developed and provided by the Influenza Division of the CDC. They were designed for detection of universal influenza A and B, and for subtype and lineage identification. All tests were performed in the investigators' laboratory at the University of Michigan School of Public Health.

5.4.3 Influenza Vaccination Status

Individuals were considered vaccinated if they had documentation or plausible self-report of influenza vaccine receipt ≥14 days before illness onset. Documented vaccination status was determined based on documentation from the EMR or state immunization registry. Plausible self-report was defined as reporting both the approximate date and location of vaccination. Individuals were considered unvaccinated if they had no evidence of documentation of vaccination and self-reported no vaccination. Participants were excluded if they had an incomplete self-report of vaccination (e.g. missing date or location) and no additional documentation or if they were vaccinated <14 days before illness onset.

5.4.4 Statistical Methods

CCI scores were categorized as 0, 1, 2, or 3 or greater; high CCI was defined as greater than 2. Frailty was defined as the presence of up to 5 dichotomized variables taken from the enrollment interview that were summed and weighted by the number of questions answered, as a few participants either refused to answer or answered "don't know" to either one or two of the frailty questions [152,153]. Total prior-year health care visits were defined as all inpatient and outpatient visits for any reason to a UM or HF Health System affiliated clinic in the previous

year. Tertiles of prior year health care visits among all participants were calculated, and the variable was expressed as either 0 visits, or visits falling into the first (1-8 visits), second (9-21 visits), or third (≥22 visits) tertile. Long length of stay was defined as length of stay of >8 days. When used as a continuous outcome, LOS was log-transformed and beta coefficients were analyzed as percent change of LOS.

Participants were compared in frequency models using Pearson χ^2 test or Fisher's exact test. Firth's penalized logistic regression models were used to predict the odds of severe illness by various risk factors. Firth's method was used to reduce small-sample bias and improve model fit in the context of quasi-separation. Hospital site (UMH or HFH), sex, age (18-49, 50-64, 65+), frailty score, and CCI>2 were included in adjusted models a priori. Tertile of prior-year health care visits was included based on their significance in univariate models; this variable was modeled categorically for the outcomes of ICU admission and hospital length of stay and ordinally for 30-day readmission due to the monotonic relationship between these variables. For analyses restricted to influenza A positive individuals, influenza A subtype, influenza vaccination were included as adjustment factors. Cox proportional hazard models, censoring on death, were used to estimate the impact of antiviral treatment on hospital length of stay. Neuraminidase inhibitor administration was modeled as a time varying covariate indicating the day in the hospital admission when participants were treated. The models were adjusted for covariates associated with increased hospital length of stay in the risk factor analysis, weighted frailty score and tertile of prior-year health care visits. All statistics were completed using SAS (release 9.4, SAS Institute). Statistical significance was defined as a 95% confidence interval that did not include the null value.

5.5 Results

5.5.1 Demographics and Outcomes by Influenza Status

We enrolled 1199 adults with ARI; 727 from the 2014-2015 season and 472 from the 2015-2016 season. Eighty (7%) hospitalizations were excluded due to missing or incomplete information on vaccination status, influenza status, or Charlson score, leaving 1119 participants in the analysis.

Two-hundred sixteen (19%) participants had PCR-confirmed influenza virus infection. Influenza-positive participants were significantly less likely to have received influenza vaccines (Table 5.1). Half of participants had a CCI >2 but this percentage was significantly lower in individuals with influenza (41.2%) compared to those testing negative (52.2%). Among influenza positive participants there were 2 deaths, 22 ICU admissions, 10 invasive ventilations and 19 instances of long LOS (>8 days); these outcomes were observed in similar frequencies between the influenza positive and negative populations. Thirty-day readmission was significantly less frequent among influenza-positive participants compared to those testing negative (Table 5.1).

One-hundred and eleven participants were infected with influenza A(H3N2) viruses, 90 with influenza A(H1N1)pdm2009 and 15 with influenza B viruses; models restricted to influenza-positive individuals excluded individuals with influenza B virus infection. There was a higher frequency of influenza A(H1N1)pdm09 infection among participants who were 18-49 years old (37% with H1N1 vs. 26% with H3N2, p=0.10), though this difference was not statistically significant (Table 5.2). CCI (p=0.02), tertile of prior year health care visits (p=0.05) and vaccination status (p=0.02) were associated with influenza A subtype; individuals with a CCI of 0, no health care visits in the prior year and who were unvaccinated were more frequently

infected with influenza A(H1N1)pdm09 (Table 5.2). A higher percentage of participants infected with H1N1 were admitted to the ICU, put on an invasive ventilator, and had LOS >8 days compared to those infected with H3N2 (Table 5.2).

5.5.2 Models Prediction Severe ARI and Influenza-associated ARI

Higher frailty and increased tertile of prior-year health care visits were associated with increased 30-day readmission among influenza-positive participants (Table 5.3). Individuals with the highest tertile of prior-year health care visits had decreased odds of ICU admission compared to those with no prior-year visits regardless of influenza status (Table 5.3). Frailty score was associated with longer LOS among all participants but not among participants with influenza-associated ARI (Table 5.3).

5.5.3 Neuraminidase Inhibitor Prescription

One hundred fourty-seven (68%) influenza-positive participants were treated with neuraminidase inhibitors. Treatment varied by enrollment hospital; over 75% of influenza-positive patients from Hospital A were treated compared to only 57% from Hospital B (p=0.01) (Table 5.4). Neuraminidase inhibitor administration also varied by time from illness onset to admission; 73% of participants admitted within two days were treated compared to 59% of those admitted later (p=0.02) (Data not shown). Median length of stay was lower among those with timely antiviral treatment (2.0 days) compared to those with late antiviral treatment (3.0 days) or no treatment (3.0), however the median length of stay did not vary significantly.

Clinical testing for influenza varied significantly by enrollment hospital, 74% of influenza-positive participants from Hospital B by research testing received a clinical influenza test compared to 90% from Hospital A. Only 10% of participants from either hospital without a clinically positive influenza test were treated with neuraminidase inhibitors (Data not shown).

The influenza-positive population was further stratified by vaccination status. Vaccinated individuals who were treated with neuraminidase inhibitors had a significantly reduced LOS ($HR_{discharge}$:1.6, 95% CI: 1.0, 2.4], p=0.04) compared to those who were untreated (Table 5.5). Other severe outcomes were not evaluated in this analysis due to insufficient sample size.

5.6 Discussion

Our study identified risk factors for severe influenza-associated ARI and all-cause ARI among hospitalized patients over two influenza seasons. Given that viral etiology is often unknown at admission when many treatment decisions are made, it is important to understand severity of ARI of all causes in the hospital. Of note, 65% of participants were tested clinically for influenza and the majority of these tests were initiated the day of or the day after hospital admission. Despite the timely testing, it may take many hours for PCR results to be available to the clinician and rapid influenza tests are known for their low specificity. For these reasons, treatment decisions should be made before viral etiology is known in most cases. Higher frailty score was associated with longer LOS, and having 0 prior-year health care visits was associated with higher odds of ICU admission. Frailty is a well-known predictor of severity and death, especially among the elderly, though many studies do not consider frailty when studying influenza severity [154–156]. The increased severity among those without prior-year health care visits may indicate that individuals who are unlikely to seek care present to the hospital with the most severe illnesses. Increased health care visits over the prior year were also associated with increased, rather than reduced, 30-day readmission indicating that 30-day readmission may be, in part, a measure of underlying chronic conditions [157].

We evaluated the impact of vaccination and neuraminidase inhibitor administration on influenza severity. Neuraminidase inhibitors were significantly associated with decreased LOS

among vaccinated individuals only after stratification by vaccination status. While the association between neuraminidase inhibitor administration and reduced influenza severity has been emphasized, the interaction between vaccination and neuraminidase inhibitors is not well documented or understood [134,158]. Though this result offers an interesting potential relationship between antiviral treatment, vaccination, and influenza severity, the extremely small sample size in this stratified population necessitates repeated demonstration of this association in larger, future studies.

In light of this result and other evidence in the literature, it is critical that hospitalized influenza-positive patients are treated with neuraminidase inhibitors [68,134]. We found that just 67% of participants with PCR-confirmed influenza were prescribed neuraminidase inhibitors though treatment is recommended for all hospitalized patients with suspected or confirmed influenza. Treatment varied significantly by enrollment hospital; over 40% of influenza-positive participants at Hospital B did not receive neuraminidase inhibitors, compared to 23% at Hospital A. While all participants are tested for influenza by our research team, not every patient receives a clinical influenza test during their hospital stay. This appeared to impact treatment decisions, as very few individuals without a clinically positive influenza test were treated despite the recommendation that hospitalized individuals with suspected influenza be treated empirically. These numbers indicate a need to continue public health messaging directed at nurses and physicians to encourage empiric treatment and to keep influenza on the list of possible diagnoses during influenza season. Additionally, participants were less likely to be treated if they were admitted to the hospital >2 days after symptom onset. This reflects the widely held opinion that antiviral drugs are only effective within 2 days of symptom onset. While studies have shown that effectiveness is higher when neuraminidase inhibitors are given promptly, there is evidence

among hospitalized patients with influenza that treatment within 5 days of symptom onset improves survival [65,134,158].

Continued interest in the potential for vaccination to reduce influenza severity stems from the vaccine effectiveness (VE) estimates from the 2014-15 influenza season, which primarily consisted of influenza A viruses that were antigenically drifted from the Northern Hemisphere vaccine strains [152,159]. VE estimates from the 2014-15 season were higher in hospital studies than in ambulatory care studies, where they were not significantly different from zero [111,152,160]. This could indicate that influenza vaccination reduces severity as well as incidence; this hypothesis has been previously evaluated but results are mixed [28,29,56,57]. We did not find an association between severity and vaccination. Observational studies of severity, such as ours, as well as evaluations of interventions such as vaccination are often impacted by confounding by indication and other challenges.

Overall, the small number of influenza-positive participants in this study led to reduced power, which may explain the few significant predictors of influenza severity. The in-hospital observational nature of the study complicated our ability to study some commonly used severity endpoints such as mechanical ventilation and death. Additionally, selection into this study depended on hospital admission prior to enrollment, potentially increasing the number of older individuals with comorbidities who are more likely to be admitted to the hospital with a less severe disease. We accounted for this in our analysis by adjusting for age, CCI, and prior-year health care visits, but residual confounding is always a concern. In addition, when calculating the tertile of prior-year health care visits, we could only access visits within the hospital study sites or their associated outpatient clinics, and the majority of individuals who had no visits did not get their regular care within these two systems. However, when the population was restricted to

those who did get regular care at our study sites in a sensitivity analysis, the trends of increased severity among those with no prior-year visits remained.

In conclusion, we identified frailty and number of prior-year health care visits as predictors of all-cause and influenza-associated ARI severity. Our finding that vaccinated patients who received neuraminidase inhibitors had decreased LOS needs confirmation from future studies, but also adds to the evidence that administration of neuraminidase inhibitors to hospitalized patients reduces influenza severity and reinforces current treatment recommendations in the hospital [[12,68,135,161]].

Table 5.1 Demographics and Outcomes of Hospitalized Adults with ARI by Influenza Status

	Total N=1119	**Influenza Positive N=216**	**Influenza Negative N=903**	**P Value[3]**
Characteristics	N (Column %)	N (Column %)	N (Column %)	
Sex				0.68
Male	501 (44.7%)	94 (43.5%)	407 (45.1%)	
Female	618(54.8%)	122 (56.5%)	496 (54.9%)	
Age				0.44
18-49	323 (28.9%)	67 (31.0%)	256 (28.3%)	
50-64	415 (37.1%)	72 (33.3%)	343 (38.0%)	
≥65	381 (34.0%)	77 (35.7%)	304 (33.7%)	
Race[1]				0.62
White (Non-Hispanic)	583 (52.7%)	114 (54.0%)	469 (52.3%)	
Black (Non-Hispanic)	392 (35.4%)	76 (36.0%)	316 (35.3%)	
Other	132 (11.9%)	21 (10.0%)	111 (12.4%)	
Site of Enrollment				0.47
Hospital A	636 (56.8%)	118 (54.6%)	518 (57.4%)	
Hospital B	483 (43.2%)	98 (45.4%)	385 (42.6%)	
Year				0.12
2014-2015	664 (59.3%)	118 (54.6%)	546 (60.5%)	
2015-2016	455 (40.7%)	98 (45.4%)	357 (39.5%)	
Charlson Score				0.01
0	119 (10.6%)	33 (15.3%)	86 (9.5%)	
1	283 (25.3%)	62 (28.7%)	221 (24.5%)	
2	157 (14.0%)	32 (14.8%)	125 (13.8%)	
≥3	560 (50.0%)	89 (41.2%)	471 (52.2%)	
Frailty Score (median(IQR))	0.25 (0.0,0.40)	0.25 (0.0-0.50)	0.40 (0.20-0.60)	0.04
BMI Category[2]				0.51
<18.5	42 (3.9%)	4 (1.9%)	38 (4.3%)	
18.5-24.9	267 (24.7%)	53 (25.7%)	214 (24.5%)	
25-29.9	284 (26.3%)	53 (25.7%)	231 (26.4%)	
30-39.9	315 (29.1%)	65 (31.6%)	250 (28.6%)	
≥40	173 (16.0%)	31 (15.1%)	142 (16.2%)	
Number of Healthcare Visits (Tertiles)				0.61
0	128 (11.4%)	28 (13.0%)	100 (11.1%)	
1	349 (31.2%)	72 (33.3%)	277 (30.7%)	
2	318 (28.4%)	60 (27.8%)	258 (28.6%)	
3	324 (29.0%)	56 (25.9%)	268 (29.7%)	
Vaccination Status				<0.01
Vaccinated	750 (67.0%)	113 (52.3%)	637 (70.5%)	
Unvaccinated	369 (33.0%)	103 (47.7%)	266 (29.5%)	
Death	15 (1.3%)	2 (1.0%)	13 (1.4%)	0.56
ICU	126 (11.3%)	22 (10.2%)	104 (11.5%)	0.58
Invasive Ventilator	48 (4.3%)	10 (4.6%)	38 (4.2%)	0.78
LOS >8 Days	108 (9.7%)	19 (8.8%)	89 (9.9%)	0.63
30 day Readmission	167 (14.9%)	16 (7.4%)	151 (16.7%)	<0.01

[1]12 individuals have missing Race information
[2]38 individuals have missing BMI information
[3]P values are from chi square tests or Fisher's exact tests when appropriate

Table 5.2 Demographics and Outcomes of Enrolled Patients Hospitalized with Influenza A Associated ARI by Subtype

	Total N=201	**H3N2 N=111**	**H1N1 N=90**	
Characteristics	N (Column %)	N (Column %)	N (Column %)	P Value[3]
Sex				0.20
Male	90 (44.8%)	43 (48.7%)	43 (47.8%)	
Female	111 (55.2%)	68 (61.3%)	47 (52.2%)	
Age				0.10
18-49 y	62 (30.8%)	29 (26.1%)	33 (36.7%)	
50-64 y	68 (33.8%)	36 (32.4%)	32 (35.6%)	
≥65 y	71 (35.3%)	46 (41.4%)	25 (35.2%)	
Race[1]				0.22
White (Not Hispanic)	106 (53.8%)	63 (57.3%)	43 (49.4%)	
Black	71 (36.0%)	34 (30.9%)	37 (42.5%)	
Other	20 (10.1%)	13 (11.8%)	7 (8.0%)	
Site of Enrollment				0.29
Hospital A	111 (55.2%)	65 (58.6%)	46 (51.1%)	
Hospital B	90 (44.8%)	46 (41.4%)	44 (48.9%)	
Charlson Score				0.02
0	31 (15.4%)	10 (9.0%)	21 (23.3%)	
1	59 (29.3%)	39 (35.1%)	20 (22.2%)	
2	27 (13.4%)	13 (11.7%)	14 (15.6%)	
≥3	84 (41.8%)	49 (44.1%)	35 (38.9%)	
Frailty Score (median(IQR))	0.25 (0.0,0.40)	0.20 (0.0-0.5)	0.40 (0.0-0.40)	0.89
BMI Category[2]				0.11
<18.5	4 (1.7%)	4 (3.6%)	0 (0.0%)	
18.5-24.9	47 (24.0%)	24 (21.8%)	23 (28.4%)	
25-29.9	49 (25.1%)	33 (30.0%)	16 (19.7%)	
≥30	91 (49.2%)	49 (44.6%)	42 (51.9%)	
Year				<0.01
2014-15	107 (53.2%)	107 (96.4%)	0 (0.0%)	
2015-16	94 (46.8%)	4 (3.6%)	90 (100.0%)	
Total Number of Healthcare Visits In the Last Year (Tertiles)				0.05
0	25 (12.4%)	8 (7.2%)	17 (18.9%)	
1	64 (31.8%)	34 (30.6%)	30 (33.3%)	
2	58 (28.9%)	34 (30.6%)	24 (26.7%)	
3	54 (26.9%)	35 (31.5%)	19 (21.1%)	
Vaccination Status				0.02
Vaccinated	106 (52.7%)	67 (60.4%)	39 (43.3%)	
Unvaccinated	95 (46.1%)	44(39.6%)	51 (56.7%)	
Death	2 (1.0%)	1 (0.9%)	1 (1.1%)	1.00
ICU	20 (10.0%)	7 (6.3%)	13 (14.4%)	0.06
Invasive Ventilator	9 (4.5%)	1 (0.9%)	8 (8.9%)	0.01
LOS >8 Days	18 (9.0%)	5 (4.5%)	13 (14.4%)	0.02
30 day Readmission	16 (8.0%)	10 (9.0%)	6 (6.7%)	0.61

[1]4 individuals are missing race information
[2]**10 individuals are missing BMI information**
[3]**P values reflect results of Pearson Chi-square tests or Fisher's exact test when appropriate. P values for continuous variables represent results of Wilcoxon tests**

Table 5.3 Predictors of Severe Disease in Participants with All-Cause ARI and Influenza A Associated ARI

	ARI[1] (N=1072)			Influenza A Positive (N=188)		
	ICU (OR, 95% CI)	**LOS (Percent Change, 95% CI)**	**30 Day Readmission (OR, 95% CI)**	**ICU (OR, 95% CI)**	**LOS (Percent Change, 95% CI)**	**30 Day Readmission (OR, 95% CI)**
Predictors						
Male Sex	1.5 (1.0, 2.2)	6.1 (-0.5, 13.1)	1.1 (0.8, 1.6)	0.7 (0.3, 1.8)	-5.0(-19.2, 11.7)	0.4 (0.1, 1.4)
Age						
18-49	1.0	0.0	1.0	1.0	0.0	1.0
50-64	1.0 (0.6, 1.6)	3.6 (-4.4, 12.3)	0.9 (0.6, 1.3)	1.1 (0.3, 3.6)	3.2 (-16.1, 26.9)	1.5 (0.3, 7.7)
≥65	1.0 (0.6, 1.6)	0.4 (-7.7, 9.1)	0.6 (0.4, 1.0)	1.0 (0.3, 3.4)	6.6 (-13.8, 31.9)	1.2 (0.3, 6.2)
Site of Enrollment						
Hospital A	1.0	0.0	1.0	1.0	0.0	1.0
Hospital B	0.8 (0.5, 1.2)	-0.7 (-7.3, 6.2)	1.4 (1.0, 2.1)	0.7 (0.2, 2.0)	-8.1 (-23.2, 9.9)	2.7 (0.9, 9.0)
Charlson Score >2	1.5 (1.0, 2.3)	21.7 (13.3, 30.7)	1.8 (1.2, 2.7)*	1.6 (0.5, 5.7)	8.5 (-11.0, 32.2)	1.1 (0.3, 4.2)
Vaccination	-	-	-	1.0 (0.3, 3.1)	-6.1 (-22.4, 13.6)	0.9 (0.3, 3.5)
Frailty Score[2]	1.5 (0.8, 3.0)	22.7 (9.3, 37.5)*	1.4 (0.8, 2.5)	1.0 (0.1, 6.1)	31.4 (-4.5, 80.9)	8.9 (1.2, 78.0)*
Total Visits[3] (Tertiles)			1.5 (1.2, 1.8)*			2.5 (1.2, 5.8)*
0	1.0	0.0		1.0	0.0	
1	0.6 (0.4, 1.1)	-18.8 (-21.1, -1.7)*		0.3 (0.1, 1.0)*	-11.7 (-33.1, 16.6)	
2	0.6 (0.3, 1.0)	-9.3 (-18.9, 1.6)		0.2 (0.0, 0.8)*	-4.2 (-28.3, 28.0)	
3	0.3 (0.2, 0.7)*	-11.2 (-21.3, 0.2)		0.1 (0.0, 0.8)*	-10.0 (-35.1, 24.9)	
Influenza A Subtype						
H3N2	-	-	-	1.0	0.0	1.0
H1N1	-	-	-	1.9 (0.7, 5.2)	11.1 (-5.7, 30.9)	0.8 (0.3, 2.4)

[1]**Adjusted models contain male sex, age group, enrollment site, Charlson score, weighted frailty score, total annual healthcare visits, and influenza status. Influenza A subtype and vaccination were also included in models restricted to influenza A positive adults.**
[2]**OR and percent changes reflect the impact of a one-unit increase in weighted frailty score.**
[3]**Total number of annual healthcare visits is modeled categorically except in models predicting 30-day readmission where it is modeled ordinally and OR represent change in odds for a one tertile increase**
***indicates significance at the 5% confidence level**

Table 5.4 Demographics by Antiviral Prescription Timing Among Participants with Laboratory Confirmed Influenza

	Timely Antivirals[1] N=86	Late Antivirals N=61	No Antivirals N=69	P Value[4]
Characteristics	N (Row %)	N (Row %)	N (Row %)	
Sex				0.75
Male	40 (42.6%)	26 (27.7%)	28 (29.8%)	
Female	46 (37.7%)	35 (28.7%)	41 (33.6%)	
Age				0.31
18-49	31 (46.3%)	21 (31.3%)	15 (22.4%)	
50-64	27 (37.5%)	21 (29.2%)	24 (33.3%)	
≥65	28 (36.4%)	19 (24.7%)	30 (39.0%)	
Race[2]				0.21
White	51 (44.7%)	32 (28.1%)	31 (27.2%)	
Black	30 (39.5%)	19 (25.0%)	27 (35.5%)	
Other	4 (19.0%)	8 (38.1%)	9 (42.9%)	
Site of Enrollment				0.01
Hospital A	54 (45.8%)	37 (31.4%)	27 (22.9%)	
Hospital B	32 (32.6%)	24 (24.5%)	42 (42.9%)	
Year				0.24
2014-2015	42 (35.6%)	33 (28.0%)	43 (36.4%)	
2015-2016	44 (44.9%)	28 (28.6%)	26 (26.5%)	
Influenza Type/Subtype				0.23
A/H3N2	37 (33.3%)	35 (31.5%)	39 (35.1%)	
A/H1N1	42 (46.7%)	24 (26.7%)	24 (26.7%)	
B	7 (46.7%)	2 (13.3%)	6 (40.0%)	
Charlson Score				0.36
0	17 (51.5%)	8 (24.2%)	8 (24.2%)	
1	17 (27.4%)	21 (33.9%)	24 (38.7%)	
2	14 (43.7%)	9 (28.1%)	9 (28.1%)	
≥3	38 (42.7%)	23 (25.8%)	28 (31.5%)	
Frailty Score	0.20 (0.0-0.40)	0.40 (0.20-0.60)	0.40 (0.20-0.60)	0.20
Obese[3]				0.22
Yes	39 (40.6%)	31 (32.3%)	26 (27.1%)	
No	43 (39.1%)	26 (23.6%)	41 (37.3%)	
Number of Health Care Visits (Tertiles)				0.52
0	11 (39.3%)	8 (28.6%)	9 (32.1%)	
1	28 (38.9%)	15 (20.8%)	29 (40.3%)	
2	23 (38.3%)	21 (35.0%)	16 (26.7%)	
3	24 (42.9%)	17 (30.4%)	15 (26.8%)	
Vaccination Status				0.85
Yes	46 (40.7%)	30 (26.6%)	37 (32.7%)	
No	40 (38.8%)	31 (30.1%)	32 (31.1%)	
Length of Stay (median, IQR)	2.0 (2.0-4.0)	3.0 (2.0-5.0)	3.0 (2.0-5.0)	0.17

[1]Timely antivirals refers to antivirals within 2 days of symptom onset
[2]5 individuals are missing race information
[3]10 individuals are missing BMI information
[4]P values are from chi square tests or Fisher's exact tests when appropriate

Table 5.5 Hazards of Discharge Related to Antiviral Treatment Timing

	Overall (N=201)		Vaccinated (N=106)		Unvaccinated (N=95)	
Predictors	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Antiviral Treatment	1.1 (0.8, 1.5)	0.44	1.6 (1.0, 2.4)	0.04	0.9 (0.5, 1.4)	0.52
Frailty Score	0.5 (0.3, 1.0)	0.04	0.6 (0.3, 1.3)	0.17	0.5 (0.2, 1.2)	0.11
Total Visits (Tertiles)						
Tertile 0	ref		Ref		ref	
Tertile 1	1.6 (1.0, 2.7)	0.05	0.5 (0.1, 1.6)	0.23	1.6 (0.9, 2.9)	0.09
Tertile 2	1.3 (0.8, 2.1)	0.32	0.3 (0.1, 1.0)	0.05	1.3 (0.7, 2.4)	0.40
Tertile 3	1.5 (0.9, 2.4)	0.13	0.4 (0.2, 1.2)	0.09	1.6 (0.8, 3.5)	0.21
[1]Models contain all predictors in the table						

Chapter 6 Summary and Conclusions

6.1 Summary of Findings

Overall, this dissertation examined two topics, the effectiveness of the influenza vaccine against hospitalization and the impact of influenza vaccination and neuraminidase inhibition on severe influenza outcomes. VE against hospitalization was explored in two ways, first influenza VE against hospitalization was studied in an understudied group, hospitalized children in Israel in chapter 2. Secondly, in chapter three, we examined a potential bias of VE studies in the hospital setting. We used alternate control groups to determine whether inclusion of individuals without a true ARI caused bias in VE estimates against hospitalization. In chapters 4 and 5 we examined predictors and prevention of influenza severity. In chapter 4 we evaluated whether obesity was a predictor of severe influenza among hospitalized adults in Detroit, and determined whether antiviral treatment practices differed for obese versus non-obese patients. Finally, in chapter 5, we examined predictors of influenza severity among hospitalized adults in one A(H1N1)pdm09 dominated season and one A(H3N2) dominated season and evaluated the ability of influenza vaccination and treatment with neuraminidase inhibitors to reduce severe ARI and influenza. The results of these studies are described below, with emphasis on their implications, strengths and limitations.

6.1.1 Aim 1

Influenza VE is frequently estimated against medically attended illness in ambulatory care centers and hospitals in many countries across the world[30,107,108,162]. In the Middle East there

are very few estimations of VE published, and VE against hospitalization has not previously been calculated. In addition, it is recommended that children under the age of 9 receive two influenza vaccines in the first season that they are vaccinated, however few studies have evaluated the impact of full vs. partial vaccination in the hospital[13,14,37]. Understanding the effectiveness of the vaccine in a setting where vaccination rates are relatively low but the vaccine is universally recommended is important for public health messaging to reinforce guidelines.

In the first analysis presented in this dissertation, we demonstrated that the influenza vaccine was effective against hospitalization in Israeli children over three influenza seasons. Consistently, influenza VE was much higher in children who were fully vaccinated, and VE was generally not significantly effective in partially vaccinated children. This result reinforces guidelines that recommend two influenza vaccines in the first season of vaccination. Despite the recommendation by the Israeli Ministry of Health that all individuals over 6 months of age be vaccine annually, only 34% of children in our analysis received a current season influenza vaccine. Special attention is frequently paid to children with comorbid conditions, as they are often at high risk for severe influenza-related outcomes. However, in our study, children with comorbidities had similarly low rates of vaccination. Our results, which demonstrate the effectiveness of influenza vaccines against severe influenza in healthy children and children with comorbidities, reinforce guidelines from the Ministry of Health and could be used to demonstrate the importance of annual vaccination.

We used retrospective medical data from hospital records through Clalit Health Services, an Israeli provider and insurer, to calculate influenza VE. Influenza vaccines in Israel are only available at scheduled primary care visits and occasional government-sponsored school vaccination events in particular age groups. In addition, very few Israelis switch health care

insurers throughout their lifetime. For these reasons, the medical records used facilitated very detailed measurement of lifetime influenza vaccination. We were able to take advantage of this very accurate exposure information to not only have an systematic measure of current season vaccination, but also vaccination since birth, which allowed us to determine full versus partial vaccination status in a more accurate manner than previous analyses[42].

Overall, we showed high VE for both influenza A and B. VE for influenza A in particular was striking; VE was 81% in 2015-16, 71% in 2016-17, and 46% in 2017-18. These estimates were higher than the estimates produced for young children in Israeli outpatient studies in this season, perhaps suggesting the superiority of the vaccine at preventing severe disease[47,48]. Our population was very young, 61% of the children in our study and 45% of the influenza positive population were under 2 years of age. Pooled VE across the three seasons was nearly identical when the population was stratified by age (<2 and ≥2 years of age). However, it appeared that children under 2 had lower vaccine effectiveness against influenza B compared to children over the age of 2. Unfortunately the sample size did not allow for further investigation of this potential difference by age. In any case, the widely significant VE among children aged 6 months through 8 years, including children with comorbidities, provides strong reinforcement for influenza vaccination in Israeli children. These data can be used for public health messaging to improve vaccination rates across Israel.

6.1.2 Aim 2

The test negative design has been used to study influenza VE for many years, and this study design has been well-validated in the outpatient setting[20,26,27]. However, there have been few evaluations of the validity of this design among inpatients[43]. In particular, the high prevalence of comorbidities among inpatient adults with symptoms that mimic ARI symptoms is

of concern. If patients with these comorbidities are likely to be enrolled in a TND study without a true ARI, it could cause significant bias in the study, particularly because these patients with numerous comorbidities may be more likely to seek care for an illness and may be more likely to be vaccinated.

In Aim 2, we evaluated whether inclusion of patients without a respiratory virus was biasing VE estimates. Specifically, we tested all samples for the 2014-15 and 2015-16 seasons of our hospital based TND study, and used three control groups when calculating VE: all influenza negative (traditional control group), non-influenza other virus positive, and pan-negative. Our results did not show a consistent difference between influenza VE by control group, indicating that the inclusion of individuals without an ARI did not bias the VE estimates. However, we did see some differences, though confidence intervals were wide, between the other virus positive and influenza negative VE estimates in the 2014-15 season.

Numerous other studies have used other virus positive and pan-negative control groups to determine the validity of VE estimates[109,114,115]. However, very few of these studies have taken place in inpatient settings, where this type of bias is more likely to occur. A recent meta-analysis by Feng et al. on this topic indicated that the past literature, consisting primarily of studies from outpatient settings, had results consistent with those reported here, providing evidence that this bias is not occurring in TND studies[25]. Interestingly, the studies with the lowest VE had the most variation in VE estimates between control groups, similar to what we observed in the 2014-15 season.

The results of this analysis point to the strengths of the HAIVEN study. In this study, patients are enrolled according to a strict case definition based on patient symptoms recorded during a review of the admission note. The results of our analysis indicate that this strategy

allows us to correctly identify patients with an ARI without biasing our results. As many studies around the world use similar methods to identify potentially eligible patients, this result is encouraging as it indicates that the TND is a valid study design for use in the inpatient setting in addition to the outpatient setting. However, this validity is not ensured in studies that do not use a strict case definition to enroll patients.

6.1.3 Aim 3

During the 2009 influenza A(H1N1) pandemic, morbidly obese individuals were at high risk for severe influenza outcomes such as ICU admission, mechanical ventilation, and death[51,125,126]. As a result of this increased severity, obesity was added to the list of high risk conditions for influenza, indicating that obese patients should be treated with neuraminidase inhibitors for any suspected medically attended influenza illness[60]. In the post-pandemic period, it has been a priority to understand whether this risk factor for severe influenza is still relevant and whether it applies to both circulating influenza A strains, or only influenza A(H1N1)pdm09.

In Aim 3, we examined whether obesity was a risk factor for influenza severity in a prospective study of influenza-positive adults hospitalized in Detroit. Participants were interviewed in the hospital and then again 30 days after discharge to get details on their disease course as well as any positive health changes that they made post-hospitalization. This study took place during the 2011-12 influenza season, which was a very mild season. Due to the low number of influenza-positive patients identified, enrollment was extended into the 2012-13 season, though only 55 individuals were enrolled. This small sample was a challenge, but we used inverse probability weighted models to adjust for confounding without compromising power.

We did not find a significant association between obesity or morbid obesity and any of the tested metrics of influenza severity, though the extremely small sample size was likely a factor. All individuals enrolled in the study were treated with neuraminidase inhibitors, but obese patients were treated significantly sooner after hospital admission. While all individuals who are hospitalized with influenza are recommended to be treated with neuraminidase inhibitors empirically, it is possible that physicians were influenced by the inclusion of morbid obesity on the ACIP list of high risk conditions for influenza and treated these patients empirically while waiting for confirmation of illness for the other patients.

Late antiviral treatment (treatment >2 days post illness onset) was significantly associated with increased odds of lower pulmonary disease. This result, despite the small size, reinforce guidelines for empiric treatment at hospital admission and mirror results from numerous studies indicating that rapid treatment with neuraminidase inhibitors is more effective than late treatment[141,142].

Antiviral treatment timing was related to both obesity status and severe influenza outcomes, and is therefore a possible confounder between obesity and severe influenza. If obese patients are routinely treated with neuraminidase inhibitors more rapidly than other patients, the relationship between obesity and severe influenza could be masked by different treatment practices. In our study, the likelihood of severe disease associated with obesity increased after adjustment with antiviral treatment, though the sample size was not sufficient to evaluate this relationship further. Future studies examining the relationship between obesity and severe influenza should take antiviral treatment practices into account.

6.1.4 Aim 4

Individuals who are hospitalized for influenza are at risk for severe outcomes beyond hospitalization, such as mechanical ventilation, ICU admission and death. Understanding risk factors for severe influenza outcomes would allow us to identify individuals for rapid administrations of antivirals and prioritization of vaccination in the case of a pandemic. In seasons when the influenza vaccine is not effective, understanding the full protection that vaccination affords beyond prevention of illness is important to effectively prevent severe disease.

In Aim 4, we used the data from the Michigan site of the HAIVEN study from the 2014-15 and 2015-16 seasons to identify predictors of severe influenza and estimate the impact of vaccination and neuraminidase inhibition on severe outcomes. As the 2014-15 influenza season was dominated by influenza A(H3N2) circulation and the 2015-16 season was dominated by influenza A(H1N1)pdm09, we were able to compare differences between hospitalized patients with each type of influenza. As previously reported, individuals hospitalized with influenza A(H1N1)pdm09 were younger and were previously "healthier" (had lower Charlson score and less past year healthcare visits)[49]. A higher percentage of individuals with influenza A(H1N1)pdm09 were also admitted to the ICU, put on a mechanical ventilator, and had a long (>8 days) hospital length of stay. This could indicate that influenza A(H1N1)pdm09 led to more severe outcomes than influenza A(H3N2), or it could indicate that the patient infected with A(H1N1)pdm09, the "healthier" younger adults, do not present to the hospital unless they have a very severe illness.

In adjusted models, higher frailty score was associated with longer LOS, and having zero prior-year health care visits was associated with higher odds of ICU admission. Frailty is a well-

established predictor of influenza severity. The association between less prior-year healthcare visits and severity may support the A(H1N1)pdm09 results, that individuals who do not frequently seek care present to the hospital with a more severe disease.

We used Cox proportional hazard models to evaluate whether neuraminidase inhibitor administration is associated with increased hospital length of stay. We found that neuraminidase inhibitor administration significantly reduced hospital length of stay among influenza –positive patients who were vaccinated, but it did not impact length of stay among unvaccinated patients. This result could indicate synergy between the impact of vaccination and antiviral treatment on influenza severity. However, it is also possible that this result is related to difference in severity at hospital admission between vaccinated and unvaccinated individuals. If vaccinated individuals also happen to be individuals who frequently seek care and present to the hospital with less severe illness it may be easier to see the impact of antivirals in this group.

6.2 Strength and Limitations

The data used for the chapter 2 came from medical records from Clalit Health Services, the largest medical insurer and provider in Israel. These data contain very complete vaccination information. All Israelis are entitled to medical care, and very few Israelis change insurers throughout their lifetime, as all insurers are geographically dispersed across the country. For this reason, we had access to incredibly complete vaccination information from birth. As confirmation of vaccination is generally a very difficult task in VE studies in countries where there is not universal medical care, having such a detailed registry of vaccination history among hospitalized patients was very valuable. In addition, we are confident that exposure misclassification was a minimal problem in this analysis.

The data source for this study, however, is also a limitation. Currently, there is not a VE network in Israel enrolling hospitalized patients. Consequently, to complete this analysis, we used a retrospective review of hospital records. For this reason we were unable to employ a case definition and relied on physician driven testing to identify our study sample. It is possible that individuals who did not have an ARI were included in our sample. If more severely ill children with more comorbid conditions were more likely to be tested for influenza without ARI symptoms and also more likely to be vaccinated, this could have biased our study and inflated our VE results. However, our results were in line with those from other studies. In addition, while influenza positivity was low in our study, it was within rates that had been previously reported.

Our use of a retrospective medical record review was appropriate for generation of the first VE estimates against hospitalization from Israel. Our encouraging results, showing significant VE for fully vaccinated children over three seasons, may encourage others to invest in a hospital network study. While our methods were appropriate for an initial assessment of VE in the region, it would beneficial to have a prospectively enrolling TND study to confirm our results.

In chapter 3, our ability to test our specimens for a variety of pathogens from the same extracted material using a multiple PCR kit allowed us to ensure that our results were comparable throughout the entire study. With the multiplex kit, we were able to test for over 20 viral pathogens using the same extraction on the same PCR plate. This was an advantage, as it ensured that different run times or freeze thaw cycles did not make identification of certain specimens more likely within a sample.

On the other hand, the study design in which these specimens were collected was designed specifically for identification of influenza. Individuals were enrolled in the study up to ten days

post illness onset and nares swabs were used to collect the specimens. While this method is appropriate for identification of influenza, some other viral pathogens do not shed as long as influenza. In addition, viral material may be difficult to isolate in sufficient titers for identification without using a nasopharyngeal swab. If this is the case than there may have been participants who had a false negative swab for respiratory viruses and were included in the pan-negative group rather than the other virus positive group erroneously. Individuals could have also been "false positives". Some viruses are shed for a long period of time even without active illness. If a participant was admitted to the hospital with respiratory symptoms that were unrelated to the virus that they tested positive for, they could have been erroneously included in the other virus positive group when they should have been in the pan-negative group. As we showed, influenza vaccination was not related to the incidence of a non-influenza virus. This potential outcome misclassification is not likely to be differential to the exposure, however it still could impact our VE results.

The other major limitation of this analysis was the inability to definitively show lack of bias through the estimates produced. Having VE estimates from different control groups that are similar to one another is good evidence that control group selection is not biasing our study, however, because our study was not powered to detect these differences, simply not having statistically significantly different VE estimates does not ensure that there is no bias. However, if this bias were consistently impacting inpatient TND design studies, we would expect consistent differences between the other virus positive and influenza negative control groups. The VE estimates from the different control groups in 2015-16 were nearly identical, indicating that this bias was not a factor in this season. The differences in VE seen in the 2014-15 season are likely

due to random variation, but a more thorough statistical test in a larger population would be necessary to provide more thorough evidence.

The study design and modeling strategy used in chapter 4 allowed for some useful conclusions to be drawn despite a very small sample size. The prospective nature of this study was a major strength; influenza positive patients were enrolled in the study and given a day 1 survey and 71% of patients who gave a day 1 survey responded to a day 30 survey as well. This design allowed for the collection of high quality data, which is important with such few participants, as even a small amount of misclassification could have large impacts on the effect estimates.

Using inverse probability weighting of propensity scores to remove confounding allowed us to reduce bias in our estimates without overfitting our models. This strategy allowed us to remove some confounding, which would not have been possible using standard statistical methods due to the extremely small sample size. Despite our efforts, confounders were not perfectly balanced between our exposed and unexposed populations. We were unable to include steroid use in our weighted model, which was a potentially important confounder. Unfortunately, in the propensity score model predicting late antiviral treatment, steroid use was collinear with other confounders and its inclusion in the model negatively impacted model fit.

Due to the in-hospital nature of the study, we are unable to confirm that our outcomes, such as ICU admission and lower pulmonary disease, occurred before antiviral treatment. In particular, it is possible that participants who came to the hospital looking more ill with lung infiltrates or hypoxia could have been given antivirals soon after admission. In addition, patients admitted straight into the ICU may have been given antivirals upon arrival. This would bias our

associations to the null, making it less likely for us to see an effect of early antiviral treatment on severity.

In chapter 5, we used data from the HAIVEN study to understand predictors and prevention of influenza severity. Using data collected from a test-negative design study had benefits. These studies are routinely collected in many settings, so if this type of analysis can be successfully conducted using these data, it could allow for rapid understanding of the effectiveness of neuraminidase inhibitors or vaccination on preventing severe influenza outcomes in the case of a pandemic. In addition, nesting this study in a test negative design study means that we had access to very detailed information about hospital course and patient characteristics. In addition to gaining access to each patient's medical record to collect information about comorbidities and disease course in the hospital, we also interviewed each patient upon enrollment. Detailed information on vaccination was collected, as this the main exposure in influenza VE studies. Having this high quality information on each patient allowed us to evaluate a variety of potential predictors for influenza severity as well as multiple outcomes including ICU admission, 30-day readmission, and hospital length of stay.

Our relatively small number of influenza positive participants limited our ability to evaluate all outcomes when stratifying by vaccination status. Consequently, we were only able to use our continuous outcomes, length of hospital stay. If individuals with serious comorbidities have extended hospitalizations due to management of their comorbidity only, then this outcome would not reflect influenza severity. Similar issues are present with other outcomes used in chapter 5 and throughout the dissertation. Thirty-day readmission was associated with comorbid conditions and correlated best with increased number of past-year health care visits while other severe outcomes were related to less past-year health care visits. It is possible that 30-day

readmission is more of a measure of health care seeking behavior then influenza severity. The advantage of 30-day readmission over other outcome measures is that it occurs well after hospital admission and well after administration of neuraminidase inhibitors. The limitation with the other in-hospital outcomes is that they take place so close to the exposure that there may not be enough time to see the impact of treatment. New outcomes that are both good measures of influenza severity and that take place well after neuraminidase inhibitor administration may be necessary to fully understand the impact of neuraminidase inhibitor administration on severe influenza.

Another challenge with this study was interpreting the result that neuraminidase inhibitor administration was only effective when stratifying by vaccination, even though vaccination was not significantly effective at reducing hospital length of stay. As mentioned, the two ways to interpret this result are that influenza vaccination and antiviral treatment act synergistically at reducing severe influenza, or that there is some sort of selection bias of confounding masking the association that is removed when stratifying by vaccination status. More work is needed to determine the cause of this observation.

6.3 Future Work

Two limitations that continued to present themselves throughout the chapters of this dissertation were the difficulty classifying participants in a hospital-based study; particularly, how do we determine the severity of their illness upon hospital admission and how can we use this information to improve our research; and how to find severe outcomes that both occur after the exposures of interest and are true measures of severity.

Both of these problems are challenging to solve, and are difficulties of hospital-based studies that have been expressed in other contexts. While every study in this dissertation has

encountered at least one of these difficulties, sometimes both, no study was designed specifically to solve these problems. Even though comorbid conditions, frailty, and older age are consistently identified as predictors of influenza hospitalization and overall likelihood of death from influenza in the literature, these same factors were not identified as consistent predictors of influenza severity in our hospital-based studies. In fact, in multiple chapters, the positive relationship between previously healthy individuals and individuals who do not frequently seek medical care and influenza severity has been highlighted. We have hypothesized that individuals who frequently seek care, and possibly have serious comorbid conditions may present and be admitted to the hospital with a less severe illness.

The same factors that are related to care seeking with a less severe illness may also be related to vaccination and antiviral treatment. In order to address this challenge, we propose quantifying and stratifying models by baseline illness severity at the time of hospital presentation. We have received all of the HAIVEN network data for the 2017-18 influenza season, which was a particularly severe season. Having all of the network data will improve our analysis by giving us increased power to detect differences in influenza severity. In addition, these data contain a variety of vitals and laboratory measures collect at hospital admission. We will consider variables such as blood pressure, respiratory rate, temperature, blood urea nitrogen, sodium levels, glucose levels, hematocrit, partial pressure of arterial oxygen, oxygen saturation, white blood cell count, platelet count, creatinine, bilirubin, lactic acid, and Glasgow Coma Scale when defining baseline severity.

In order to quantify baseline severity, either regression shrinkage methods (LASSO, ENET) or latent variable modeling will be used. Latent variable modeling would allow us to use indicator variables, such as the severity variables, to categorize our enrollees into groups based

on an unmeasured latent variable. We could then stratify by this latent variable when examining the impact of vaccination and antiviral treatment on severe outcomes.

Initial examination of the 2017-18 data provides early indication that we will need to quantify baseline severity to understand the biases present in this analysis. When we first analyzed the 2017-18 data we found that vaccine receipt appeared to be associated with a reduction in odds of ICU admission among influenza positive participants (Table 6.1), however, this also was the case for influenza negative participants. When we stratified by time from illness onset to hospital admission, a measure that could be related to health care seeking behavior and severity upon admission, we saw that VE against ICU admission increased among influenza positive patients who had onset more than three days prior to hospitalization, but decreased for influenza negative individuals (Table 6.2). More evaluation is needed to understand these results.

Beyond measuring baseline severity, another future direction of this research is to find outcomes that more accurately reflect disease severity. When conducting an in-hospital study, the outcomes that can be used are limited and none are ideal. ICU admission, which is often common enough to use as a severe endpoint, depends on availability of ICU beds in addition to physician discretion, which may be related more to other comorbid conditions that the patient has than their acute illness. Hospital length of stay, again, may reflect management of comorbid conditions, which may or may not be related to the current illness.

To address this limitation, in the future we would like to conduct follow up surveys and detailed assessments of HAIVEN enrollees. We could use survey results to ask questions about maximum severity of symptoms, time removed from normal activities, length of symptoms, and changes in functional status from admission to a month post-discharge. Receiving more detailed outcome information from patients would allow us to have a more detailed understanding of

disease course. In addition, we could evaluate which in-hospital outcomes correlate best with more robust outcomes to assess whether this extra effort is truly necessary whenever evaluating influenza severity among inpatients.

6.4 Conclusions

This dissertation examined two main topics, influenza vaccine effectiveness among inpatients, and predictors and prevention of severe influenza outcomes. In terms of influenza VE in the hospital, we found that the influenza vaccine was effective at preventing influenza among children who were fully, but not partially, vaccinated. This evidence supports current guidelines recommending that children aged 6 months through 8 years receive two influenza vaccines in their first season of vaccination. In addition, we found no evidence of bias due to control group selection in test negative design studies among inpatients, supporting the validity of VE estimates in this setting. When examining the predictors and prevention of influenza severity through vaccination and neuraminidase inhibitor administration, we saw that hospitalized patients with obesity were treated with neuraminidase inhibitors sooner after admission than other patients, potentially impacting the measured relationship between obesity and influenza severity in the hospital. We also saw that in the HAIVEN study, neuraminidase inhibitor administration was associated with reduced hospital length of stay among vaccinated, but not unvaccinated hospitalized adults. These results lead us to our upcoming analyses, a goal of understanding whether this stratification by vaccination status is necessary to assess the impact of neuraminidase inhibitor administration on influenza severity due to underlying differences in disease severity at hospital admission. These analyses should allow for a better understanding of the reduction of influenza severity afforded by vaccination and neuraminidase inhibitor

administrations, leading to rapid assessment of new interventions in a pandemic scenario and improvement of current interventions.

Table 6.1 VE against ICU admission, stratified by influenza positivity

	Influenza Positive		Influenza Negative	
	VE	95% CI	VE	95% CI
Unadjusted	33.6%	(-5.1%, 58.0%)	27.2%	(8.2%, 42.0%)
Fully Adjusted	38.3%	(-1.1%, 62.3%)	29.3%	(9.8%, 44.5%)
Firth's corrected models. Fully adjusted models are adjusted for sex, age (continuous), race group, enrollment site, frailty score (cont), charlson category (1-3, cont), self reported health (poor/fair vs excellent/very good/good)				

Table 6.2 Influenza VE against ICU Admission, Stratified by Influenza Positivity and Time from Illness Onset to Hospital Admission.

	Influenza Positive		Influenza Negative	
Time from Onset to Admission<=3 days	VE (%)	95% CI	VE (%)	95% CI
Unadjusted	8.8%	(-71.3%, 50.6%)	33.3%	(14.0%, 50.8%)
Fully Adjusted	6.7%	(-81.7%, 51.2%)	37.0%	(15.1%, 53.0%)
Time from Onset to Admission>3 days				
Unadjusted	54.9%	(10.1%, 77.9%)	9.1%	(-37.4%, 39.1%)
Fully Adjusted	65.4%	(25.1%, 84.4%)	10.8%	(-37.6%, 41.5%)
Firth's corrected models. Fully adjusted models are adjusted for sex, age (continuous), race group, enrollment site, frailty score (cont), charlson category (1-3, cont), self reported health (poor/fair vs excellent/very good/good)				

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