INFLUENZA INFECTION IN HUMANS: PREDICTORS AND CONSEQUENCES OF DISEASE OUTCOMES

by

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(Under the Direction of Andreas Handel)

ABSTRACT

This dissertation covers a wide range of topics from describing factors related to within-host infection outcomes to the population-level effects of disease severity as well as the clinical epidemiology of influenza. We address four different problems in the subsequent studies. First is the development of a clinical prediction rule for use in a non-hospitalized population since, currently, such a score has not been developed or validated. Then we explore the impact inoculum dose has on infection outcomes using data from challenge studies. We are specifically looking at the within-host viral dynamics, immune response, and symptoms. In the same study we explore the implications of using non-parametric methods for some infection outcomes. We then explore the impact symptoms have on the transmission through reduction of activity and the possible impacts they can have on population-level disease transmission. Finally, we look at a new PCR point of care test and see if the viral load at diagnosis can help predict the clinical outcomes of the patients. All these manuscripts provide additional knowledge to a wide range of topics related to human influenza.

INDEX WORDS: Influenza, Virulence Trade-offs, Inoculum dose, Clinical Epidemiology, Infection outcomes

INFLUENZA INFECTION IN HUMANS: PREDICTORS AND CONSEQUENCES OF DISEASE SEVERITY

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DEDICATION

To my wife, Allison and my three wonderful children, Audrey, Carder, and John. For all the love and support they have shown me through this process. To Kathy without her loving care of our children none of this would have been possible. Finally to my parents for the lifetime of guidance and love.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The research presented in this dissertation covers a wide range of topics relating to influenza infection in humans, from within host dynamics to population-level impacts. Specifically, the development of a clinical prediction rule using signs and symptoms, investigating the impact inoculum dose has on infection outcomes, the impact symptoms have on transmission, and exploring the clinical relevance of viral load at diagnosis.

1.2 Summary of Objectives

Chapter 2

Currently, only severe complications are investigated when developing a clinical prediction, and the patient populations they can be applied to are limited [1]. The plan to develop and validate a clinical prediction rule for both severe and less severe complications in an outpatient setting has the potential to help a much larger number of patients. The less severe complications of influenza are those that require an antibiotic such as bacterial sinus infections or ear infections. These complications occur at a much higher rate of around 10% [2] compared to the severe complications that occur in 1-2% of those infected [3,4]. These less severe outcomes do not require the same level of care as the severe outcomes, but since they occur at a significantly higher rate, they are important in the context of a pandemic. Decision aids, such as clinical prediction rules have been shown to have a positive impact on clinical care [5] and are used to help physicians in

a wide variety of clinical settings [6]. The application of clinical prediction rules also helps doctors avoid both under and overestimating the risk of complications, which can occur when using clinical judgment alone [7].

Chapter 3

Understanding the relationship between inoculum dose, viral dynamics, and infection outcomes in humans infected with influenza are critical to creating effective control measures and identifying important clinical aspects of the disease. Previous studies have explored the natural history of the disease, the relationship between viral load and symptoms scores as well as infectiousness [8,9]. However, the correlation between many infection outcomes and inoculum dose has not been explored. Through analysis of the challenge study data, this study hopes to provide a better understanding of the impact that influenza inoculum dose has on disease outcomes related to within-host viral dynamics, host immune response, and morbidity associated with influenza infection.

Chapter 4

There is very little data for human pathogens that can be used to investigate the relationship between symptoms and transmission. Influenza induces symptoms in around 84% of infected individuals [10]. Some of the symptoms, such as coughing and sneezing, likely enhance transmission by increasing the infectiousness of a host. A recent study provided estimates for the transmission potential of symptomatic versus asymptomatic individuals and found that individuals with symptomatic infections are about 3-12 times as infectious as persons with asymptomatic infections [11]. Other symptoms, such as fever, body aches, and general malaise, are more likely to lead to a reduction in transmission by reducing host activity. A previous study on influenza in 146 adults and children in the United Kingdom found that healthy individuals had a mean of 12.72

contacts per day, while sick individuals only had a 3.58 [12]. The study also showed that the number of contacts decreased as the number of symptoms increased. These studies suggest that there might be a trade-off between infectiousness and activity for influenza, which together determines overall transmission. We plan to investigate this relationship.

Chapter 5

Currently, there are no studies of viral load at diagnosis in an outpatient setting using a point of care PCR test. We plan to conduct an analysis of PCR results from the Cobas Liat POC test to determine if viral load measurement provided useful additional information about a patient's disease progression or recovery. Our study is unique in that our study population was from a primary care setting, use of a POC PCR test, and the inclusion of outcomes for disease resolution five days after the patients visit. The goal of our analysis was to describe the relative viral load at diagnosis based on POC PCR and its potential relevance to physicians.

1.3 Seasonal and Pandemic Influenza

The seasonality of influenza infections is one of the diseases most prominent features. In temperate regions, "seasonal" outbreaks occur each winter while seasonality is less pronounces in the tropical and subtropical areas. In temperate regions, the flu seasons begins in November and diminish in April and May. There is a significant year to year variation, and the exact cause of the seasonality is not apparent [13,14]. In southern temperate regions, the timing of seasonality is reversed and equally hard to predict. In tropical and subtropical regions seasonality is less clear, and transmission seems to be related to different climate factors and possibly altitude [15]. Flu season often starts with type A causing most infections with type B taking over towards the end. Understanding the seasonality of influenza infection has allowed modelers to access the impact of influenza on excess hospitalizations and death without requiring laboratory confirmation of cases.

The epidemiology of influenza is determined by the virus currently in circulation [16]. Each virus variant is capable of causing a varying degree of disease and factors associated with risk of infection or severity are also variant specific. Pandemic influenza is distinguished from seasonal influenza by existing prevalence and geographic spread of the infectious agent [17]. Generally, seasonal outbreaks are caused by circulating A and B strains from previous years or previous pandemics. During seasonal outbreaks, it is common for more than one strain to be co-circulating, which is not the case during a pandemic caused by a novel strain. The seasonal strains slowly change over the years. When the changes in the surface proteins of the virus are small, it is referred to as antigenic "drift". This process allows the virus to at least partially evade host immunity and is why a strain can circulate year after year in the same host population. Significant antigenic drift is cited as the cause of the more severe flu season in 2003 [18]. When a drastic change occurs, it is referred to as antigenic "shift". A shift occurs when an entirely new gene segment or segments are added through re-assortment. When the resulting novel variant is introduced to a population, and there is little to no pre-existing immunity and sets the stage for a severe outbreak with global consequences.

Severe pandemics are generally caused by a novel type A virus and are generally zoonotic in origin. In some cases, even though the virus is considered novel the predecessors of the novel virus have been in circulation for years or decades. As a result, some hosts have been exposed to the proceeding virus may have some level of protection [19,20]. This is thought to affect the attack rate in certain age groups. The strain that causes the pandemic becomes the seasonal strain in future flu seasons. 2009 H1N1 is a perfect example of this process. With each passing year, the epidemiology of the virus changes as immunity builds in the population. Since the 1918 pandemic, the characteristics and severity of each subsequent pandemic have varied widely. It is important to

note that while pandemics are a significant public health concern seasonal flu is responsible for the majority of morbidity and mortality. Efficient transmission is often the first hurdle for novel flu types. Influenza A(H5N1) has been considered a significant threat for a severe pandemic. Currently, only sporadic H5N1 infections have been reported in Asia and Egypt [21]. There is little evidence to support human-to-human transmission. H5N1 has been studied intensely, and many of the viral determinants of severity are well understood as well as the relatively few mutations required to allow for probable human-to-human transmission [23,24]. The variability of the virus requires constant surveillance since the consequences of not being prepared could be grave.

1.4 Clinical Prediction Rules

In most cases of disease in a healthy person the infected will resolve without any complications. Due to the large numbers of infections each year there is also a considerable amount of morbidity and excess mortality that is attributed to influenza. Estimates of the number of infections, hospitalization, and deaths have a huge range. One study estimates that in the US over five flu seasons (2010-2015) 9.2 million to 35.6 million experienced illness related to Influenza [25]. The same study estimated that during the same time period there were 139,000 to 708,000 flu related hospitalizations and 4,000 to 56,000 deaths depending on the complications considered [25]. The most commonly reported estimate is that influenza causes 36,000 deaths and 50,000 to 400,000 hospitalizations annually [26,27]. Despite the indirect methods used to generate the estimates most remain relatively consistent between different models [28].

The severe complications of influenza have been divided into 4 categories; primary viral pneumonia, secondary bacterial pneumonia, pneumonia due to unusual pathogens, and finally exacerbation of underling pulmonary disease [3,4,29]. These complications often result in hospitalizations and deaths. During seasonal epidemics and pandemics the ability to provide the

best care often requires choices to maximize the utility of limited resources to treat these severe cases [30]. Current methods of triage of patients utilize clinical prediction rules adapted for triage of patients with pneumonia such as the SOFA, CURB-65, or PMEWS scores [7,31–33]. There are a number of clinical prediction rules (CPRs) that have been explored for severe influenza complications such as hospitalization, ICU admission, and death among children, patients already hospitalized, and patients presenting to the ER [34,35], and summarized in previous reviews [1,36]. Among the studies included in these reviews only one looked patients who presented to the emergency department [34]. The study population was mostly pediatric patients and 70% of them had some underlying illness and 39% were hospitalized. The outcomes of interest for all the CPRs included in the review are rare and severe such as hospitalization, ICU admission or death [1].

Complications related to influenza have been associated with a number of host factors and are used to access the risk of a having a severe outcome. Age is one of the most commonly discussed predictors but, it is important to realize that there are a number of chronic medical conditions independent of age that increase the risk of hospitalizations and death [27,37,38]. Beyond chronic conditions there are also those who are immune suppressed such as pregnant women, patients infected with human immunodeficiency virus (HIV), and transplant recipients are all at greater risk [39–45]. Those infected by HIV are not only at greater risk of infection but the influenza vaccine does not provide them with same level of protection even when the HIV is well managed [44,46]. Transplant recipients seem to be especially susceptible to viral or secondary bacterial pneumonia [39,47,48]. There is also some evidence that sex may be related to the risk of severe complication is some instances [36,49–51]. During epidemic the resources need to provide care for critically ill patients are often stretched thin [52] and the outpatient system will experience similar choices about who should receive what level of care.

1.5 Inoculum Dose

Inoculum dose refers to the quantity of pathogens a host is exposed to at the beginning of an infection. It plays an important role in infection outcomes. Inoculum dose can effect the within host dynamics of the pathogen once they are infected. Such as the peak levels, and the duration of time it takes to reach the peak [53–57]. Beyond the pathogen dynamics the host immune response is affected as well [58–61]. The immune response plays a role in morbidity and mortality experience by a host. [62–68]. A study has shown that an increase in exposure of the amount of wild-type influenza virus leads to an increase in infection rate for an individual[8], not much is known about the impact virus dosage has on the other infection outcomes in humans.

1.6 Virulence Trade-off

Many infectious diseases cause symptoms in at least some of their hosts. Often, those symptoms increase the host's infectiousness and facilitate the transmission of the pathogen [12,69,70]. Coughing and sneezing for respiratory infections are prime examples. On the other hand, symptoms that are too severe may reduce host activity or in extreme cases cause host death, reducing transmission opportunities. The trade-off hypothesis describes the relationship between virulence and transmission potential [71–77] and predicts that an intermediate level of virulence leads to maximum fitness (usually quantified by the reproductive number) for the pathogen. At such an optimal level of virulence, the pathogen maximizes transmission by inducing symptoms that increase a host's infectiousness, while minimizing transmission-reducing morbidity symptoms. The optimal virulence level can depend on both population-level and within-host level processes, the implications of which have been theoretically explored previously [71,72,79–89].

The most commonly discussed and studied trade-off is between increasing transmission potential due to increased host infectiousness and decreasing transmission potential due to host

mortality [72]. While, this likely applies to many animal diseases and some human diseases (e.g., viral hemorrhagic diseases [90]), for most human pathogens mortality is low, and it is more likely that increased virulence leads to reduced host activity and thus reduced transmission opportunities. Sub-lethal impacts such as weight loss and effects on host fitness have been suggested [72,73,91,92], and interactions between symptoms, activity, and transmission potential have been recognized [93]. Despite this, there is very little data available for human pathogens. One study on Plasmodium falciparum infections in humans showed an increase in transmission potential as virulence, quantified by mortality, increased, with no apparent trade-off [94]. A study in HIV infected individuals showed a negative relationship between duration of asymptomatic infection and viral load and a positive relationship between infectiousness and viral load with optimal transmission potential occurring at an intermediate viral load [95]. As far as we are aware, no studies for any other human pathogens have examined data to directly determine the relationship between virulence and transmission.

1.7 Viral Load at Diagnosis

Diagnostic polymerase chain reaction (PCR) tests are a sensitive and specific method for determining the presence of many pathogens. Until recently, PCR methods were expensive, time-consuming, and required specialized equipment and staff. As a result, the application of PCR tests for diagnostic purposes is limited. There are two Clinical Laboratory Improvement Amendments (CLIA)-waived point-of-care (POC) PCR systems, Xpert Xpress by Cepheid, and cobas Liat by Roche [96,97], available to physicians. These systems can provide highly accurate results in 20-30 minutes without the need for a laboratory or highly trained staff. As the price decreases and the number of pathogens that can be detected increases, these systems will likely have a positive impact on the care of patients.

Currently, the cobas Liat system is only used to produce a qualitative result based on the internal threshold of optical brightness. The system provides the result as either positive (present) or negative (absent) for the pathogen. While these systems are not currently used to estimate the viral load in the sample, it is possible to estimate the viral load using the number of cycles required to generate a positive test, with more cycles associated with a lower viral load [98–100]. This quantitative measurement could potentially give a physician additional information that could help determine the appropriate treatment and advice regarding prognosis for patients. For both influenza and other pathogens, the pathogen load correlates with factors such as disease severity, treatment success, and risk of transmission [9,101–108].

Previous studies have looked at the relationship of a single measure of viral load at diagnosis and the characteristics of the disease and patients with seasonal influenza [99,109–113]. The results of these studies have been mixed with some reporting associations [109–111,113,114], and others reporting no associations with clinical characteristics of disease [99,112]. The time since onset of disease and the viral load has been explored in 5 studies [99,109–111,113], and all but one found a relationship [113]. Only one study has looked at disease outcomes of hospitalized patients with influenza [113]. Analyses from other seasonal influenza infection studies based on repeated measurement of viral load show a reduction of viral load correlates with a decrease in symptoms as well as other clinical outcomes [114–119]. All of the previous studies relied on standard quantitative PCR methods that require significant resources to implement.

CHAPTER 2

CLINICAL PREDICTION RULES TO PREDICT COMPLICATIONS AMONG PATIENTS WITH INFLUENZA LIKE ILLNESS AND INFLUENZA[1]

[1] McKay B, Ebell M, Shen Y, and Handel A. To be submitted to *Journal of the American Board of Family Medicine.*

2.1 Abstract

Background: Currently, there are not clinic prediction rules that predict influenza complications in the outpatient setting. Implementation of a valid prognostic score in this setting could help identify patients most in need of treatment.

Methods: We used data from 4103 patients with influenza-like illness (ILI) enrolled in 11 clinical trials from 1997-2001 to develop prognostic scores for three composite complication outcomes: 1) serious complications (hospitalization, pneumonia, or sepsis) 2) complications that can be treated with antibiotics and 3) complications that required additional treatment. Multivariate logistic regression was used to identify independent predictors of influenza complications. Scores were developed based on the multivariate models for patients with ILI and for the subset that were PCR positive (FLU) for influenza. Finally, we used fast and frugal trees to see if a straightforward model could be created that would be simple to use in a clinical setting.

Results: Using a simple score based clinical prediction rule (CPR), we were able to create low, moderate, and high risk groups for both the FLU and ILI populations. The score for serious complications was able to place 19% of FLU and 33.9% of ILI patients in low risk groups who could be reassured. In general, the scores showed consistent performance with likelihood ratios of less than 1 for the low-risk group and more than 1 in the high risk groups. The decision trees developed performed well in both populations for the serious complications capturing 66% of patients with a complication with 32% of the ILI and 28% FLU patients classified as high risk.

Conclusions: We have developed and tested the internal validity of 6 clinical prediction scores that successfully classifies patients as being at low, moderate, and high risk for three

complications, as well as fast and frugal decision trees. Further work is need to determine the clinical impact of the scores and decision trees through prospective validation.

2.2 Introduction

In most cases of influenza in a healthy person, the infection will resolve without any complications. However, due to the large numbers of infections, each year, influenza causes a considerable amount of morbidity and excess mortality. One study estimates that in the United States over five flu seasons (2010-2015), 9.2 million to 35.6 million experienced illness related to influenza [25]. Of those infected it is estimated that during the same time period there were 139,000 to 708,000 flu related hospitalizations and 4,000 to 56,000 deaths [25]. Given the common nature of flu, it would be very useful to have an easy and accurate method to categorize patients based on their expected risk to develop complications, so low risk patients can be reassured while high risk patients can be monitored more closely.

Decision aids, such as clinical prediction rules (CPRs) have been shown to have a positive impact on clinical care [5] and are used in a variety of clinical settings [6]. Several clinical prediction rules (CPRs) for severe influenza outcomes such as hospitalization, ICU admission, and death among children, hospitalized patients, and ICU patients have been developed [34,35], and summarized in previous reviews [1,36]. Among the studies included in these reviews only one looked patients who presented to the emergency department [34]. The study population was mostly pediatric patients and 70% of them had some underlying illness and 39% were hospitalized. The outcomes of interest for all the CPRs included in the review are rare and severe such as hospitalization, ICU admission or death [1]. A CPR that could be used to triage patients and focus resources on those most likely to develop complications and need further attention would be very valuable [30]. Conversely, identifying patients at low risk of complications can help clinicians

reassure these patients and avoid over-treatment. Current methods of triage for patients with ILI utilize CPRs adapted for triage of patients with pneumonia, such as the SOFA, CURB-65, or PMEWS scores [7,31–33]. These CPRs have been shown to be more accurate than clinical judgment alone for the prediction of complications [7].

Less severe complications that require additional treatment or those that can be treated with an antibiotic occur in approximately 10% of those infected with influenza [2]. Currently, there are no CPRs to help identify adult patients in the outpatient setting who are likely to have complications, or a low risk who are likely to do well. The purpose of this study is to develop CPRs based on logistic regression and simple heuristic decision trees to create a set of tools that can effectively triage adult patients with ILI and confirmed influenza in the outpatient setting.

2.3 Methods

Data

This study will develop and validate a clinical prediction rule using data from 11 published and unpublished clinical trials (Table 2.1). All studies obtained institutional review board approval. We received the data from Roche through ClinicalStudyDataRequest.com, a registry of individual patient level data from clinical trials. All of the studies are randomized, blinded, placebo-controlled, phase III trials for oseltamivir (Tamiflu). The studies were conducted in outpatient settings, and enrollment was open to individuals presenting within 36 hours of the onset of influenza-like illness. We reviewed the study protocol for data collection before pooling the data for the analysis.

Outcomes of Interest

There are three outcomes of interest that we hope to predict using a CPR (Table 2.1). The first is a serious complication, the second are complications of the ears or respiratory tract that can

be treated with an antibiotic, and the third is complications of the ears or respiratory tract that required follow up treatment as a result of the complication.

Predictor Variables

Since no previous CPR's have tried to predict the outcomes of interest in an outpatient population, we initially considered all biologically plausible demographics, signs, symptoms, and elements of the medical history that would be available or could be easily obtained by a doctor or nurse during an initial visit or possibly a phone or telemedicine-based triage (SM Table 2.2) [120,121]. As a result, data from laboratory tests (i.e., white blood cell count) were not considered for use as predictors. To ensure that the score would be practical, variables that were poorly defined or judged to be overly subjective were not considered. We included predictor variables with data collected at the baseline visit for each of the 11 trials. Symptom variables are reported by the patients using a severity score. The score values ranged from 0 to 3, with 0 being absent and 3 being severe. Two new variables were created by dichotomizing the scores. The first is absent (score 0) or present (score 1-3), and the second is absent/mild (scores of 0 or 1) or moderate/severe (scores of 2 or 3).

Populations of Interest

A CPR for each outcome will be created for two populations, patients presenting with influenza-like illness (ILI), and the subset of ILI patients who were PCR confirmed (FLU). Patients with missing values for the 28 baseline variables were excluded from the analysis. Additionally, patients who received the 150mg dose twice daily of Tamiflu are also excluded. This dose has been recommended for hospitalized patients [122–124] and is not representative of the treatment a patient would have received in an outpatient setting.

Internal Validation

The data for ILI and FLU patients were both randomly split into two independent samples. The training data set consists of 70% of the original data set, with the remaining 30% used as the test data set to internally validate the models [125]. The selection of predictors for inclusion, the final model selection, and the selection of cut points for the low, moderate, and high risk groups were all completed in the training data sets. The models, scores, and trees were then applied to the test data to validate the models.

Data analysis

The bivariate regression analysis included all of the eligible predictors in both groups of patients for each of the three outcomes. All variables with $p<0.2$ were considered for inclusion in the multivariate model. The treatment variable (Tamiflu 75mg vs Placebo) was forced into the multivariate model, regardless of statistical significance.

We constructed a logistic regression model for each population and outcome using stepwise backward elimination based on AIC [126]. The cut off for the risk groups was created using two methods. First, a data-driven approach was used where high or low-risk groups were determined by the point that minimizes the distance between the ROC curve and the top left corner (point(0,1)) of the ROC plot [127,128] using the OptimalCutpoints package in R [129]. The second method determined the cut points based on clinical considerations and created high, moderate, and low-risk groups. We did not create high, moderate, and low-risk groups if the distribution of predicated risk was too narrow to make such distinctions meaningful. For each group we report the likelihood ratios and prevalence on the outcomes for each The calibration of the model was tested using the Hosmer and Lemeshow (HL) test and plotting the observed vs the model predictions [130,131]. The model we developed in the training data set was applied to the test data

set and checked for differences in performance. Confidence intervals for the AUC and comparison between AUCs were calculated using the Delong non-parametric methods [132,133].

We produced 6 CPRs based on scores generated using the final regression model for each of the three outcomes for both populations. The scores are based on regression beta coefficients of the variables included in the final model. The coefficients were converted to points using the Sullivan scoring system [134,135], where the beta coefficients are divided by the smallest absolute value of the regression coefficient and rounded to the nearest integer. If any continuous variables were included in the regression model, the scores were created based on categorization based on clinical considerations to make the score simple to implement in clinical settings. The cut points for the risk groups will be determined using the same methods described above. The high, moderate, and low-risk categories corresponded to the clinical decision thresholds of no intervention required, more information required, and consider empiric therapy based on expert clinical opinion and previous studies of test and treatment thresholds [136]

In addition to the logistic model-based scores, a simple heuristic fast and frugal decision tree will also be generated using the ifan algorithm [137] using the variables indicated in the univariate analysis. Fast and frugal trees rarely overfit the data [138] and are easy to interpret and implement in clinical practice [139,140]. The R package FFTrees [137] was used to generate decision trees. All analyses were performed in R (version 3.4.3).

2.4 Results

Of the 4287 patients with ILI enrolled in the studies, 453 received the 150mg dose of Tamiflu and were excluded. Of the remaining 3834 patients, a total of 3684 had complete baseline data for the variables of interest of which 2394 were PCR confirmed. The data for both the PCR confirmed flu and ILI patients was randomly split 70/30 into training and test data sets,

respectively. The distribution of variables between the training and test data sets is similar for both populations (SM Table 2.1). The number of observations and variable distributions contributed from each study are shown in the supplementary material (SM Table 2.2 and 2.3).

Bivariate Analysis

The odds ratios for all of the candidate variables for each of the three outcomes are in the supplementary material (SM Table 2.4 - 2.9). Variables with $p<0.2$ for each population and outcome are used to generate the full model. The treatment variable indicating if a patient received placebo or oseltamivir 75mg was forced into every final model to account for any effects of the treatment. The following abbreviations were used to when referring to different patient populations or outcomes: PCR confirmed patients (FLU), patients with influenza like illness (ILI), serious complications (C-S), complications requiring an antibiotic (C-AB), complications requiring further treatment (C-FT).

Regression Models

Serious Complications

Very few FLU patients had serious complications with a prevalence of 1.8% in the training data and 2.5% in the test data. Among the ILI patients serious complications occurred in 2.4% of patients in the train data and 2.7% of patient in the test data. The FLU C-S final logistic regression model developed in the training data included 4 of the 6 variables indicated by the bivariate analysis (SM Table 2.10). The ILI C-S final logistic regression model developed in the training data included all the variables indicated by the bivariate analysis (SM Table 2.10).

The FLU C-S model performed similarly in the test (AUC=0.77, 95%CI (0.67, 0.87)) and train (AUC=0.69, 95%CI (0.59, 0.79)) data and there was not an indication of a significant difference between the AUCs using DeLong nonparametric test (D=-0.256, df=1496.6, p=0.79).

The cut off associated with the point on the curve closest to the top left corner is a probability of a serious complication greater than 1.7%. Detailed results for the FLU C-S model performance in the test and training data are provided in the supplement (SM Table 2.10-2.11). In the ILI patients the ILI C-S model performed similarly in the test (AUC=0.69, 95%CI (0.59, 0.79)) and train (AUC=0.66, 95%CI (0.59, 0.73)) data and there was no indication of a significant difference between the AUCs (D= 0.49, df= 2202.1, p = 0.62) (SM Table 2.11). The cut associated with the point on the curve closest to the top left corner is a probability of a serious complication greater than 2.8%. Detailed results for ILI C-S model performance in the test and training data are provided in the supplement (SM Table 2.10-2.11).

Among the FLU patients the distribution of predicted probabilities was wide enough that we were able to divide the population into low, moderate, and high-risk groups for C-S. For the ILI patients the predicted probability range was narrow (0.011 to 0.069), and we could only create a low and high risk group. Since this outcome is serious it is important that most of the cases are caught. We therefore chose the cut offs to ensure that as few of the complications would be included in the low risk group as possible favoring sensitivity over specificity. Posttest probabilities and likelihood ratios for the training and test data are shown in Table 2.3.

Complications requiring an antibiotic

Complications requiring an antibiotic (C-AB) occurred more often than the more serious complications with a prevalence of 4.8% in the train data and 5.8% in the test data for FLU patients. Among ILI patients the prevalence was 5.1% in the train data and 7.0% in the test data. Among the FLU patients the final model (FLU C-AB) developed in the training data included 7 of the 11 variables indicated by the bivariate analysis (SM Table 2.12). For the ILI patients the final model developed in the training data included 6 of the 13 variables indicated (SM Table 2.12).

The FLU C-AB model performed similarly in the test (AUC=0.64, 95%CI (0.55, 0.73)) and train (AUC=0.657, 95%CI (0.59, 0.72)) data and there was not an indication of a significant difference between the AUCs (D=-0.261, df=1526.8, p=0.794). The cut off associated with the point on the curve closest to the top left corner is a probability of a complication of 4.7% of greater. Detailed results for the FLU C-AB model performance in the test and training data are provided in the supplement (SM Table 2.6-2.7, Figure 2.5-2.6). In the ILI patients the ILI C-AB model performed similarly in the test (AUC=0.59, 95%CI (0.53, 0.66)) and train (AUC=0.642, 95%CI (0.59, 0.69)) data and there was not an indication of a significant difference between the AUCs (D = -1.09, df = 2316.6, p = 0.27) (SM Table 2.12). The cut associated with the point on the curve closest to the top left corner is a probability of a complication of 4.8% or higher. Detailed results for the ILI C-AB model performance in the test and training data are provided in the supplement (SM Table 2.12-2.13, SM Figure 2.7-2.8).

The predicted probability was wide enough for both the FLU and ILI patients and we divided the populations into low, moderate, and high risk groups. Posttest probabilities and likelihood ratios for the training and test data are shown in Table 2.4.

Complications requiring further treatment

Among the FLU patients, complications that require further treatment occurred more often than any of the other composite outcomes with a prevalence of 15.8% in the training data and 17.0% in the test data. In the ILI population the prevalence was nearly identical with 15.7% in the train data and 16.6% in the test data. Among the FLU patients the final model (FLU-FT) developed in the training data included 10 of the 16 variables indicated by the bivariate analysis (SM Table 2.14). For the ILI patients the final model (ILI-FT) included 9 of the 14 variables indicated by the bivariate analysis (SM Table 2.14).

The FLU-FT model performed similarly in the test (AUC=0.65, 95%CI (0.60, 0.71)) and train (AUC=0.66, 95%CI (0.63, 0.70)) data and there was not an indication of a significant difference between the AUCs (D= -0.253, df= 1372.7, p= 0.799). The cut off associated with the point on the curve closest to the top left corner is a probability of a complication greater than 16.8%. Detailed results for the FLU-FT model performance in the test and training data are provided in the supplement (SM Table 2.14-2.14, SM Figure 2.9-2.10). In the ILI patients the ILI-FT model performed similarly in the test (AUC=0.63, 95%CI (0.59, 0.68)) and train (AUC=0.63, 95%CI (0.60, 0.66)) data and there was not an indication of a significant difference between the AUCs (D = -0.0117, df = 2177.4, p = 0.99) (SM Table 2.15). The cutoff associated with the point on the curve closest to the top left corner is p>=0.156. Detailed results for the ILI-FT model performance in the test and training data are provided in the supplement (SM Table 2.14-2.15, SM Figure 2.11-2.12).

The range of predicted probability was wide enough for both the FLU and ILI patients and we divided both populations into low, moderate, and high-risk groups. Posttest probabilities and likelihood ratios for the training and test data are shown in Table 2.5.

Clinical Scores

Hospitalization, Sepsis, or Pneumonia

Using the beta coefficients of FLU C-S and ILI C-S models we developed a score for both patient populations (Table 2.6). Use the training data we created low, moderate, and high-risk groups based on the scores. Posttest probabilities and likelihood ratios for the training and test data are shown in Table 2.7.

Complications Requiring an Antibiotic

Using the beta coefficients of FLU C-AB and ILI C-AB models we developed a score for both patient populations (Table 2.8). Use the training data we created low, moderate, and high-risk groups based on the scores. Posttest probabilities and likelihood ratios for the training and test data are shown in Table 2.9.

Complications Requiring Further Treatment

Using the beta coefficients of FLU C-FT and ILI C-FT models we developed a score for both patient populations (Table 2.10). Use the training data we created low, moderate, and high-risk groups based on the scores. Posttest probabilities and likelihood ratios for the training and test data are shown in Table 2.11.

Fast and Frugal Decision Trees

Hospitalization, Sepsis, or Pneumonia

Among FLU patients the FLU C-S-tree developed in the training data was able to identify over half of the patients with complications while greatly reducing the number patients at risk. Among the 476 high risk patients the proportion of complication was 3.9% compared to 1.0% in the low risk group (SM Figure 2.12). The FLU C-S-tree performed similarly well in the test data capturing a third of patient with complications (Figure 2.2). Among the 204 patients identified as high risk the proportion of complications is 6.25% compared to 1.1% among the 514 classified as low risk.

The ILI C-S tree developed in the training data performed similarly well, identifying over half of the patients with a complication and reducing the number of patients at risk. Among the 834 patients identified as high risk the proportion with complication was 4.5% compared to 1.3% in the low risk group (SM Figure 2.14). The ILI C-S tree performed equally well in the test data, correctly classifying more than half of the patients with complications as high risk and greatly

reducing the number of patients to be considered. Among the 356 high risk patients, the proportion of complications is 5.6% compared to 1.3% among 749 patients in the low risk group (Figure 2.2).

Complications requiring an antibiotic

The FLU C-AB-tree developed in the PCR patient training data performed poorly misclassifying over half of those with complications (SM Figure 2.15). Among the high risk the proportion of complication is 8.7% compared to 3.3% in the low risk group. The tree also performed poorly in the test data missing more than half of the complications (SM Figure 2.16).

The ILI C-AB-tree developed in the training data was able to greatly reduce the number of patients that would need to be followed up. Out of 2579 patients it classified 920 as high risk. Among those high risk patients the proportion of complications was 7.8% compared to 3.6% in the low risk group. Unfortunately it still missed nearly half of the patients with these complications (SM Figure 2.17). The ILI C-AB-tree performance in the test data was poor. The tree was only marginally better than guessing with the high risk group having 7.5% complications compared to 6.5% in the low risk group (SM Figure 2.18).

Complications requiring further treatment

The FLU-FT tree developed in the training data performed poorly in overall accuracy missing classifying about half of patients. Among those identified as high risk the proportion of complications is 22.2% compared to 12.2% in the high risk group (SM Figure 2.19). Performance in the test data was similar to the train data. Of those identified as high risk 24.1% had a complication compared to 13.1% in those that are low risk (SM Figure 2.20).

The ILI-FT tree developed in the training data for the ILI population misclassified more 60% of the outcomes. Among the patients classified as high risk the proportion of complications is 24.7% compared to 12.9% in the low risk group (SM Figure 2.21). The ILI-FT tree performance

in the training data was similar to the training data. Again more than 60% of the complications are misclassified. Among the high risk patients the proportion of complication is 23.7% compared to 14.2% in the low risk group (SM Figure 2.22).

2.5 Discussion

Using data from 3684 patients with influenza-like illness, we were able to develop clinical prediction scores that allowed us to stratify non-hospitalized adult patients into low, moderate, and high-risk groups for all three composite complication outcomes that we considered. Finally, we developed a simple decision tree for each outcome in both populations that classified patients as high or low risk. We included the ILI population since it is a common presenting complaint, and allows the score to be used in the absence of a confirmatory test. The PCR confirmed population (FLU) represents the best-case scenario, which are patients with ILI who have their diagnosis confirmed with a PCR test. The score in the FLU patients will still have practical applications, especially as the availability of highly accurate point of care PCR tests for influenza increases. The results were generally better in the PCR population compared to the ILI population. The performance of the models, scores, and trees was similar in the testing and training populations, indicating good internal validity. The calibration of the models varied between the outcomes. For complications requiring an antibiotic or further treatment the lack of calibration may be due to patient demand and rather than proper application of antibiotic treatment based on suspected bacterial infection [141].

The final models for the three outcomes in both patient populations included predictors previously shown to have associations with influenza complications such as age, asthma, COPD, and sex [3,4,7,36,49–51], but many of the clinical signs and symptoms included have not previously been explored. Throat related signs and symptoms have not been described as a risk

factor for the serious (C-S) complications we explored, but all of the final models except one had at least one predictor related to throat symptoms or signs. Throat related symptoms are present in many cases of influenza, and it has been shown to be associated with a reduced odds of actually having influenza [142,143]. Sore throat and the associated signs of inflammation are also commonly associated with bacterial infections [144]. In our study, this may indicate the presence of a bacterial co-infection, which could lead to the complications we included in our analysis. The other symptom-based predictors of cough, myalgia, chills/sweats, and fatigue are all common symptoms associated with influenza infection and are used in many clinical decision rules for the diagnosis of influenza, but unlike sore throat, they are all positively associated with influenza infection [142,143,145]. All of the models were forced to include the treatment variable since there is evidence that treatment with Tamiflu helps prevents some but not all complications [3,4,7,146–149].

The clinical prediction rules for serious complications (C-S) can accurately identify a subset of patients as being high risk. Using the FLU C-S score in the training data was able place 19% of patients in a low risk group who can be reassured, while 36% of the patients in the high risk group are identified as likely benefiting from a follow up or being encouraged to seek care is they get worse. The moderate-risk group for FLU consisted of 45% of the patients who had a post-test probability nearly the same as the pretest. Using the ILI C-S score in the training data was able to place 34% of patients in the low risk group, and 43% in the high risk group that require future follow up or should seek care if they began to feel worse. The ILI C-S score placed 23% of patients in the moderate risk group who had a post-test risk approximately the same as the pretest. In both patient populations, the score reduced the number of patients that should be followed up by more than half.

Complications that can be treated with antibiotics and those that require further treatment are more common than the serious and severe complications that are generally included in the clinical prediction rules. These complications also represent a significant burden to the outpatient system, and being able to limit the number of patients targeted for follow up could help improve the efficient use of resources. Unfortunately both the ILI and FLU C-FT and C-AB scores lacked the ability to create low, moderate, and high-risk groups with a meaningful difference in risk. The use of high and low risk groups for maybe better based on the similarity of the risk in the low and moderate groups.

The trees that we developed may be simpler than the scores for clinicians use, and could be built into a telephone triage system for prioritizing access to an outpatient visit during a pandemic. For the serious outcomes, the same cues were used for both FLU C-S and ILI C-S. Only the order of the cues was different. The FLU C-S tree correctly captured 66% the patients with a serious complication, with only 204 of 718 patients in the test data set being classified as high risk. The ILI C-S tree successfully captured 66% of the patients with a serious complication, with only 356 of 1105 patients in the test data classified as high risk. Unfortunately, the trees for outcomes C-AB and C-FT could not reliably classify patients as high or low risk.

Out study has several limitations. First, while we did test the internal validity by randomly splitting the data into a training and testing data set, we could not complete an external validation. Additionally, we combined data from 11 studies, and while they all had remarkably similar inclusion criteria and protocols, the primary goal of the analysis was not to study the outcomes we examined in our analysis. We did benefit from the fact that the data was collected over four different flu seasons (1997-2001) from more than five different countries. Finally, the data used in this analysis consisted of patients 18 and older who presented for care within 36 hours of symptom

onset. Therefore our results may not apply to adult patients presenting much later in the course of their disease or in younger patients.

2.6 Conclusion

We have developed and test the internal validity of a clinical prediction score that successfully classifies patients as being at low, moderate, and high risk for three complications associated with influenza infection. These scores are based on simple questions that can easily be assessed in a clinical setting to identify patients' risk. We also developed fast and frugal trees for serious complications based on three simple questions that were able to capture the majority of the complications while greatly reducing the number of patients at risk. Based on the results of this study both the FLU and ILI C-S score and tree warrant further study and prospective validation.

2.7 Figures and Tables

Table 2. 1 Description of the included clinical trials of Tamiflu included in the analysis.

Roche Trial ID	Study Location	Data Collection Dates	Study Inclusion Criteria	Patients Enrolled
M76001	USA	24 December 1998 to 19 February 1999	Adults age 13 to 80 with ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	1459
WV15670 (Nicholson et al.)	Europe, China, and Canada	12 December 1997 to 18 April 1998	Adults age 18 to 65 with ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	726
WV15671 (Treanor et al.)	USA	23 December 1997 to 20 April 1998	Adults age 18 to 65 with ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	629
WV15707	Australia, South Africa, and South America	20 July 1998 to 16 November 1998	Elderly adults over 65 years of age with ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	27
WV15730	Australia, and South Africa	1 July 1998 to 21 September 1998	Adults age 18 to 65 with ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	60
WV15812	USA, Canada, and Europe	5 January 1999 to 12 April 1999	Adults age 13+ with chronic cardiac or respiratory disease and ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	304
WV15819	Europe, USA, Canada, and Israel	1998 to 1999 (exact dates are not provided)	Elderly adults age 65 or older with ILI (fever ≥ 37.5°C + one respiratory and one constitutional symptom) presenting within 36 hours.	168
WV15872	Australia, New Zealand and South Africa	2 June 1999 to 2 October 1999	Adults age 13+ with chronic cardiac or respiratory disease and ILI (fever + one respiratory and one constitutional symptom) presenting within 36 hours.	100
WV15876	South Africa, New Zealand and Australia	1999 (exact dates are not provided)	Elderly adults age 65 or older with ILI (fever ≥ 37.5°C + one respiratory and one constitutional symptom) presenting within 36 hours.	99
WV15978	Europe, USA, and Canada	1999 to 2000 (exact dates are not provided)	Elderly adults age 65 or older with ILI (fever ≥ 37.5°C + one respiratory and one constitutional symptom) presenting within 36 hours.	468
WV16277	Europe	4 January 2001 to 23 March 2001	Age ≥ 13 years (or ≥ 18 years in countries with local IRB requirements) Sudden onset of fever (≥37.8°C) and at least two of the following symptoms: nasal congestion, sore throat, cough, myalgia, fatigue, headache, chills/sweats presenting within 36 hours.	451

Table 2. 2 Definition for the three complications used in the study.

Outcome	Outcome definitions
Complications requiring an antibiotic (AB)	Among patients with a complication of the ears, lower or upper respiratory who receive a diagnosis of; sinusitis, tonsillitis, bacterial pharyngitis, otitis media, pneumonia, sinus pain, peritonsillar abscess, mycoplasma infection, streptococcal infection, lower respiratory tract infection, sepsis, or crepitation. Only diagnosis that occurred on or after study day 1 are counted. (note: Symptoms reported by physicians during follow up were indicated as not being related to suspected flu infection.)
Complications requiring follow up treatment (FT)	Among patients with a complication of the ears, lower or upper respiratory who received additional treatment as a result of the complication. Treatment was not necessarily with an antibiotic. Only complications and treatments that occurred on or after study day 1 are counted.
Serious complications (C-S)	Patients with a diagnosis of pneumonia or sepsis or were hospitalized. Only complications and treatments that occurred on or after study day 1 are counted.

Table 2.3 Accuracy of CPRs for predicting serious complications in the FLU and ILI patients

FLU C-S Model				
Training Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.01)	5	569	0.87% (34.3%)	0.46
Moderate (.011-0.03)	11	829	1.31% (50.1%)	0.70
High (> 0.03)	15	247	5.72% (15.6%)	3.22
Test Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.01)	1	295	0.33% (41.2%)	0.13
Moderate (.011-0.03)	6	231	2.53% (33.0%)	1.01
High (> 0.03)	11	174	5.94% (25.8%)	2.45
ILI C-S Model				
Training Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.028)	19	1479	1.27% (58.1%)	0.53
High (>0.028)	42	1039	3.88% (41.9%)	1.66
Test Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.028)	13	802	1.59% (73.8%)	0.58
High (>0.028)	17	273	5.96% (26.2%)	2.23

Table 2.4 Accuracy of CPRs for predicting complications requiring an antibiotic in the FLU and ILI patients

FLU C-AB Model				
Training Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.045)	27	909	2.88% (55.9%)	0.59
Moderate (.0451-0.055)	12	228	5.00% (14.4%)	1.05
High (> 0.055)	41	459	8.20% (29.9%)	1.78
Test Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.045)	9	296	2.95% (42.5%)	0.48
Moderate (.0451-0.055)	4	82	4.65% (11.9%)	0.78
High (> 0.055)	29	298	8.86% (45.5%)	1.56
ILI C-AB Model				
Training Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.025)	8	283	2.74% (11.3%)	0.52
Moderate (0.0251-0.06)	65	1509	4.12% (61.0%)	0.79
High (> 0.06)	59	655	8.26% (27.7%)	1.66
Test Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.025)	0	0	NA (NA)	NA
Moderate (0.0251-0.06)	26	491	5.02% (46.8%)	0.70
High (> 0.06)	51	537	8.67% (53.2%)	1.26

Table 2.5 Accuracy of CPRs for predicting complications requiring additional treatment in the FLU and ILI patients

FLU-FT Model				
Training Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.10)	36	401	8.97% (26.1%)	0.47
Moderate (.10-0.20)	106	697	15.2% (47.9%)	0.80
High (> 0.20)	123	313	39.2% (26.0%)	2.09
Test Data				
Risk Group (Probability complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.10)	11	108	9.24% (16.6%)	0.49
Moderate (.10-0.20)	55	349	13.6% (56.3%)	0.76
High (> 0.20)	56	139	28.7% (27.2%)	1.96
ILI-FT Model				
Training Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.07)	3	93	3.12% (3.7%)	0.17
Moderate (0.07-0.14)	141	1091	11.4% (47.8%)	0.69
High (> 0.14)	261	990	20.8% (48.5%)	1.41
Test Data				
Risk Group (Probability of complication 0-1.0)	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-0.07)	1	25	3.84% (2.4%)	0.20
Moderate (0.07-0.14)	51	387	11.6% (39.6%)	0.66
High (> 0.14)	131	510	20.4% (58.0%)	1.29

Table 2.6 Model coefficients and corresponding point value for the clinical prediction score

Model and Included Variables	**Beta Coefficients**	**Points**
FLU C-S Score		
Tamiflu = No	0.65	3
Asthma = Yes	1.04	4
Sore Throat = Severe	0.61	3
Age (40,65]	0.24	1
Age (65,100]	0.98	4
ILI C-S Score		
Tamiflu = No	0.24	1
COPD = Yes	0.93	4
Asthma or COPD Rx = Yes	0.67	3

Table 2.7 Score cut points developed in training data then applied to the test data for FLU and ILI-models.

FLU C-S Likelihood Ratios				
Training Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-1 points)	2	323	0.61% (19.4%)	0.32
Moderate (2-4 points)	9	696	1.27% (42.1%)	0.68
High (>4 points)	20	626	3.09% (38.5%)	1.69
Test Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-1 points)	0	137	0% (19.1%)	0
Moderate (2-4 points)	8	315	2.47% (45.0%)	0.98
High (>4 points)	10	248	3.87% (35.9%)	1.56
ILI C-S Likelihood Ratios				
Training Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0 points)	9	854	1.04% (33.5%)	0.43
Moderate (1-3 points)	10	625	1.57% (24.6%)	0.66
High (>4 points)	42	1039	3.88% (41.9%)	1.66
Test Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0 points)	4	371	1.06% (33.9%)	0.38
Moderate (1-3 points)	5	251	1.95% (23.2%)	0.71
High (>4 points)	21	453	4.43% (42.9%)	1.66

Table 2.8 FLU C-AB and ILI C-AB model coefficients and corresponding point value for the clinical prediction score.

Model and Included Variables	**Beta Coefficients**	**Points**
FLU C-AB Score		
Tamiflu = No	0.05	1
Throat Physical = Abnormal	0.47	8
Asthma = Yes	0.65	11
Nasal Symptoms = Severe	0.51	9
Myalgia = Severe	0.67	12
Age (40,65]	0.36	6
Age (65,100]	0.51	9
Sex = Female	0.42	7
ILI C-AB Score		
Tamiflu = No	0.10	1
Sex = Female	0.59	5
Throat Physical = Abnormal	0.34	3
Asthma or COPD Rx = Yes	0.73	7
Sore Throat = Absent	0.39	4
Cough = Absent	0.46	4
Fatigue = Severe	0.50	5

Table 2.9 FLU C-AB and ILI C-AB score cut points developed in training data then applied to the test data.

FLU C-AB Score Likelihood Ratios				
Training Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-18 points)	7	246	2.76% (15.1%)	0.56
Moderate (19-28 points)	22	626	3.39% (38.7%)	0.70
High (>28 points)	51	724	6.58% (46.2%)	1.40
Test Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-18 points)	3	102	2.85% (14.6%)	0.47
Moderate (19-28 points)	15	260	5.45% (38.3%)	0.92
High (>28 points)	24	314	7.10% (47.1%)	1.23
ILI C-AB Likelihood Ratios				
Training Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-4 points)	1	122	0.81% (4.8%)	0.15
Moderate (5-10 points)	42	1241	3.27% (49.7%)	0.62
High (>10 points)	89	1084	7.58% (45.48%)	1.52
Test Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-4 points)	3	47	6.00% (4.5%)	0.85
Moderate (5-10 points)	35	516	6.35% (49.9%)	0.90
High (>10 points)	39	465	7.73% (45.6%)	1.11

Table 2.10 FLU-FT and ILI-FT model coefficients and corresponding point value for the clinical prediction score.

Model and Included Variables	Beta Coefficients	Points
FLU-FT Score		
Tamiflu = No	0.26	1
Throat Physical = Abnormal	0.34	1
Asthma = Yes	0.52	2
Nasal Symptoms = Severe	0.36	1
Sore Throat = Absent	0.54	2
Cough = Severe	0.33	1
Fatigue = Severe	0.44	2
Chills or Sweats = Severe	0.28	1
Myalgia = Severe	0.48	2
Age (40,65]	0.32	1
Age (65,100]	0.98	4
ILI-FT Score		
Tamiflu = No	0.21	2
Sex = Female	0.20	2
Asthma = Yes	0.32	3
COPD = Yes	0.35	3
Asthma or COPD Rx = Yes	0.45	4
Nasal Symptoms = Severe	0.16	1
Sore Throat = Absent	0.41	4
Fatigue = Severe	0.69	6
Age (40,65]	0.11	1
Age (65,100]	0.24	2

Table 2.11 FLU-FT and ILI-FT score cut points developed in training data then applied to the test data.

FLU-FT Likelihood Ratios				
Training Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-5 Points)	18	232	7.20% (14.9%)	0.41
Moderate (6-8 Points)	115	807	12.47% (55.0%)	0.75
High (>8 Points)	132	372	26.19% (30.1%)	1.88
Test Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-5 Points)	8	86	8.51% (13.1%)	0.45
Moderate (6-8 Points)	68	363	15.77% (60.0%)	0.91
High (>8 Points)	46	147	23.83% (26.9%)	1.52
ILI-FT Likelihood Ratios				
Training Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-4 points)	3	96	3.03% (3.8%)	0.16
Moderate (5-10 points)	125	1003	11.08% (43.7%)	0.66
High (>10 points)	277	1075	20.48% (52.5%)	1.38
Test Data				
Risk group	Complication	No Complication	Complication % (% of patients in risk group)	Likelihood ratio
Low (0-4 points)	2	37	5.12% (3.5%)	0.27
Moderate (5-10 points)	69	428	13.88% (45.0%)	0.81
High (>10 points)	112	457	19.68% (51.5%)	1.23

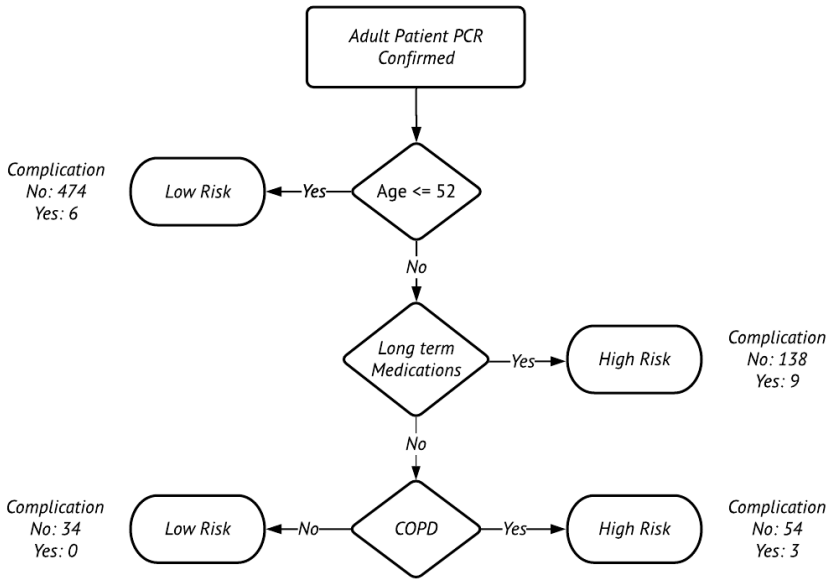

Figure 2.1 FLU C-S decision tree for hospitalization, pneumonia, and sepsis in PCR test data.

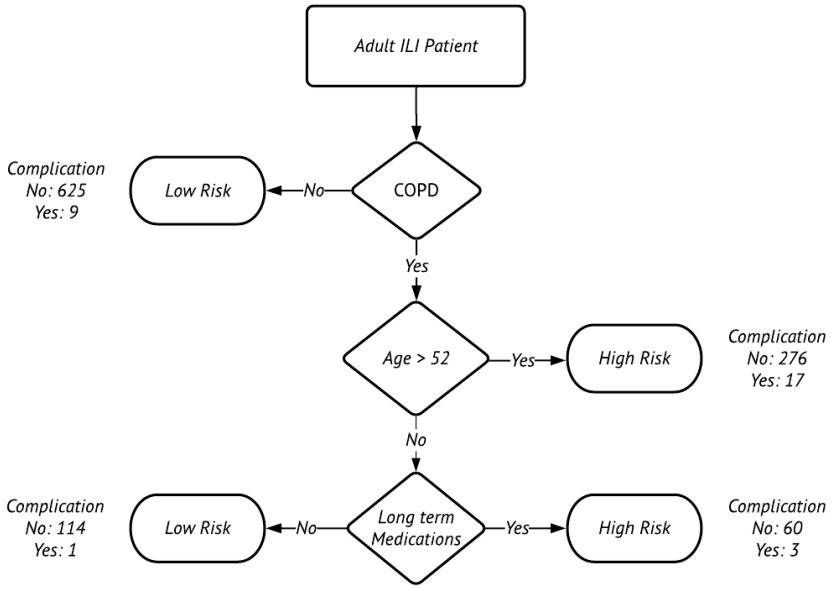

Figure 2.2 ILI C-S decision tree for hospitalization, pneumonia, and sepsis in test data.

CHAPTER 3

THE IMPACT OF INOCULUM DOSE ON INFECTION AND IMMUNITY OUTCOMES FOR INFLUENZA VIRUS[2]

[2] McKay B, Ebell M, Shen Y, and Handel A. To be submitted to *American Journal of Epidemiology*.

3.1 Abstract

Introduction: Inoculum dose is the quantity of pathogens a host is exposed to at the beginning of an infection. Understanding the relationship between inoculum dose, viral dynamics, and infection outcomes in humans infected with influenza is critical to creating effective control measures and identifying important clinical aspects of the disease.

Methods: We completed a systematic literature review to identify influenza challenge studies conducted in humans. Data on the dose, viral, and symptom outcomes were abstracted. Using the data gathered we described the relationship between the inoculum dose and outcomes related to within host viral dynamic, immune response, and symptoms using traditional dose response models as natural splines so that non-monotonic non-linear trend could be detected.

Results: We identified 149 influenza challenge studies conducted in 7821 individual volunteers published between 1943 and 2016. We found that dose response was similar to a past study in regards to probability of infection. Surprisingly we did find a number of decreasing trends for both mean viral peak and proportion of individuals with systemic symptoms. For immune response there was a clear increasing trend for the proportion of individuals with a significant increase in HAI titers but when looking as the ratio of before and after there did not seem to be any relationship with the dose.

Conclusion: Parametric dose response models are biologically based and their use for modeling the probability of infection is justified, but for some outcomes a function that assumes an increasing relationship may be misleading. The inoculum dose does play a role in infection outcomes and a greater understanding of the effects will lead to the creation of more effective controls.

3.2 Introduction

Inoculum dose is the quantity of pathogens a host is exposed to at the beginning of an infection. Inoculum dose can affect the within host dynamics of the pathogen once they are infected, such as the peak levels and the duration of time it takes to reach the peak [53–57]. Beyond the pathogen dynamics the host immune response can also be affected [58–61]. There are also changes in the immune response as well as the impact they have on the morbidity and mortality experience an infected individual. [62–68].

Understanding the relationship between inoculum dose, viral dynamics, and infection outcomes in humans infected with influenza is critical to creating effective control measures and identifying important clinical aspects of the disease. The application of sigmoidal functions in dose response analysis has clear biologic meaning in terms of infection. The exponential, Weibull, and other functions commonly used assume that when no virus is present, infection cannot occur and as virus increases so does the probability of infection until saturation [150]. In the case of infection these assumptions are reasonable, but for other outcomes related to viral dynamics, immune response, and morbidity increasing the dose may not always lead to an increase in the outcome [152–156]. Modeling these infection outcomes using the traditional dose response models may miss important relationships between the dose and outcome.

Influenza infections are common around the world and are often characterized by sudden onset of symptoms such as fever, myalgia, and headache [145]. The natural history and the within host viral dynamics influenza have been studied [9,157,158]. As well as the role inoculum dose plays in the probability of infection [8]. The objective of this meta-analysis, using results from human influenza challenge studies, is to determine the impact inoculum dose has on influenza viral dynamics, within host immune response, and symptom outcomes in healthy humans. Not much is known about the impact the dose has on the symptom outcome [8]. We considered data from

challenge studies that were not included in previous studies [8,9] where individuals are challenged by live attenuated influenza virus often used in influenza vaccines [159]. By fitting nonlinear models to data we hope to provide a better understanding of the impact inoculum dose has on disease outcomes beyond the probability of infection.

3.3 Methods

Systematic Review

Following PRISMA guidelines we conducted a systematic literature search for human influenza challenge studies published from January 1st, 1946 to January 15th, 2017 was carried out using the PubMed, and Web of Science databases. Studies included met the following criteria: Study was conducted in human volunteers, a living virus was used, and data are reported for at least one infection outcome. Studies were not excluded based on language unless reasonable attempts to have it translated failed. Citations of all included articles and reviews identified in the search were hand searched for additional articles that should be considered for inclusion. Only published data was used; we did not request access to the original data for any of the included studies. Study titles were initially screened by two researchers independently. The abstracts and full text of the remaining studies where then reviewed for final inclusion by two researchers independently. Any disagreement was resolved by consensus.

Data Abstraction

Data were abstracted from each study by two researchers independently and then compared for agreement. Any disagreement was resolved by consensus after reviewing the full text of the study in question. Data for the following variables were collected: year of publication, size of the study, age range, mean age, median age, proportion male, proportion female, virus name, viral preparation, viral subtype, viral type, pre-challenge HAI, per-challenge NAI, inoculum dose,

inoculum dose units, inoculum volume, inoculum route, and many others. Outcomes were recorded by challenge group where the group was defined by the dose and the virus given.

Data Processing

Data was cleaned and variables correctly formatted before being analyzed. A single data set was created and included all the outcomes. Meta-data such as study title, publishing journal, general comments, and others were removed. Variables were then formatted as appropriate as numeric, character, or categorical. Missing data for each variable was investigated to ensure they were not the result of data entry error. Only data from studies that reported an inoculum dose and outcome of interest were included in the quantitative analysis. Once the data set was cleaned subsets were created based on virus preparation (wild type or attenuated) and then further stratified by subtype.

Statistical Analysis

The data was pooled with careful consideration for differences in the virus prep. The proportion infected data was initially fitted using a two parameter exponential as well as a two parameter approximate beta-Poisson [150,160,161]. Model parameters were selected using NLOPTR, a non-linear optimization package in R [162]. To explore the trends observed in the outcomes a generalized additive model using natural splines was used [163]. The degrees of freedom for the splines were tuned using Monte Carlo cross validation with 25 re-samples splitting the data in to 75% train and 25% test using the caret package in R [164,165]. In the stratified analysis of virus subtype the strata was only included if 5 or more observations where present. Time trends were also assessed using linear models.

3.4 Results

Systematic Review

Our search results included 1351 unique results with 134 full text articles being included after reviewing 378 full texts. Review of the included texts citations yielded an additional 15 studies (Figure 1.1).

Data Description

In the 149 included studies there were 495 challenge groups consisting of 7821 individual volunteers these studies were published between 1943 and 2016. Median tissue culture infectious dose (TCID50) and median egg infectious dose (EID50) were the unit used to verify viral titer in 61% and 36% of challenge groups respectively. The route used to inoculate volunteers was almost exclusively described as intranasal (Table 1). The volume used for inoculation was 0.5 ml for 44% and 1.0 ml in 19% of the groups. Infection was the most commonly reported outcome and was reported in all but 16 of the challenge groups. The mean peak titer was reported for 176 challenge groups of which 164 used log10 TCID50/ml as the unit. The presence of any systemic symptom was reported in 204 challenge groups. Increase in antibodies to hemagglutinin (HA) was reported in 403 groups. The use of the 4 fold change in the pre and post hemagglutination inhibition (HAI) assay was used in 343 of the challenge groups. In 59 studies the patients were classified based on a "significant" change and the definition of significant was not always apparent.

Infection Outcomes

We investigated the impact of inoculum dose on different infection outcomes. We looked at the proportion infected, the mean peak viral titer, the proportion of patients with systemic symptoms, and immune response in regards to the change in HAI and NAI titers. Table 3.2 summarizes the number of observations that are included in the analysis for each of the different outcomes.

Proportion Infected

The range of inoculum doses was narrow. There seemed to be a clear difference between attenuated and wild type clear (Figure 3.2). Differences between the subtypes of both attenuated and wild type viruses were less apparent (Figures 3.3-3.4)

Mean peak titer

The general additive model fit with two degrees of freedom for both the attenuated and wild type viruses. The fit for these were only marginally better than a simpler linear model. In the case of the wild type virus the impact of the dose seems to reverse directions while for the attenuated there is a steady increase (Figure 3.5). For the wild type H1N1 virus the model used three degrees of freedom and indicated a negative trend (Figure 3.6). The wild type H3N2 model used 2 degrees of freedom and while it initially increases it levels off as dose increases (Figure 3.6). Both of the subtypes for attenuated show increasing trends (Figure 3.7)

Proportion with Systemic Symptoms

The models for both attenuated and wild type used 2 degrees of freedom and show a negative trend (Figure 3.8). For the wild type subtypes H1N1 and H3N2 a model with 1 degree of freedom was selected by cross validation and both so a negative trend (Figure 3.9). Similarly the subtypes for the attenuated viruses also fit with a single degree of freedom and no trends were apparent (Figure 3.10).

Immune Response

The models for both the attenuated and wild type fit best with a single degree of freedom and while the wild type indicated a strong positive correlation the wild type showed almost none in either direction (Figure 3.11). The both of the wild type subtypes indicated almost no effect at different doses (Figure 3.12). In the case of the attenuated subtypes a strong positive correlation

was observed (Figure 3.13). We also looked at the ratio between pre and post vaccine HAI titers. In all cases dose seemed to have little to no effect on the outcome (SM Figures 3.17-3.19).

Change over time

There has been an increase in the inoculum dose used over time for challenge with wild-type virus, and while there is a slight upward trend in the attenuated virus it is not present when stratified by the subtypes (SM Figure 3.20). The mean peak titers have little to no change among wild type virus either as a group or when stratified by subtype (SM Figure 3.21). On the other hand there is a strong decline among the attenuated group which is unsurprising since the goal of live vaccine is to not be transmittable. In regards to the proportion of patients with systemic symptoms there is a negative trend in both the wild type and attenuated viruses that is still present when stratified by subtype (SM Figure 3.22). Again for the attenuated group this is expected as improvements to live vaccines were being made over time. In proportion with 4 fold increase there is not much change in the wild type virus but interestingly there is in the attenuated (SM Figure 3.23).

3.5 Discussion

We completed an extensive systematic literature review and created a data base of published challenge studies published from 1943 to 2016. Using this data we hope to further our understanding of the impact inoculum dose has on infection outcomes in humans.

We did find some evidence that for some of the outcomes a sigmoidal curve would not be able to detect relevant relationships in the data. In the case of dose and mean peak titer for wild-type influenza there does seem to be an initial increase but it does not continue as the dose increases. In the case of the attenuated virus there is a general increasing trend as dose increases. When both the wild-type and attenuated viruses are stratified by subtype the same general trends

between the dose and mean viral peak. For among patients challenged with a wild-type virus the proportion with systemic symptoms had a negative relationship with dose. The same trends were observed when the wild-type virus was stratified by subtype. A previous study that looked a similar outcome fitted a beta-Poisson model which would be unable to detect a negative trend in the data [8]. The existence of a negative relationship between dose and fever, which is the primary systemic symptom has been observed in the past [9]. The trend between dose and proportion of patients challenged for the attenuated virus was less clear. When stratified by subtype there did not appear to be any trend at all.

Understanding the impact the inoculum dose has on the immune response is important as we work to develop better vaccines [166–168]. In particular for live vaccines, knowing the right balance between enough inoculum to trigger a robust immune response, and low enough inoculum to prevent potential side effects is crucial [169]. Our results show that increasing dose does lead to an increase in the proportion of patients with a significant increase in HAI titers when using attenuated viruses. In the case of the wild type virus there seems to be no change as dose increases. It is important to note that the range of doses is limited ranging from approximately 3 to 8 log10 TCID50 for the attenuated viruses and 3 to 7 log10 TCID50 for the wild-type.

We did find some evidence that the wild-type strains used for challenge studies are becoming less virulent over time. With a general trend of increasing dose and decreasing measures of viral load and symptoms. It is interesting to note that in terms of immune response there seems to be very little change. This is not too surprising since the development of wild type challenge stains does require the virus have a moderate pathogenicity while still being infective [9,170]. Decrease in the morbidity associated with attenuated viruses was expected since the goal is to produce a vaccine with as few side effects as possible while still eliciting an immune response.

Further analysis and modeling of this data to provide a better understanding of the impact that influenza inoculum dose has on disease outcomes and can provide important information to the optimal dosage for maximizing immune response while minimizing symptom outcome in in future vaccine development.

3.6 Figures and Tables

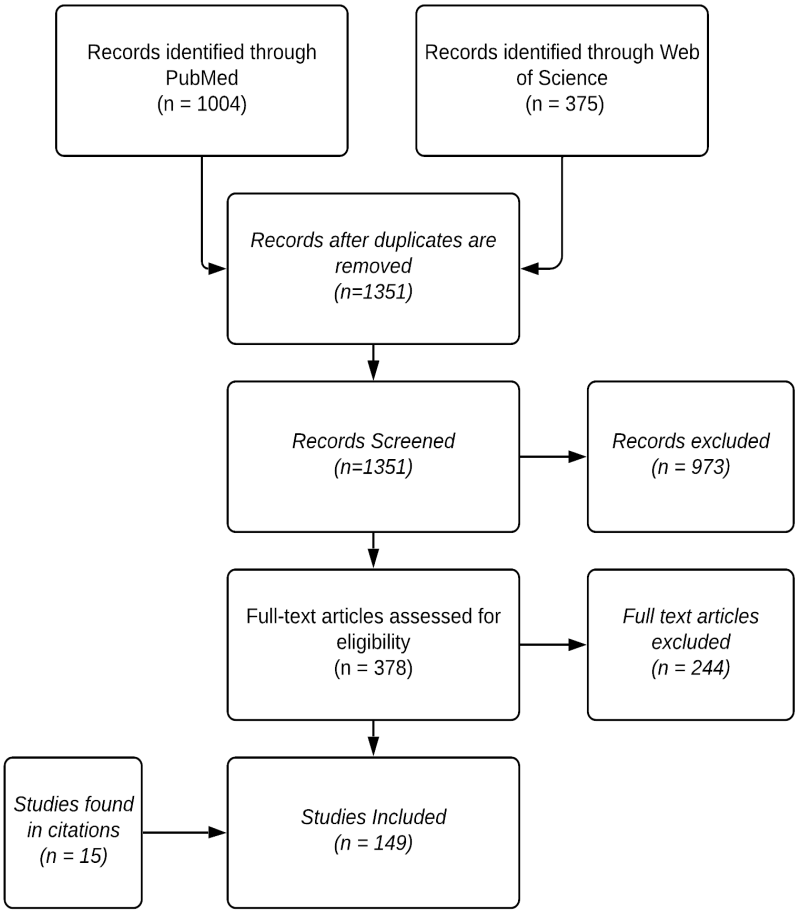

Figure 3.1: PRISMA flowchart for systematic literature review.

Table 3.1: Summary of the exposures across all groups

Challenge Groups		495
Group size (mean (SD))		15.80 (16.71)
Inoculum Dose (mean (SD))		5.91 (1.47)
[Log10 TCID50 or EID50]		
Inoculum Volume (mean (SD))		0.72 (0.46)
Route of inoculation (%)	aerosol	1 (0.2)
	inhalation	1 (0.2)
	intranasal	411 (93.0)
	intranasal/oral	14 (3.2)
	nasopharyngeal	12 (2.7)
	oral	3 (0.7)
Virus (%)	Attenuated	362 (73.1)
	Wild-Type	133 (26.9)
Virus type (%)	A	458 (92.5)
	B	37 (7.5)

Table 3.2: Summary for each outcome

Outcomes	Proportion Infected	Mean Viral Peak	Proportion Systemic	Significant increase HAI titer
Challenge Groups	479	162	204	188
Mean group size (mean (SD))	15.35 (16.05)	15.26 (9.24)	15.80 (11.99)	14.58 (8.91)
Mean inoculum dose (mean (SD)) [Log10 TCID50 or EID50]	5.93 (1.48)	6.15 (1.25)	6.03 (1.27)	6.17 (1.21)
Inoculum volume (mean (SD))	0.73 (0.46)	0.53 (0.12)	0.68 (0.52)	0.65 (0.38)
Route of inoculation (%)				
inhalation	1 (0.2)	0 (0.0)	1 (0.5)	0 (0.0)
intranasal	396 (93.0)	151 (100.0)	182 (93.3)	175 (98.3)
intranasal/oral	14 (3.3)	0 (0.0)	6 (3.1)	0 (0.0)
nasopharyngeal	12 (2.8)	0 (0.0)	4 (2.1)	3 (1.7)
oral	3 (0.7)	0 (0.0)	2 (1.0)	0 (0.0)
Virus preparation (%)				
Attenuated	349 (72.9)	105 (64.8)	139 (68.1)	153 (81.4)
Wild-Type	130 (27.1)	57 (35.2)	65 (31.9)	35 (18.6)
Virus Type (%)				
A	446 (93.1)	148 (91.4)	197 (96.6)	182 (96.8)
B	33 (6.9)	14 (8.6)	7 (3.4)	6 (3.2)

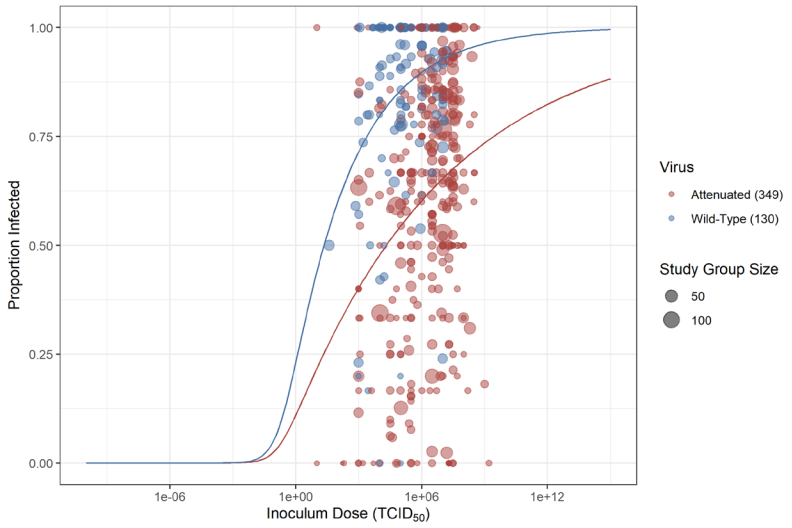

Figure 3.2: Impact of Inoculum Dose on proportion infected stratified by wild-type and attenuated. Weighted fit using approximate beta Poison function.

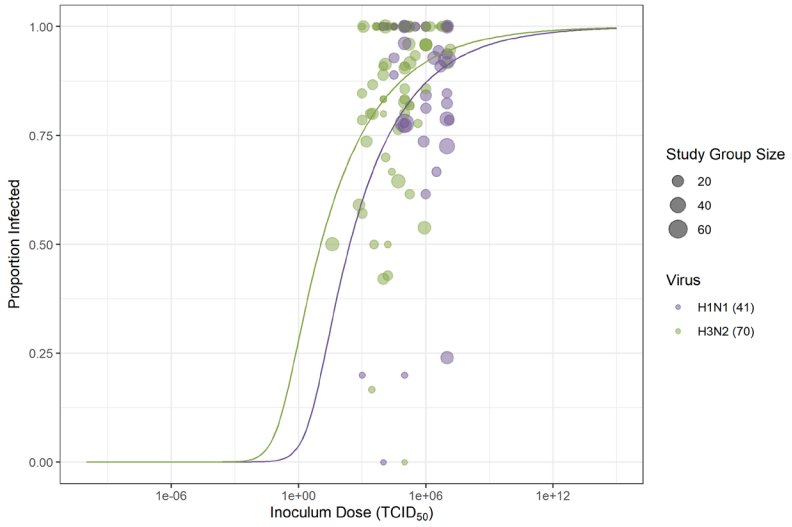

Figure 3.3: Impact of Inoculum Dose on proportion infected wild-type stratified by subtype. Weighted fit using approximate beta Poison function.

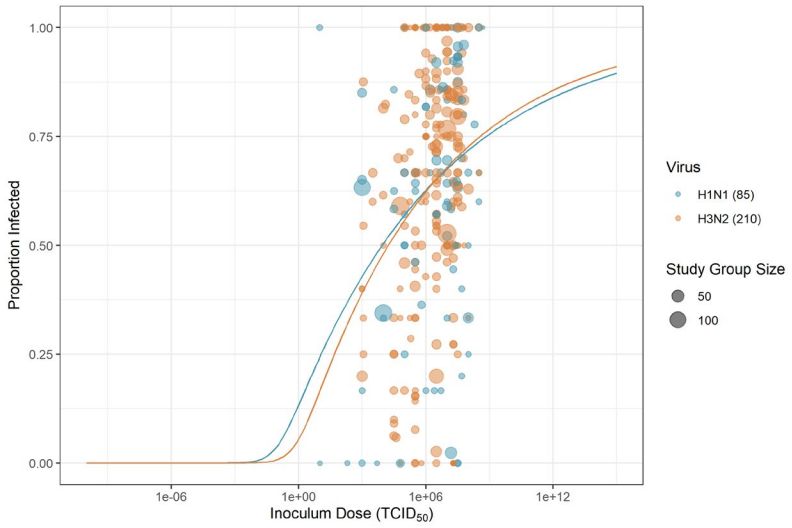

Figure 3.4: Impact of Inoculum Dose on proportion infected wild-type stratified by subtype. Weighted fit using approximate beta Poison function.

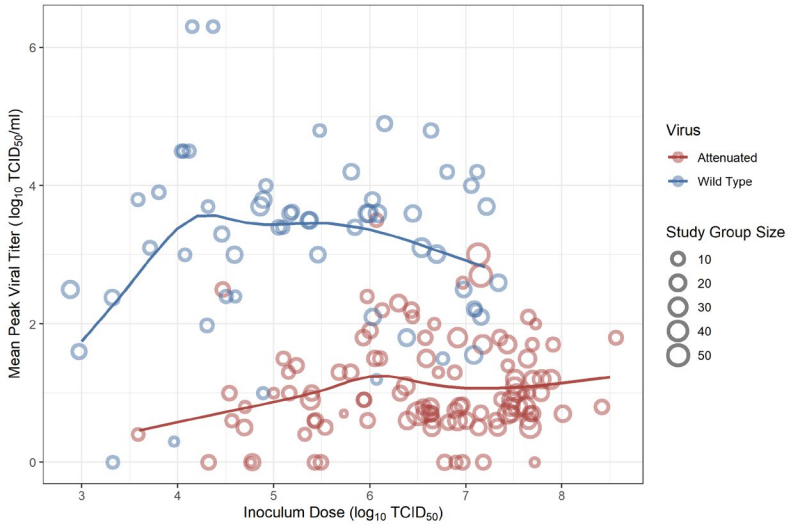

Figure 3.5: Impact of Inoculum Dose on mean peak viral titer Weighted. Stratified by wild type vs attenuated

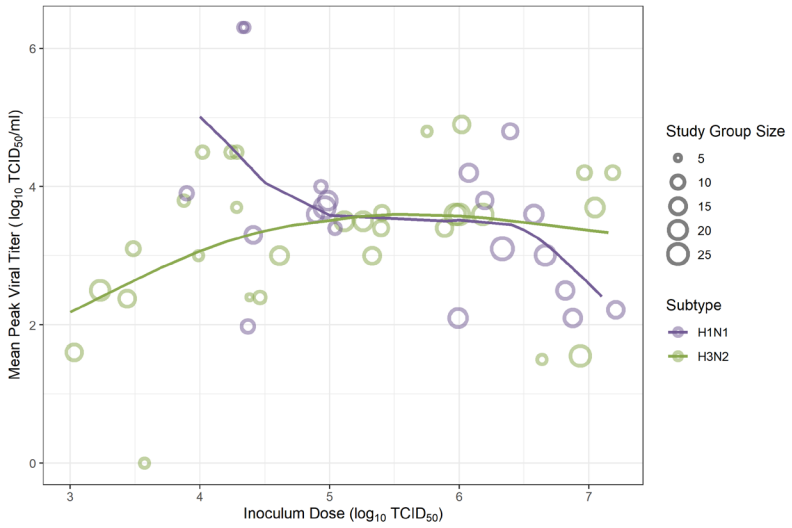

Figure 3.6: Impact of Inoculum Dose on mean peak viral titer Weighted. Wild type stratified by subtype

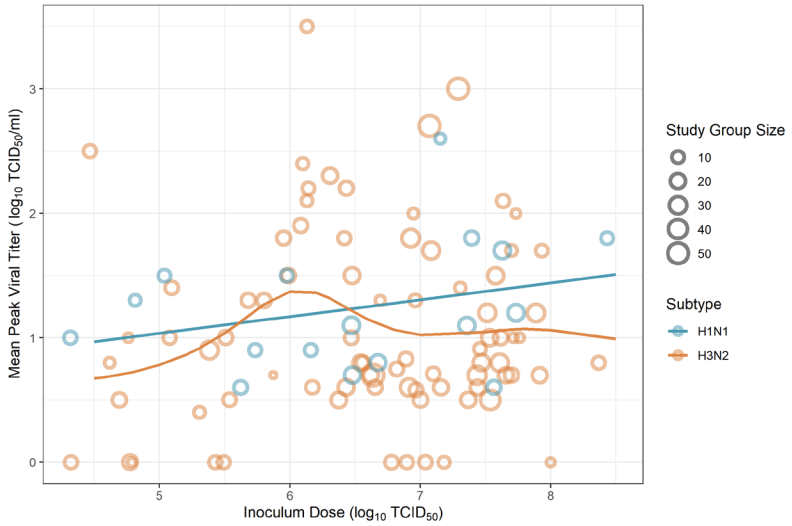

Figure 3.7: Impact of Inoculum Dose on mean peak viral titer Weighted. Attenuated stratified by subtype

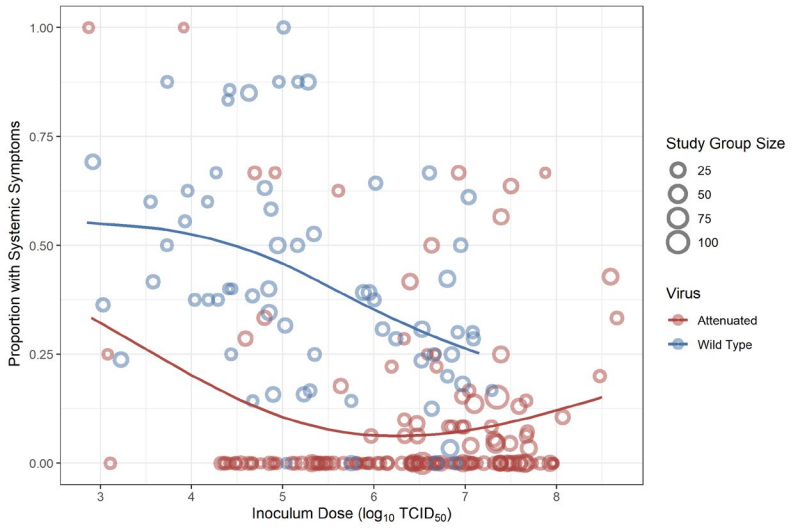

Figure 3.8: Impact of Inoculum Dose on Proportion Systemic Weighted. Stratified by wild type vs attenuated.

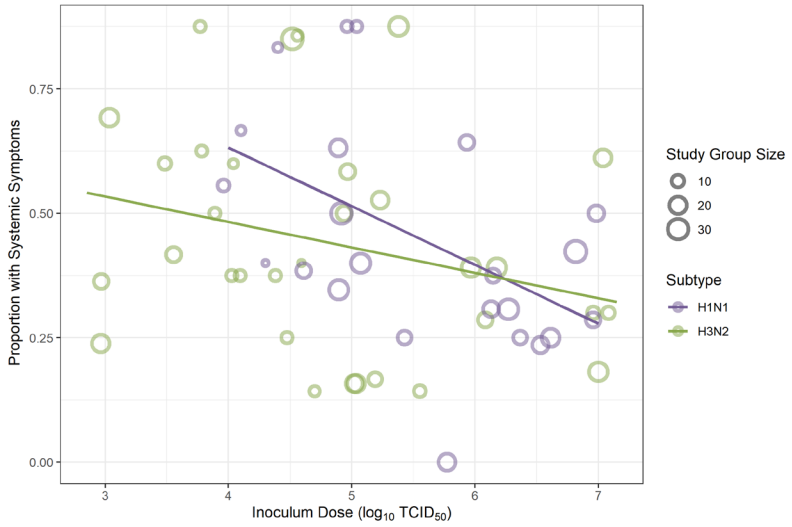

Figure 3.9: Impact of Inoculum Dose on Proportion Systemic Weighted. Wild type virus stratified by subtype.

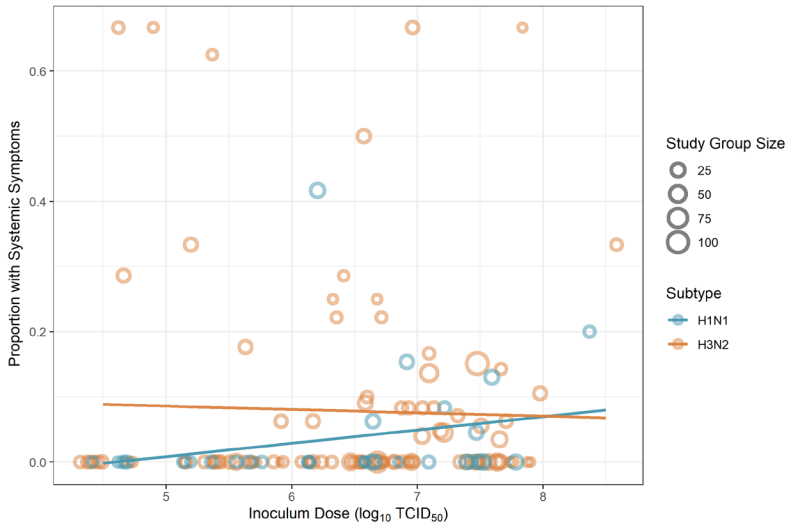

Figure 3.10: Impact of Inoculum Dose on Proportion Systemic Weighted. Attenuated virus stratified by subtype.

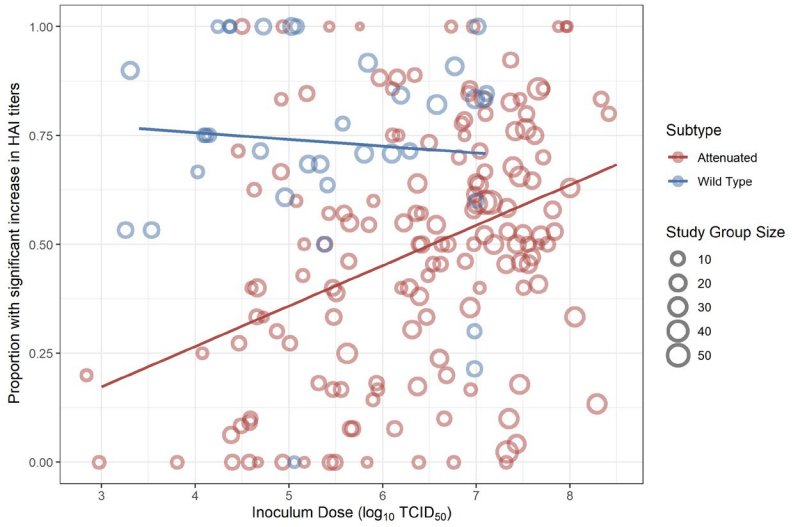

Figure 3.11: Impact of Inoculum Dose on proportion of patients with 4-fold or significant increase in HAI. Stratified by wild type vs attenuated.

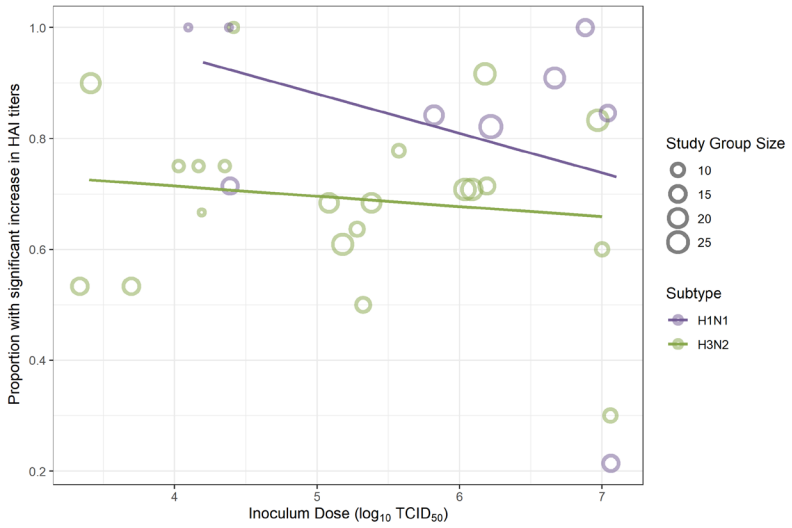

Figure 3.12: Impact of Inoculum Dose on proportion of patients with 4-fold or significant increase in HAI. Stratified by wild type vs attenuated

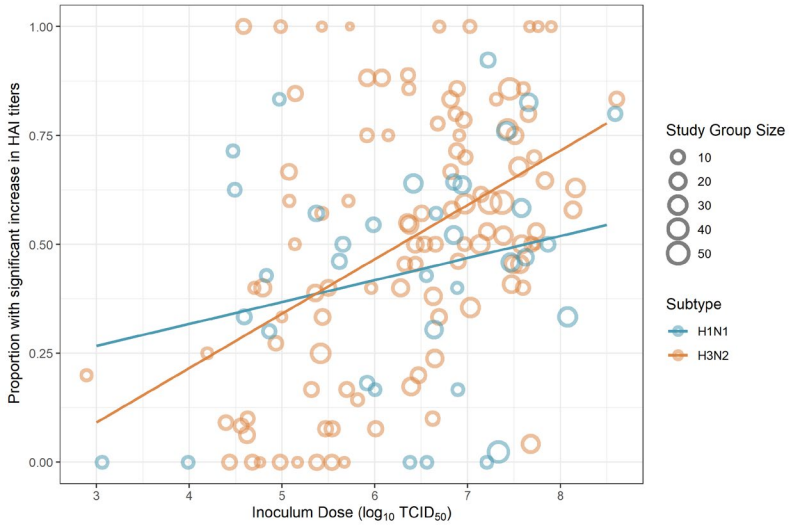

Figure 3.13: Impact of Inoculum Dose on proportion of patients with 4-fold or significant increase in HAI. Wild type virus stratified by subtype

CHAPTER 4

VIRULENCE-MEDIATED INFECTIOUSNESS AND ACTIVITY TRADE-OFFS AND THEIR IMPACT ON TRANSMISSION POTENTIAL OF PATIENTS INFECTED WITH INFLUENZA[3]

[3] McKay B, Ebell M, Dale AP, Shen Y, and Handel A. Submitted to *Proceedings of the Royal Society B*, 08/27/19.

4.1 Abstract

Most communicable diseases have some amount of virulence that induces infectiousness-enhancing symptoms. However, too much virulence can cause host morbidity and a reduction in transmission potential. For human diseases, the reduction in transmission opportunities is commonly caused by reduced activity. There is limited data regarding the potential impact of virulence on transmission potential. We analyzed data of 326 influenza patients at a university health center during the 2016/2017 influenza season. We classified symptoms as infectiousness-related or morbidity-related and calculated two scores. The scores were used to explore the relationship between infectiousness, morbidity, and activity levels. We found a decrease in activity levels with increasing morbidity scores. There was no consistent pattern between activity level and infectiousness score. We also found a positive correlation between the morbidity and infectiousness scores. Our results provide evidence that for influenza, increasing virulence leads to increased infectiousness and reduced activity. This trade-off determines the transmission potential. Our findings suggest that a reduction of systemic symptoms may increase host activity without reducing infectiousness. Therefore interventions should target both systemic and infectiousness related symptoms to reduce overall transmission potential. Our findings can also inform simulation models to investigate the impact of different interventions on transmission.

4.2 Introduction

Many infectious diseases cause symptoms in at least some of their hosts. Often, those symptoms increase the host's infectiousness and facilitate the transmission of the pathogen [12,69,70]. Coughing and sneezing for respiratory infections are prime examples. On the other hand, symptoms that are too severe may reduce host activity or in extreme cases cause host death, reducing transmission opportunities. The trade-off hypothesis describes the relationship between

virulence and transmission potential [71–77] and predicts that an intermediate level of virulence leads to maximum fitness (usually quantified by the reproductive number) for the pathogen. At such an optimal level of virulence, the pathogen maximizes transmission by inducing symptoms that increase a host's infectiousness, while minimizing transmission-reducing morbidity symptoms. The optimal virulence level can depend on both population-level and within-host level processes, the implications of which have been theoretically explored previously [71,72,79–89].

The most commonly discussed and studied trade-off is between increasing transmission potential due to increased host infectiousness and decreasing transmission potential due to host mortality [72]. While, this likely applies to many animal diseases and some human diseases (e.g., viral hemorrhagic diseases [90]), for most human pathogens mortality is low, and it is more likely that increased virulence leads to reduced host activity and thus reduced transmission opportunities. Sub-lethal impacts such as weight loss and effects on host fitness have been suggested [72,73,91,92], and interactions between symptoms, activity, and transmission potential have been recognized [93]. Despite this, there is very little data available for human pathogens. One study on *Plasmodium falciparum* infections in humans showed an increase in transmission potential as virulence, quantified by mortality, increased, with no apparent trade-off [94]. A study in HIV infected individuals showed a negative relationship between duration of asymptomatic infection and viral load and a positive relationship between infectiousness and viral load with optimal transmission potential occurring at an intermediate viral load [95]. As far as we are aware, no studies for any other human pathogens have examined data to directly determine the relationship between virulence and transmission.

Here, we investigate this relationship for influenza. Influenza induces symptoms in around 84% of infected individuals [10]. Some of the symptoms, such as coughing and sneezing, likely

enhance transmission by increasing infectiousness of a host. A recent study provided estimates for the transmission potential of symptomatic versus asymptomatic individuals and found that individuals with symptomatic infections are about 3-12 times as infectious as persons with asymptomatic infections [11]. Other symptoms, such as fever, body aches, and general malaise are more likely to lead to a reduction in transmission by reducing host activity. A previous study on influenza in 146 adults and children in the United Kingdom found that healthy individuals had a mean of 12.72 contacts per day, while sick individuals only had a 3.58 [12]. The study also showed that the number of contacts decreased as the number of symptoms increased. These studies suggest that there might be a trade-off between infectiousness and activity for influenza, which together determines overall transmission. In this study, we investigate this relationship.

4.3 Methods

Data Collection

Our patient population consisted of students who made an appointment at the university health center of a large research university from December 2016 to February 2017. The study participants were selected sequentially and included all patients with a primary complaint of respiratory infections. All participants were required to fill out an electronic questionnaire. The questionnaire collected data about their current symptoms and activity level. A response was required for all symptom-related questions when they scheduled their appointments. We included all symptoms collected by the questionnaire in this analysis. The complete questionnaire is available in the supplementary material.

For the symptoms of weakness and body aches, the patient graded the severity of the symptom as none, mild, moderate, and severe. The patient recorded all other symptom data as present or absent. The patient also reported any changes in their normal behavior. Patients describe

their activity level as a number between 0 and 10, with 10 indicating no change in regular activity and 0 being bedridden.

The study population includes all patients with a diagnosis of influenza. The data and results presented in the main text includes patients diagnosed with a rapid antigen or rapid PCR test. To address the impact of the influenza diagnosis method we performed the same analyses for all patients diagnosed with influenza regardless of the method used. The results are in the supplementary material.

The institutional review board approved the study protocol. Data on PCR results for patients is from a study funded by Roche Diagnostics.

Data Cleaning

We cleaned the data to format the variables and to check for variables with potential errors or missing entries. During the cleaning process, we removed uninformative variables which we defined as any symptoms found to occur in less than 5% of patients. The symptoms of blurred vision and hearing loss both had a prevalence of less than 5%, so they were not considered for further analysis. To allow easy comparison of all symptom variables, we dichotomized weakness and body aches to "absent" or "present".

Analysis

We assessed the univariate relationships between activity and each symptom using linear regression treating activity level as a continuous variable. We also performed multiple linear regression. We determined the variables to include in our final model with a sequential forward floating selection, minimizing the root mean square error (RMSE) on test data through a 5-fold cross validation (20 times repeated) [171].

Next, we constructed two cumulative scores, one for overall infectiousness and one for overall morbidity. To that end, we divided all symptoms into those related to infectiousness and those related to morbidity. We defined morbidity symptoms as symptoms that influence overall feelings of well-being but are not associated with infectiousness. Infectiousness symptoms are any symptoms that could plausibly contribute to passing the virus from an infected host to another. Importantly, the grouping of variables to either one of these categories and inclusion of symptoms in the scores was based on *a priori* medical and biologic considerations, independently of any observed correlation with activity level. Doing so prevents any circular reasoning since only including symptoms correlated with activity would, of course, generate a score which would match the impact on activity level. These scores are similar to systemic and respiratory scores used in past studies [9,108].

To prevent redundant variables from being included in the score, we calculated Yule's Q between symptoms within each category [172]. Only one of a pair of symptoms was incorporated in the score if the correlation coefficient was higher than 0.9 [173]. We also performed a sensitivity analysis using 0.75 as the cut off for identifying redundant symptoms. The results of this sensitivity analysis is in the supplementary material.

For highly correlated symptom pairs, we included the one in the score with the best balance (closest to 50%) of symptom presence or absence. We summed the symptoms in each category based on absence or presence, creating two scores. Correlations between the infectiousness score, morbidity score, and activity were assessed using Spearman correlation [174,175] and the generalized Mantel–Haenszel procedure [176,177]. Linear regression lines are included in the plots to help visualize the relationships.

All analyses were completed using R (version 3.5.3). We used the mlr package for cross-validation [178], vcdExtra to compute Yule's Q and the CHM trend test [179], DescTools to compute Spearman's rank correlation coefficient and corresponding confidence intervals [180].

4.4 Results

Study Population

During the study period, 2326 patients had a respiratory complaint and filled out the questionnaire. Among those, 326 had a lab-based diagnosis of influenza (PCR or rapid antigen). The following analyses focus on those patients since they are most likely to actually be infected with influenza. For analyses of patients who received a flu diagnosis with either the tests or empirically from a physician, see the supplemental material.

Those patients with influenza reported activity levels ranging from 0 to 10 with a median of 4 (SM Figure 4.1). All of the patients reported symptoms, with only 14% reporting 10 or fewer (out of a total of 25). The most common symptom was coughing and the least common was abdominal pain (Table 4.1).

Univariate and subset selection

We assessed correlations between activity level and each symptom in a univariate linear analysis (Table 4.2). All of the statistically significant symptoms had a negative correlation with activity level (Table 4.2). Next, we considered a multi-variable regression model and performed variable selection based on cross-validated minimization of RMSE. We found that the best performing model was one that included chest congestion, headache, sleeplessness, subjective fever, vomiting, and weakness (Table 4.2). While vomiting is not a common symptom of influenza, in those patients who did report vomiting it lead to major reductions in their activity.

Computation of Infectiousness and morbidity scores

We divided symptoms into infectiousness-related and morbidity-related and used them to construct an infectiousness and morbidity score. To prevent circular reasoning regarding associations between those scores and activity, the division and potential inclusion of symptoms into each score was done based purely on biological considerations, without regard for any associations with activity found in the previous analysis. We classified coughing, chest congestion, sneezing, runny nose, and nasal congestion as infectiousness related symptoms. The symptoms of subjective fever, having chills and or sweats, body aches, weakness, headache, fatigue, sleeplessness, breathlessness, wheezing, chest pain, sore throat, abdominal pain, diarrhea, nausea, vomiting, ear pain, tooth pain, eye pain, itchy eyes, and swollen lymph nodes were classified as morbidity related symptoms.

Among the symptoms related to infectiousness only cough and chest congestion correlated with each other at a level of greater than 0.9 (SM Figure 4.2). We kept chest congestion since it was more balanced then cough, which was present in 94% of patients. Among the morbidity symptoms, only vomiting and weakness correlated greater than 0.9 (SM Figure 4.3). Vomiting was included in the score since it was more balanced then weakness, which was present in 94% of patients. For the results of the sensitivity analysis using 0.75 as the cut off for identifying redundant symptoms, see the supplementary material.

The infectiousness score included all the candidate symptoms except cough, and the morbidity score included all the candidate symptoms except weakness. Each symptom present in a patient, contributed one point to its respective score. The calculated infectiousness score had a possible range of 0 to 4, and the morbidity score had a possible range of 0 to 19.

The median infectiousness score was 3. Only 17 patients had an infectiousness score of 0, 39% had a score of 2 or less, and 29% of patients had the maximum possible score of 4 (Figure 4.1A). The mean morbidity score was 8.6, and no patients had a morbidity score of 0, 1, 18 or 19 (Figure 4.1B). The centered distribution was expected since all the patients felt sick enough to seek medical care, but none were sick enough to require urgent care or hospitalization.

Impact of Infectiousness Score on Activity

Analysis of the association between the infectiousness score and the patient's self-reported activity level suggests that the value of this score has a small impact on the activity level of a patient, with higher infectiousness correlating with reduced activity. Spearman's rank correlation indicates negative relationship (r= -0.18 (95% CI: -0.28, -0.07)) and the Cochran-Mantel-Haenszel trend test is statistically significant (χ^2= 8.56, df= 1, $p < 0.01$) (Figure 4.2). Note however that the data suggest that the relationship between infectiousness and activity is not linear, but instead curved, with lower activity at both the low and high infectiousness score and maximum activity at intermediate infectiousness. We cannot think of a biological mechanism that might lead to this pattern. The reason the overall trend is negative is likely due to the larger sample sizes for infectiousness scores 2-4. Given that the observed negative trend is small and doesn't show a monotone decline, it is most reasonable to assume based on this data that there is no meaningful relationship between infectiousness score and activity level.

Impact on Morbidity Score on Activity

Analysis of the association between the morbidity score and the patient's self-reported activity level suggests that higher morbidity score is associated with reduced activity levels. Spearman's rank correlation indicates negative relationship (r= -0.33 (95% CI: -0.42, -0.23)) and the Cochran-Mantel-Haenszel trend test is statistically significant (χ^2= 39.34, df= 1, $p < 0.01$)

(Figure 4.3). The observed pattern is consistent and clear, with a reduction of 85% in mean activity level going from the lowest to the highest morbidity score.

Impact of Morbidity Score on Infectiousness Score

Analysis of the relationship between the morbidity and infectiousness scores show a positive correlation. Spearman's rank correlation indicates positive relationship (r= 0.28 (95% CI: 0.17, 0.37)) and the Cochran-Mantel-Haenszel trend test is statistically significant (χ^2= 24.45, df= 1, $p < 0.01$) (Figure 4.4). Apart from the mean activity levels for very low morbidity score values (with very small sample sizes), the pattern is consistent and clear, with an increase of 33% in the mean infectiousness score going from the lowest to the highest morbidity score.

Conceptualizing Our Results

The hypothesis of virulence-transmission trade-off as explained in the introduction assumes that increasing levels of virulence lead initially to an increase in transmission-enhancing symptoms, but at some point, virulence leads to transmission-reducing symptoms, with an optimum for the pathogen at some intermediate level. One can quantify this by considering overall transmission potential, T, to be proportional to the product of per-contact transmission potential, p, contact-rate among infected and susceptible, c, and the duration of infectiousness d. All 3 quantities can potentially be impacted by virulence, v i.e. $T \sim p(v) \times c(v) \times d(v)$. Unfortunately, for our study we do not have information on the duration of infectiousness. While it is quite likely that virulence can impact the duration of infectiousness, for the following discussion we assume d to be constant. In that case we have $T \sim p(v) \times c(v)$. Overall transmission potential is optimized when $p \times c$ is maximized. Figure 4.5 illustrates graphically a relation for contact rate, per-contact transmission potential and overall transmission potential as a function of virulence.

4.5 Discussion

We believe that this is the first study that investigates a trade-off between contact-rate and per contact transmission potential for influenza in humans [72,73,181,182]. We showed that for our population, activity decreased as the morbidity score increased, and we found a positive association between morbidity and infectiousness symptoms.

Limitations of the study include not knowing the flu sub-type for those infected. The type and sub-type of the virus can affect the epidemiological features of the disease [16]. Based on influenza surveillance data for the 2016/17 season only 22.1% was influenza B with 77.9% influenza A with the subtype H3N2 making up 97.2% with H1N1 making up the remaining 2.8% [183]. Additionally, we only collected data on individuals who were experiencing symptoms severe enough to seek care. As a result, we do not have data on individuals with low virulence infections. As explained above, such data would allow for a complete exploration across the full range of virulence and to determine relationships between transmission, morbidity, and infectiousness. Finally, our study population was made up of college students, i.e., generally young and healthy individuals. As such their symptoms, infectiousness, and activity behavior distributions might not fully apply to a more general population.

Despite these potential limitations, our study provides valuable information that can be useful to inform current and future interventions targeting influenza. For example, our results suggest that a treatment that only reduces those symptoms that are part of our morbidity score, without affecting symptoms that make up our infectiousness score, could lead to increased transmission. While from the perspective of a patient or clinician a reduction in any symptom may be viewed as a positive, such an intervention might lead to worse outcomes on the population level. Current FDA approval of anti-influenza drugs rely on showing an impact on the symptoms, with

a focus on more severe and systemic (i.e., morbidity) symptoms [143,184,185]. From a population perspective, it is essential that such drugs also reduce host infectiousness [185–187]. Some evidence for this has been found in previous studies[187–190] as well as being explored in mathematical models [191,192].

Population-level control of infectious diseases makes increasing use of mathematical models [193]. The need for these models to be accurate is critical. Researchers have increasingly recognized that capturing human behavior changes during an infectious disease outbreak, both for uninfected and infected individuals is relevant [194,195]. As far as we are aware, only one previous modeling study for influenza has tried to capture the impact of infection on behavior [196]. Previous studies have shown that symptoms aid infectiousness and impact the number of contacts [11,12]. In our analysis, we found an 85% reduction in mean activity as a result of increased morbidity. Using data from our study and past studies [11,12] is a starting point for future models that can explore the impacts of infectiousness and contact behavior of infected hosts [93].

4.5 Figures and Tables

Table 4.1. Symptoms of the 326 patients. The table shows the number of patients who reported having the following symptoms and the corresponding percentage.

Symptom Present	**n (%)**
Abdominal Pain	39 (12.0)
Breathlessness	131 (40.2)
Chest Congestion	197 (60.4)
Chest Pain	110 (33.7)
Chills/Sweats	287 (88.0)
Cough	308 (94.5)
Diarrhea	40 (12.3)
Ear Pain	59 (18.1)
Eye Pain	47 (14.4)
Fatigue	304 (93.3)
Headache	272 (83.4)
Itchy Eyes	73 (22.4)
Myalgia	290 (89.0)
Nasal Congestion	257 (78.8)
Nausea	119 (36.5)
Runny Nose	235 (72.1)
Sleeplessness	183 (56.1)
Sneeze	179 (54.9)
Sore Throat	268 (82.2)
Subjective Fever	242 (74.2)
Swollen Lymph Nodes	131 (40.2)
Tooth Pain	60 (18.4)
Vomiting	44 (13.5)
Weakness	307 (94.2)
Wheezing	106 (32.5)

Table 4.2. Results of the univariate and multivariate linear regression of symptoms and activity. The coefficients are the estimated effect on activity when the symptom is present. The multivariate model was selected with a sequential forward floating selection, minimizing the RMSE on test data through a 5-fold cross validation (20 times repeated). 95%CI = The 95% confidence interval for the coefficient.

	Univariate analysis	**Multivariate analysis model**
Symptom	**Coefficient (95%CI, P value)**	**Coefficient (95%CI, P value)**
Abdominal Pain	-1.02 (-1.91 to -0.14, p=0.023)	
Breathlessness	-0.22 (-0.81 to 0.37, p=0.466)	
Chest Congestion	-0.72 (-1.31 to -0.14, p=0.016)	-0.54 (-1.08 to 0.01, p=0.052)
Chest Pain	-0.43 (-1.05 to 0.18, p=0.162)	
Chills/Sweats	-1.66 (-2.53 to -0.78, p<0.001)	
Cough	0.10 (-1.17 to 1.37, p=0.877)	
Diarrhea	-0.72 (-1.60 to 0.15, p=0.106)	
Ear Pain	-0.69 (-1.44 to 0.06, p=0.070)	
Eye Pain	0.17 (-0.66 to 0.99, p=0.689)	
Fatigue	-1.67 (-2.81 to -0.53, p=0.004)	
Headache	-1.57 (-2.33 to -0.81, p<0.001)	-1.15 (-1.89 to -0.42, p=0.002)
Sleeplessness	-1.17 (-1.74 to -0.60, p<0.001)	-0.93 (-1.47 to -0.40, p=0.001)
Itchy Eyes	-0.74 (-1.43 to -0.05, p=0.035)	
Myalgia	-1.24 (-2.15 to -0.32, p=0.008)	
Nasal Congestion	-0.24 (-0.95 to 0.47, p=0.507)	
Nausea	-1.06 (-1.65 to -0.47, p<0.001)	
Sore Throat	-0.37 (-1.13 to 0.38, p=0.330)	
Runny Nose	-0.55 (-1.20 to 0.09, p=0.091)	
Sneeze	-0.71 (-1.29 to -0.14, p=0.015)	
Subjective Fever	-1.32 (-1.96 to -0.67, p<0.001)	-0.93 (-1.56 to -0.30, p=0.004)
Swollen Lymph Nodes	-0.54 (-1.13 to 0.05, p=0.073)	
Tooth Pain	-0.28 (-1.03 to 0.47, p=0.463)	
Vomiting	-1.67 (-2.49 to -0.84, p<0.001)	-1.46 (-2.24 to -0.68, p<0.001)
Weakness	-2.46 (-3.67 to -1.26, p<0.001)	-1.40 (-2.57 to -0.23, p=0.019)
Wheezing	-0.54 (-1.16 to 0.08, p=0.085)	

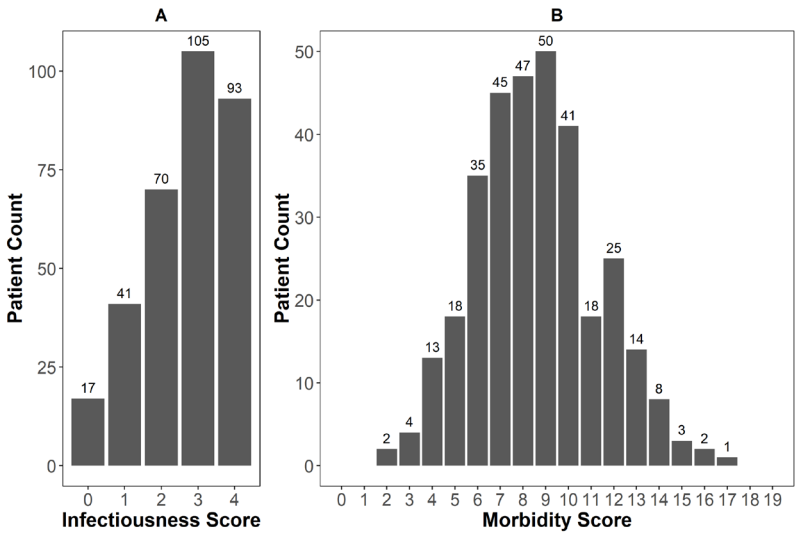

Figure 4.1: (A) The distribution of infectiousness score with counts for each level. (B) The distribution of the morbidity score with counts for each level. There are no patients with a score of 0, 1, 18, and 19.

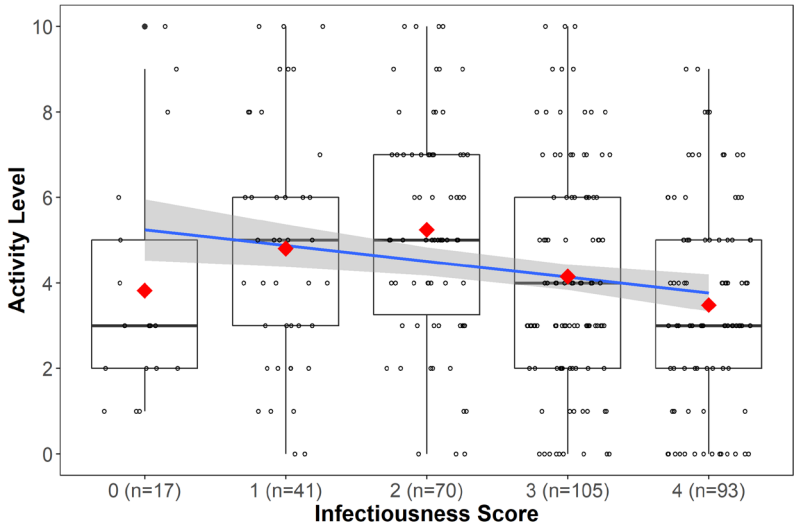

Figure 4.2: Activity level for each level of the infectiousness score. Red diamonds indicate the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

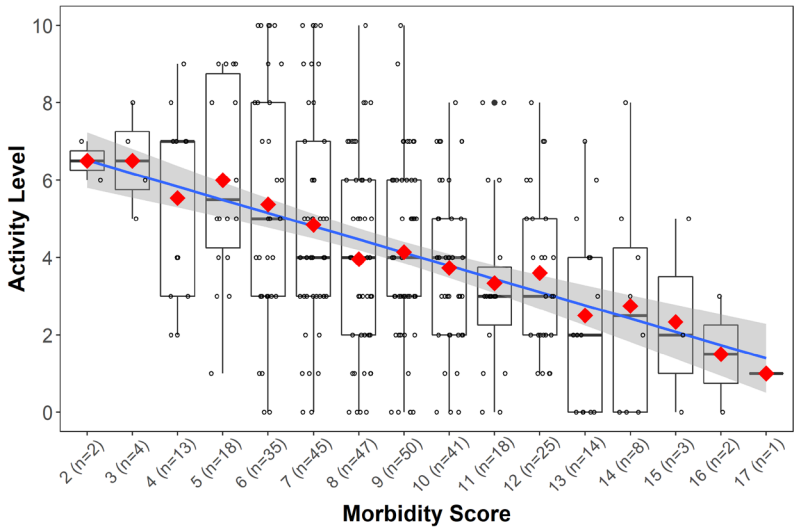

Figure 4.3: Activity level for each level of the morbidity score. Red diamonds indicate the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

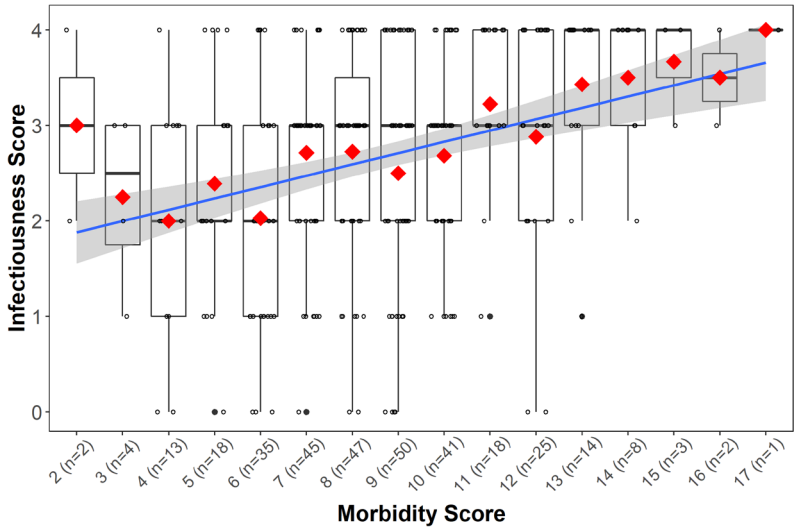

Figure 4.4: Infectiousness score for each level of the morbidity score. Red diamonds indicate the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

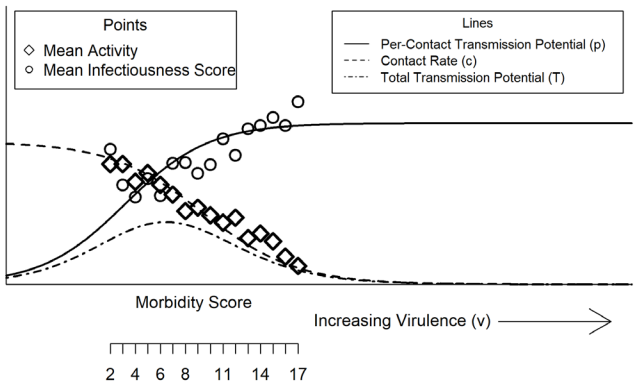

Figure 4.5: This figure illustrates conceptually the hypothetical impact of virulence on total transmission potential (T) resulting from a trade-off between per-contact transmission potential (p) and contact-rate (c). The lines are for illustrative purposes only and not fitted to the data. We are using morbidity as a proxy for virulence. We placed our data in the middle of the full virulence scale since we did not capture anyone not sick enough to seek care nor did we capture anyone who was so ill they were hospitalized or died. The values for infectiousness and activity are re-scaled to allow better visualization. The actual mapping between our measured quantities and the theoretical contact rate and per-contact infectiousness are not known, but based on past research it is feasible to expect that a proportional relationship exists.

CHAPTER 5

ASSOCIATIONS BETWEEN RELATIVE VIRAL LOAD AT DIAGNOSIS AND INFLUENZA A INFECTION SEVERITY AND RECOVERY[4]

[4] McKay B, Ebell M, Billings WZ, Dale AP, Shen Y, and Handel A. To be submitted to *Journal of Clinical Virology*

5.1 Abstract

Introduction: Rapid point of care PCR diagnostic tests are more accurate than current antigen-based tests. Currently, these tests provide a qualitative result of positive or negative, but additional information about the relative viral load could be calculated. Such quantitative information might be useful for making treatment decisions. We perform an analysis to evaluate the viral load from a rapid PCR test at diagnosis in non-hospitalized patients to predict symptom resolution and disease impact.

Methods: We sequentially enrolled 300 students at a university health center who presented with cough and one additional flu-like symptom from December 2016 to February 2017. Data were collected before, during, and five days after the clinic visit. All those enrolled in the study received a point of care PCR test (cobas Liat) to determine the presence or absence of influenza (A or B). The relative viral load was calculated for patients with a positive test for influenza A. We then assessed the relationship between the relative viral load and patient-reported activity, symptom scores, fever, duration of fever, improvement in cough, days of work or class missed, and duration of symptoms.

Results: Of the 289 students with a valid test, 136 were positive for influenza A. We found a positive correlation between viral load and body temperature at the clinic visit. The duration of symptoms appeared to have a negative correlation but was not statistically significant likely due to a potential lack of power. We did not find any correlation between viral load and patient-reported activity, symptom scores, duration of fever, improvement in cough, or days of work or class missed.

Discussion: While we found a correlation between relative viral load and body temperature, overall, for our study population of young, overall healthy adults, we did not find that viral load provided additional information that could help in determining treatment and disease outcome. It is important to note that this may not generalize to other populations. It could be that viral load contains important independent information for specific groups of patients, like young children or older adults. Further studies on those populations are warranted.

5.2 Introduction

Diagnostic polymerase chain reaction (PCR) tests are a sensitive and specific method for determining the presence of many pathogens. Until recently, PCR methods were expensive, time-consuming, and required specialized equipment and staff. As a result, the application of PCR tests for diagnostic purposes is limited. There are two Clinical Laboratory Improvement Amendments (CLIA)-waived point-of-care (POC) PCR systems, Xpert Xpress by Cepheid, and cobas Liat by Roche [96,97], available to physicians. These systems can provide highly accurate results in 20-30 minutes without the need for a laboratory or highly trained staff. As the price decreases and the number of pathogens that can be detected increases, these systems will likely have a positive impact on the care of patients.

Currently, the cobas Liat system is only used to produce a qualitative result based on the internal threshold of optical brightness. The system provides the result as either positive (present) or negative (absent) for the pathogen. While these systems are not currently used to estimate the viral load in the sample, it is possible to estimate the viral load using the number of cycles required to generate a positive test, with more cycles associated with a lower viral load [98–100]. This quantitative measurement could potentially give a physician additional information that could help determine the appropriate treatment and advice regarding prognosis for patients. For both influenza

and other pathogens, the pathogen load correlates with factors such as disease severity, treatment success, and risk of transmission [9,101–108].

Previous studies have looked at the relationship of a single measure of viral load at diagnosis and the characteristics of the disease and patients with seasonal influenza [99,109–113]. The results of these studies have been mixed with some reporting associations [109–111,113,114], and others reporting no associations with clinical characteristics of disease [99,112]. The time since onset of disease and the viral load has been explored in 5 studies [99,109–111,113], and all but one found a relationship [113]. Only one study has looked at disease outcomes of hospitalized patients with influenza [113]. Analyses from other seasonal influenza infection studies based on repeated measurement of viral load show a reduction of viral load correlates with a decrease in symptoms as well as other clinical outcomes [114–119]. All of the previous studies relied on standard quantitative PCR methods that require significant resources to implement.

We set out to study outpatient based PCR results from the cobas Liat POC test to determine if viral load measurement provided useful additional information about a patient's disease progression or recovery. Our study is unique in that our study population was from a primary care setting, use of a POC PCR test, and the inclusion of outcomes for disease resolution five days after the patients visit. The goal of our analysis was to describe the relative viral load at diagnosis based on POC PCR and its potential relevance to physicians.

5.3 Methods

Data Collection

The study used a prospective, non-randomized, sequential-patient design. Participants were recruited from patients who scheduled a clinical appointment due to an upper respiratory complaint at the student health center at the University of Georgia during the 2016-17 flu season from

December 2016 to February 2017. Patients eligible for the study had an upper-respiratory chief complaint before their clinic visit, exhibited cough and one other symptom of influenza-like illness, and were seen at the clinic within a week of symptom onset. If all criteria were met and patients gave informed consent, they were enrolled in the study at the start of their clinic visit. The enrolled patients received a POC PCR (Roche cobas Liat) diagnostic test for influenza. Study inclusion and exclusion criteria have been previously published [197]. All eligible patients were enrolled in the study sequentially until 300 study participants were enrolled. The study population for our analysis consists of the 136 patients from the study who had a positive PCR test for influenza A.

We obtained data from patients at the time they scheduled an appointment, during their visit, and five days after their visit. Patients with an upper respiratory chief complaint who tried to make an appointment with the health center were required to fill out a survey before a clinic visit. Responses were required for all the survey questions, and once submitted, the answers were captured in the patient's electronic health record. During the clinical visit, a healthcare provider recorded signs and symptoms, lab results, diagnosis, and prescribed treatments in the patient's electronic health record (EHR). Finally, five days after the clinic visit, each patient was sent a link to a follow-up survey (the link closed 24 hours after the email was sent). All PCR results were joined to the EHR and follow-up survey data using an anonymized identifier, which was unique to every clinical visit. Copies of the redacted data collection forms are available in the supplementary material (SM).

Data Cleaning

Of the 300 patients enrolled, 289 had valid PCR test results. For this analysis, only data from the 136 participants with a positive PCR result for influenza A are included. One patient's

test was run twice for confirmation, and since both results were identical, we removed one. All of the variables recorded by the previsit survey were considered for inclusion. For the data collected during the clinical visit, only variables regarding symptoms and disease characteristics are included. Symptoms of rash and tooth pain were never recorded as being present and were removed. We only included the three variables from the follow-up survey that each deal with symptom resolution or disease impact. In total, we included 49 variables that measure disease characteristics and patient outcomes.

PCR Data Checking and Processing

We completed univariate analyses of all included variables. The results between the two PCR machines used and the two lots of sample tubes were compared to ensure no artifacts were introduced into the data. The cycle threshold (CT) is the number of amplification cycles the machine ran before a sample was judged to be positive can be used to estimate the viral load from the sample [98,100]. The CT values are inversely proportional to the amount of RNA target present in the sample. The Roche cobas Liat machine performs a set number of amplification cycles; therefore, each patient's relative viral load was calculated using the equation $2^{(x-ct)}$ (x was provided by Roche). All comparisons were made against the base-10 logarithm of the relative viral load (RVL), as it spans multiple orders of magnitude.

Constructing Symptom Scores

As a measure of disease severity, we constructed a total symptom score [108]. Two versions of the total symptom score were created. One of the scores used the patient-reported data from the pre-visit questionnaire. The second score used the symptoms noted by the physicians during the visit. A single point is added for each symptom that was recorded as present. For the patient based score 27 symptoms were considered, and for the physician based score 29 symptoms

were considered resulting in maximum scores of 27 and 29, respectively. Physicians were required to provide an answer for some but not all symptoms. As a result, we classified symptoms as reported or not reported. We calculated the two total symptom scores, one based on the number of symptoms reported by the patient and the other based on the symptoms reported by the physician for each patient.

To account for the potential of strong correlations between symptoms and the 'double counting', we also performed a sensitivity analysis for which we computed the total symptom scores in a somewhat more complicated manner. Details are provided in the SM.

Statistical Analysis

To determine the relationship between numeric variables and the relative viral load, we used simple linear regression to look for trends. Difference between the relative viral load of different categorical variables was assessed with ANOVA. For dichotomous variables, the difference in mean viral load was assessed with a *t*-test. All analyses were completed in R version 3.6.0.

5.4 Results

Study Population

All participants enrolled in the study were college students, age 18 to 25 years, at a major public university. Data were collected at three different times. First patients completed a previsit electronic survey, then data from the visit was recorded in the electronic health record, and finally, a post-visit survey was sent five days after the visit. For our analysis, only patients with a positive test result for influenza A were included, resulting in 136 observations. Out of the 136 records we included in our analysis, 123 had complete data for the pre-visit survey. Thirteen patients enrolled in the study did not fill out the previsit survey when they made their appointment. The enrollment

of these 13 patients likely the result of including patients with two influenza-like symptoms instead of cough plus one additional influenza-like symptom. Second patients may have reported cough verbally to the enrollment staff but not to the physicians. Data recorded during the visit was available for all 136 patients. Finally, 115 out of the 136 completed the post-visit survey. Among the positive patients, the survey had a response rate of 84.6%. Complete tables for each point of data collection are provided in the SM (SM Table 5.1-5.3).

Correlation of Viral Load with Activity Level

There is no relationship between the relative viral load at diagnosis and the patient's level of activity reported on the pre-visit survey (reported between 1-24 hours before the visit) (Figure 5.1). The linear model between the relative viral load at diagnosis and activity level did not indicate any statistically significant trends (β= 0.01 (95% CI: -0.07, 0.09), p= 0.88).

Correlation of Viral Load with Total Symptom Scores

We use the total symptom scores we constructed as a measure of overall disease severity. Since the symptoms, the doctor asks a patient about is not always exhaustive, we use patient-reported symptom data from the pre-visit as well as doctor reported symptoms from the visit data to create two scores.

Total Symptom Scores

We created the total symptom scores as a measure of disease severity. One is based on the symptoms reported by the physician at the time, the diagnostic test was given, and the other based on the patients' self-reported symptoms (1-24 hours) before the diagnostic test was given.

The patients reported scores are on average higher than those reported by the physician with means of 13.45 points and 11.54 points, respectively. Based on visual inspection, there was no apparent relationship between RVL and either of the scores (Figure 5.2).

The linear regression for the physician score did not show any significant trends (β= 0.03 (95% CI: -0.03, 0.09), p= 0.36). Similarly, there was no apparent relationship between the patient reported symptom score and RVL (β= 0.01 (95% CI: -0.05, 0.07), p= 0.78).

The sensitivity analysis of the symptom scores which did not include correlated symptoms showed the same results. Detailed results are shown in the SM.

Correlation of Viral Load with Fever

A previous study showed a relationship between viral load and subjective fever [111]. No subsequent studies have included subjective fever, so we included it see if a relationship would be present in our data. Similarly, a previous study investigated the relationship between RVL and actual body temperature dichotomized as fever or no fever [117]. So, we looked if there was a trend present using body temperature as a continuous value.

There is a positive relationship between the patient temperature taken during the clinic visit and the log10 relative viral load (Figure 5.3). The linear model indicated a statistically significant trend (β= 0.25 (95% CI: 0.10, 0.40), p= 0.001). In the pre-visit survey, patients also reported subjective fever. Mean relative viral load in those with or without subjective fever are 5.18 and 5.55 (log10 RVL), respectively, and the difference is not statistically significant (t= 1.6, df= 54.52, p= 0.12).

Correlation of Viral Load with Symptom Resolution and Disease Impact

Arguably the most useful information would be if the relative viral load as obtained from the PCR test at the visit was predictive of disease progression and outcomes and could provide the physician with additional useful prognostic information. To investigate this, we explored if the relative viral load was predictive of disease impact as well as symptom resolution, using the data from the post-visit survey.

There was no clear relationship between the days of work or class missed by a patient and their relative viral load (Figure 5.4A). The linear regression did not indicate a statistically significant trend (β= -0.17 (95% CI: -0.93, 0.59), p= 0.40).

There was no relationship between patient reported cough recovery and relative viral load at diagnosis (Figure 5.4B). The ANOVA did not have a significant F-test result (F= 0.4976, p= 0.61).

Finally, there was no relationship between a patient's relative viral load and the number of days the patient reported a subjective fever on the follow-up questionnaire. (Figure 5.4C). The linear regression did not indicate a statistically significant trend (β= 0.01 (95% CI: -0.15, 0.18), p= 0.87).

Correlation of Viral Load and Duration of Symptoms

Previous studies have shown a reduction in average viral load as days since symptom onset increases. We see a similar pattern in our data based on visual inspection (Figure 5.5). The linear model did not indicate a statistically significant negative trend (β= -0.15 (95% CI: -0.30, 0.01), p= 0.07). The lack of statistical significance is possibly due to a lack of power.

5.5 Discussion

In both seasonal and experimental infection studies, relationships between viral load and disease caused by influenza have been identified, but the utility of viral load at diagnosis is less clear. Our study is the first we are aware of to use the internal data of a CLIA-waived, POC PCR assay to assess the relative viral load in patients seeking care in a primary care setting. By using a POC PCR assay, it was possible to conduct our study and provide results in a clinically relevant time frame in a primary care facility, for otherwise healthy individuals. Our study is also notable

for the investigation of novel outcomes related to symptom resolution, impact on the days of missed work or class, and patient activity.

Among the new outcomes we investigated, none had a statistically significant correlation with the relative viral load at diagnosis. We found a positive correlation between the relative viral load and body temperature, which has been shown previously for body temperature dichotomized as febrile and afebrile [117]. For subjective fever, we did not see a statistically significant difference in viral load, which had been found in a previous study [111]. The negative relationship between how long a patient has had symptoms and viral load has been shown previously [115–117], while there does seem to be a trend in the figure we did not find a statistically significant negative trend. We also saw that there was not a relationship between relative viral load and the patients' total symptom score regardless if the symptoms were reported by the patient or physician. The relationship between symptom scores and viral load has been demonstrated in both experimental and natural infection studies [9,118].

There are limitations to our current study. The study population only included students who are in general healthier than other individuals the same age. While the study was conducted in a primary care setting, only students enrolled in the university could use the facilities, which could introduce a healthy work type bias. We also do not know the sub-type of the viruses. Our estimation of relative viral load is based on the assumption that everyone was infected with the same sub-type. The sub-type likely has an impact on the cycle time, but we do not have the information required to make these adjustments. Based on national and state surveillance, we can be reasonably confident that the majority of our patients were infected with the same sub-type. During the 2016/17 influenza season, CDC surveillance found that 97.2% of the samples sub-typed were H3N2 [183]. In the state of Georgia, surveillance up to week 8 of the flu season showed

that among all the samples positive for influenza A 97.8% were H3N2 [198]. Another limitation, we were not able to make any assessment of the sample quality, which can impact the number of cycles required to reach a positive threshold [99]. Samples of inferior quality may result in an artificially reduced estimate of viral load. The final limitation of our analysis is that the data was not collected with the primary goal of performing an analysis of the viral load. As such, the post-visit questions were not as detailed and focused as they could have been if the data was collected primarily for the analysis of clinical outcomes. The results of the primary analysis are published [197].

Regardless of the utility of viral load at diagnosis, POC PCR testing is vastly superior to the current rapid antigen tests for accurately diagnosing influenza. These tests are currently more expensive, but the cost is likely to decline in the future. The high sensitivity and specificity of the tests qualitative results provided to the clinical staff can help improve physicians' confidence in their diagnosis and hopefully increase the chance of proper treatment [197,199]. Based on our analysis, it seems that providing quantitative data in the form of the viral load from these tests might not provide useful additional information for the physician. However, it is important to note that our findings may not generalize to other populations. It could be possible that viral load contains important independent information for specific groups of patients like young children or older adults. Further studies on those populations are warranted.

5.6 Figures and Tables

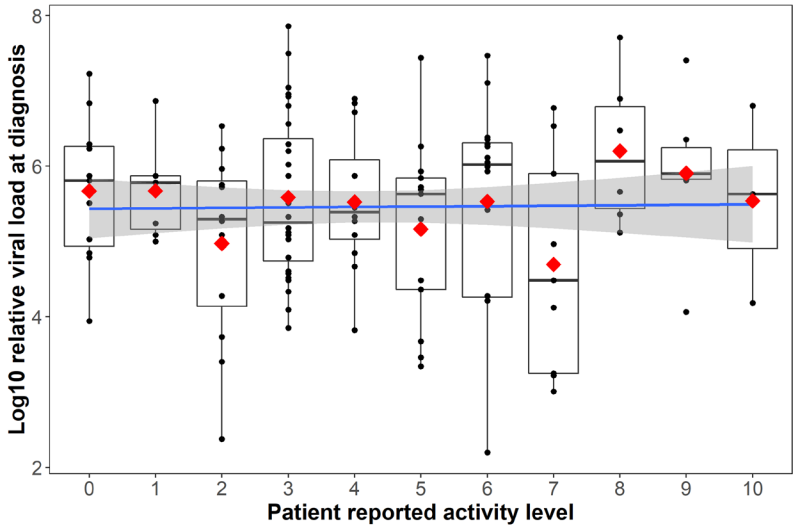

Figure 5.1: Distribution of log10 relative viral load for varying patient-reported activity levels within 24 hours of the test.

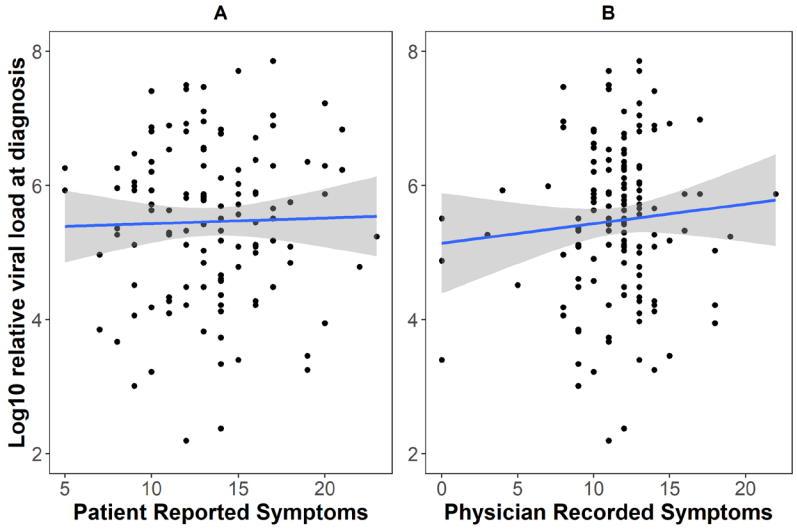

Figure 5.2: A: Relationship between the log10 relative viral load at diagnosis of the patients and the calculated total symptom scores, using symptoms reported by the patient. B: Relationship

between the log10 relative viral load at diagnosis of the patients and the calculated total symptom scores, using symptoms reported by the physician.

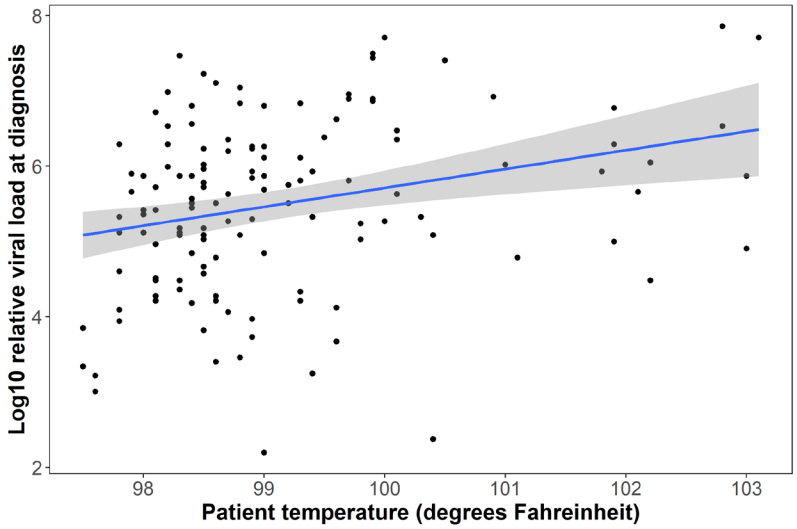

Figure 5.3: Relationship between log10 relative viral load and patient temperature at the clinic visit.

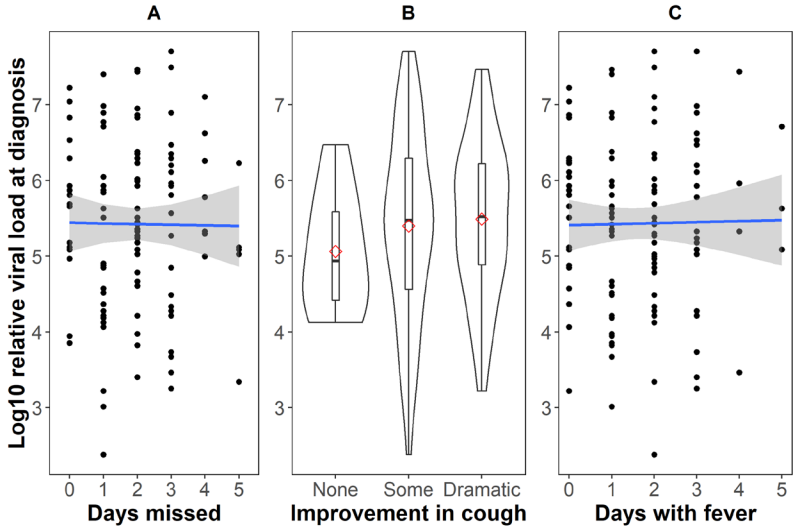

Figure 5.4: A: Relationship between log10 relative viral load and days of work or class missed. B: Relationship between log10 relative viral load and reported recovery from cough five days

after the clinic visit. C: Relationship between log10 relative viral load and reported days fever was present five days after the clinic visit.

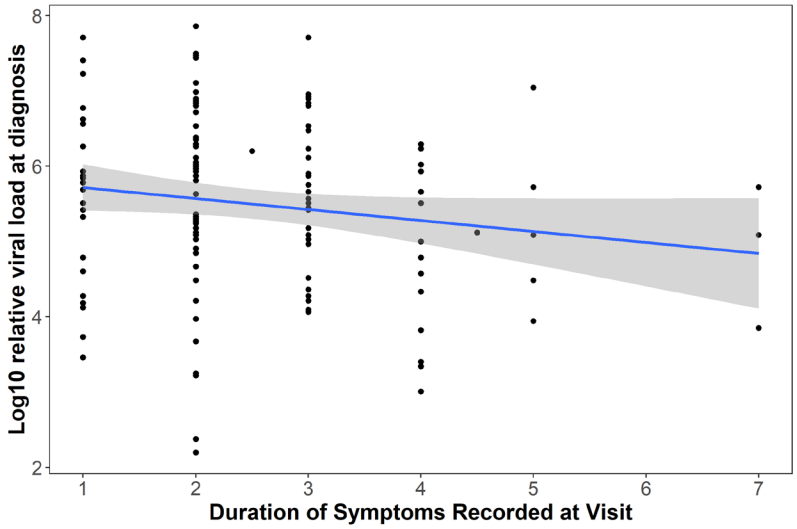

Figure 5.5: Duration of symptoms the patient reported during the visit.

CHAPTER 6

DISSERTATION CONCLUSIONS

This chapter summarizes the conclusions of the research topics covered in the dissertation. No additional results are presented below which have not been covered in the preceding chapters. This is a required summary of the results of the analysis presented in this dissertation.

Chapter 2

A clinic prediction rule did not exist for predicting influenza complications in the outpatient setting. We used data from 4103 patients with influenza-like illness (ILI) enrolled in 11 clinical trials from 1997-2001 to develop prognostic scores for three composite complication outcomes: 1) serious complications (hospitalization, pneumonia, or sepsis) 2) complications that can be treated with antibiotics and 3) complications that required additional treatment.

The scores we developed were based on the multivariate models for patients with ILI and for the subset that were PCR positive for influenza. We also developed fast and frugal trees since they generally the simplest to implement in a clinical setting.

The simple score based clinical prediction rule (CPR), we developed was able to create low, moderate, and high risk groups for both the FLU and ILI populations. The score for serious complications was able to place 19% of FLU and 33.9% of ILI patients in low risk groups who could be reassured of their low risk of complications. The scores showed consistent performance with likelihood ratios of less than 1 for the low-risk group and more than 1 in the high risk groups. The decision trees developed performed well in both populations for hospitalization, pneumonia, and sepsis capturing 66% of patients with a complication with 32% of the ILI and 28% FLU

patients classified as high risk. We have developed and tested the internal validity of 6 clinical prediction scores that successfully classifies patients as being at low, moderate, and high risk for three complications, as well as fast and frugal decision trees.

Further work is need to determine the clinical impact of the scores and decision trees through prospective validation.

Chapter 3

We performed a systematic review of the literature to identify published challenge studies and collect data that would help increase our understanding of the relationship between inoculum dose, viral dynamics, and infection outcomes in humans infected with influenza is critical to creating effective control measures and identifying important clinical aspects of the disease.

We identified 149 influenza challenge studies conducted in 7821 individual volunteers published between 1943 and 2016. We fit both parametric and non-parametric models to the data. To find our best fit parameters we used nonlinear optimization to and cross validation was used to identify the best tuning values for our non-parametric models.

We found that dose response was similar to past studies in regards to probability of infection. Surprisingly we did find a number of decreasing trends for both mean viral peak and proportion of individuals with systemic symptoms when using non-parametric methods. For immune response there was a clear increasing trend for the proportion of individuals with a significant increase in HAI titers but when looking as the ratio of before and after there did not seem to be any relationship with the dose.

Parametric dose response models are biologically based and their use for modeling the probability of infection is justified, but for some outcomes a function that assumes an increasing

relationship may be misleading. The inoculum dose does play a role in infection outcomes and a greater understanding of the effects will lead to the creation of more effective controls.

Chapter 4

Most communicable diseases have some amount of virulence that induces infectiousness-enhancing symptoms. However, too much virulence can cause host morbidity and a reduction in transmission potential. For human diseases, the reduction in transmission opportunities is commonly caused by reduced activity. There is limited data regarding the potential impact of virulence on transmission potential. We analyzed data of 326 influenza patients at a university health center during the 2016/2017 influenza season. We classified symptoms as infectiousness-related or morbidity-related and calculated two scores. The scores were used to explore the relationship between infectiousness, morbidity, and activity levels.

We found a decrease in activity levels with increasing morbidity scores. There was no consistent pattern between activity level and infectiousness score. We also found a positive correlation between the morbidity and infectiousness scores. Our results provide evidence that for influenza, increasing virulence leads to increased infectiousness and reduced activity. This trade-off determines the transmission potential. Our findings suggest that a reduction of systemic symptoms may increase host activity without reducing infectiousness. Therefore interventions should target both systemic and infectiousness related symptoms to reduce overall transmission potential. Our findings can also inform simulation models to investigate the impact of different interventions on transmission.

Chapter 5

There has been as significant increase in the availability of rapid point of care PCR diagnostic tests are more accurate than current antigen-based tests. Currently, these tests provide

a qualitative result of positive or negative. We wanted to investigate if any additional information about the relative viral load could be calculated. Since in the case of other disease quantitative information about the viral load is used in making treatment decisions and understanding disease severity. We perform an analysis to evaluate the viral load from a rapid PCR test at diagnosis in non-hospitalized patients to predict symptom resolution and disease impact.

We sequentially enrolled 300 students at a university health center who presented with cough and one additional flu-like symptom from December 2016 to February 2017. Data were collected before, during, and five days after the clinic visit. All those enrolled in the study received a point of care PCR test (cobas Liat) to determine the presence or absence of influenza (A or B). We calculated the relative viral load was calculated for patients with a positive test for influenza A using information provided by Roche diagnostics. We then assessed the relationship between the relative viral load and patient-reported activity, symptom scores, fever, duration of fever, improvement in cough, days of work or class missed, and duration of symptoms.

Of the 289 students with a valid test, 136 were positive for influenza A. We found a positive correlation between viral load and body temperature at the clinic visit. The duration of symptoms appeared to have a negative correlation but was not statistically significant likely due to a potential lack of power. We did not find any correlation between viral load and patient-reported activity, symptom scores, duration of fever, improvement in cough, or days of work or class missed.

While we found a correlation between relative viral load and body temperature, overall, for our study population of young, overall healthy adults, we did not find that viral load provided additional information that could help in determining treatment and disease outcome. It is important to note that this may not generalize to other populations. It could be that viral load

contains important independent information for specific groups of patients, like young children or older adults. Further studies on those populations are warranted.

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APPENDIX A

CHAPTER 2 SUPPLEMENTAL MATERIAL

CLINICAL PREDICTION RULE FOR COMPLICATIONS AMONG PATIENTS WITH INFLUENZA LIKE ILLNESS AND INFLUENZA POSITIVE PATIENTS

SM Table 2.1 Description of study population and predictor variables.

Variable	FLU (PCR confirmed infection)		ILI (Influenza like illness)	
	Test Data N=718	Train Data N=1676	Test Data N=1105	Train Data N=2579
Age (mean (sd))	45.02 (18.71)	45.33 (18.66)	45.59 (18.96)	45.62 (18.68)
Sex				
Female	380 (52.9)	787 (47.0)	603 (54.6)	1403 (54.4)
Male	338 (47.1)	889 (53.0)	502 (45.4)	1176 (45.6)
Tamiflu Treatment				
Placebo	316 (44.0)	759 (45.3)	486 (44.0)	1144 (44.4)
75 mg	402 (56.0)	917 (54.7)	619 (56.0)	1435 (55.6)
Outcome: Hospitalization, Sepsis, or Pneumonia (C-S)				
Yes	18 (2.5)	31 (1.8)	30 (2.7)	61 (2.4)
No	700 (97.5)	1645 (98.2)	1075 (97.3)	2518 (97.6)
Outcome: Complications requiring an antibiotic (C-AB)				
Yes	42 (5.8)	80 (4.8)	77 (7.0)	132 (5.1)
No	676 (94.2)	1596 (95.2)	1028 (93.0)	2447 (94.9)
Outcome: Complications requiring follow up treatment (C-FT)				
Yes	122 (17.0)	265 (15.8)	183 (16.6)	405 (15.7)
No	569 (83.0)	1411 (84.2)	922 (83.4)	2174 (84.3)
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	316 (44.0)	754 (45.0)	506 (45.8)	1173 (45.5)
Severe	402 (56.0)	922 (55.0)	599 (54.2)	1406 (54.5)
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present)				
Absent	85 (11.8)	223 (13.3)	147 (13.3)	346 (13.4)
Present	633 (88.2)	1453 (86.7)	958 (86.7)	2233 (86.6)
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	366 (51.0)	815 (48.6)	516 (46.7)	1232 (47.8)
Severe	352 (49.0)	861 (51.4)	589 (53.3)	1347 (52.2)
Sore Throat (0 = Absent, 1, 2 or 3=Present)				
Absent	133 (18.5)	316 (18.9)	195 (17.6)	475 (18.4)
Present	585 (81.5)	1360 (81.1)	910 (82.4)	2104 (81.6)
Cough (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	120 (16.7)	305 (18.2)	293 (26.5)	665 (25.8)
Severe	598 (83.3)	1371 (81.8)	812 (73.5)	1914 (74.2)
Cough (0 = Absent, 1, 2 or 3=Present)				
Absent	30 (4.2)	56 (3.3)	84 (7.6)	204 (7.9)
Present	688 (95.8)	1620 (96.7)	1021 (92.4)	2375 (92.1)
Myalgia (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	124 (17.3)	275 (16.4)	179 (16.2)	465 (18.0)
Severe	594 (82.7)	1401 (83.6)	926 (83.8)	2114 (82.0)
Myalgia (0 = Absent, 1, 2 or 3=Present)				
Absent	40 (5.6)	87 (5.2)	54 (4.9)	138 (5.4)
Present	678 (94.4)	1589 (94.8)	1051 (95.1)	2441 (94.6)
Fatigue (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	70 (9.7)	164 (9.8)	115 (10.4)	255 (9.9)
Severe	648 (90.3)	1512 (90.2)	990 (89.6)	2324 (90.1)
Fatigue (0 = Absent, 1, 2 or 3=Present)				
Absent	17 (2.4)	22 (1.3)	19 (1.7)	43 (1.7)
Present	701 (97.6)	1654 (98.7)	1086 (98.3)	2536 (98.3)
Headache (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	205 (28.6)	503 (30.0)	322 (29.1)	802 (31.1)
Severe	513 (71.4)	1173 (70.0)	783 (70.9)	1777 (68.9)
Headache (0 = Absent, 1, 2 or 3=Present)				
Absent	68 (9.5)	181 (10.8)	127 (11.5)	273 (10.6)

Present	650 (90.5)	1495 (89.2)	978 (88.5)	2306 (89.4)
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe)				
Moderate	100 (13.9)	298 (17.8)	208 (18.8)	491 (19.0)
Severe	618 (86.1)	1378 (82.2)	897 (81.2)	2088 (81.0)
Chills/Sweats (0 = Absent, 1, 2 or 3=Present)				
Absent	23 (3.2)	60 (3.6)	42 (3.8)	122 (4.7)
Present	695 (96.8)	1616 (96.4)	1063 (96.2)	2457 (95.3)
Mean Body Temperature (SD)	101.00 (1.01)	100.99 (0.97)	100.86 (1.01)	100.85 (0.98)
Physician Reported Signs: Ears				
Normal	648 (90.3)	1515 (90.4)	1004 (90.9)	2336 (90.6)
Abnormal	70 (9.7)	161 (9.6)	101 (9.1)	243 (9.4)
Physician Reported Signs: Nose				
Normal	455 (63.4)	1084 (64.7)	714 (64.6)	1613 (62.5)
Abnormal	263 (36.6)	592 (35.3)	391 (35.4)	966 (37.5)
Physician Reported Signs: Throat				
Normal	341 (47.5)	831 (49.6)	540 (48.9)	1237 (48.0)
Abnormal	377 (52.5)	845 (50.4)	565 (51.1)	1342 (52.0)
Physician Reported Signs: Lymph node				
Normal	636 (88.6)	1468 (87.6)	960 (86.9)	2270 (88.0)
Abnormal	82 (11.4)	208 (12.4)	145 (13.1)	309 (12.0)
Medical History Question: Asthma				
No	665 (92.6)	1549 (92.4)	1016 (91.9)	2387 (92.6)
Yes	53 (7.4)	127 (7.6)	89 (8.1)	192 (7.4)
Medical History Question: COPD				
No	445 (62.0)	1043 (62.2)	634 (57.4)	1517 (58.8)
Yes	273 (38.0)	633 (37.8)	471 (42.6)	1062 (41.2)
Medical History Question: Any Allergies or Atopies				
No	647 (90.1)	1499 (89.4)	999 (90.4)	2316 (89.8)
Yes	71 (9.9)	177 (10.6)	106 (9.6)	263 (10.2)
Medical History Question: Taking Medication for Asthma or COPD				
No	643 (89.6)	1509 (90.0)	989 (89.5)	2305 (89.4)
Yes	75 (10.4)	167 (10.0)	116 (10.5)	274 (10.6)
Medical History Question: Taking Medication for Diabetes				
No	701 (97.6)	1624 (96.9)	1068 (96.7)	2486 (96.4)
Yes	17 (2.4)	52 (3.1)	37 (3.3)	93 (3.6)
Medical History Question: Any Current Prescriptions				
No	503 (70.1)	1173 (70.0)	764 (69.1)	1762 (68.3)
Yes	215 (29.9)	503 (30.0)	341 (30.9)	817 (31.7)

SM Table 2.2 FLU Population outcomes and predictors stratified by study

Study ID	M76001	WV15670	WV15671	WV15707	WV15730	WV15812	WV15819	WV15872	WV15876	WV15978	WV16277
Study size	894	318	241	12	38	193	116	50	44	300	188
Age (mean (sd))	37.82 (13.46)	38.37 (11.78)	32.51 (10.64)	70.67 (5.07)	34.47 (10.40)	52.91 (15.72)	73.29 (6.31)	47.20 (19.25)	73.48 (6.06)	71.97 (5.91)	34.04 (11.19)
Sex											
Female	477 (53.4)	158 (49.7)	122 (50.6)	4 (33.3)	17 (44.7)	119 (61.7)	65 (56.0)	19 (38.0)	28 (63.6)	166 (55.3)	94 (50.0)
Male	417 (46.6)	160 (50.3)	119 (49.4)	8 (66.7)	21 (55.3)	74 (38.3)	51 (44.0)	31 (62.0)	16 (36.4)	134 (44.7)	94 (50.0)
Tamiflu Treatment											
Placebo	303 (33.9)	159 (50.0)	124 (51.5)	6 (50.0)	19 (50.0)	103 (53.4)	65 (56.0)	29 (58.0)	19 (43.2)	158 (52.7)	90 (47.9)
75 mg	591 (66.1)	159 (50.0)	117 (48.5)	6 (50.0)	19 (50.0)	90 (46.6)	51 (44.0)	21 (42.0)	25 (56.8)	142 (47.3)	98 (52.1)

Outcome: Hospitalization, Sepsis, or Pneumonia											
Yes	15 (1.7)	2 (0.6)	0 (0.0)	2 (16.7)	0 (0.0)	7 (3.6)	6 (5.2)	4 (8.0)	2 (4.5)	9 (3.0)	2 (1.1)
No	879 (98.3)	316 (99.4)	241 (100.0)	10 (83.3)	38 (100.0)	186 (96.4)	110 (94.8)	46 (92.0)	42 (95.5)	291 (97.0)	186 (98.9)
Outcome: Complications requiring an antibiotic											
Yes	68 (7.6)	2 (0.6)	3 (1.2)	1 (8.3)	0 (0.0)	20 (10.4)	7 (6.0)	0 (0.0)	5 (11.4)	11 (3.7)	5 (2.7)
No	826 (92.4)	316 (99.4)	238 (98.8)	11 (91.7)	38 (100.0)	173 (89.6)	109 (94.0)	50 (100.0)	39 (88.6)	289 (96.3)	183 (97.3)
Outcome: Complications requiring follow up treatment											
Yes	175 (19.6)	10 (3.1)	9 (3.7)	1 (8.3)	1 (2.6)	55 (28.5)	26 (22.4)	14 (28.0)	15 (34.1)	67 (22.3)	14 (7.4)
No	719 (80.4)	308 (96.9)	232 (96.3)	11 (91.7)	37 (97.4)	138 (71.5)	90 (77.6)	36 (72.0)	29 (65.9)	233 (77.7)	174 (92.6)
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	368 (41.2)	163 (51.3)	100 (41.5)	6 (50.0)	18 (47.4)	81 (42.0)	54 (46.6)	21 (42.0)	15 (34.1)	161 (53.7)	83 (44.1)
Severe	526 (58.8)	155 (48.7)	141 (58.5)	6 (50.0)	20 (52.6)	112 (58.0)	62 (53.4)	29 (58.0)	29 (65.9)	139 (46.3)	105 (55.9)
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present)											
Absent	77 (8.6)	49 (15.4)	19 (7.9)	4 (33.3)	5 (13.2)	26 (13.5)	29 (25.0)	6 (12.0)	7 (15.9)	57 (19.0)	29 (15.4)
Present	817 (91.4)	269 (84.6)	222 (92.1)	8 (66.7)	33 (86.8)	167 (86.5)	87 (75.0)	44 (88.0)	37 (84.1)	243 (81.0)	159 (84.6)
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	425 (47.5)	143 (45.0)	107 (44.4)	5 (41.7)	18 (47.4)	111 (57.5)	61 (52.6)	20 (40.0)	25 (56.8)	168 (56.0)	98 (52.1)
Severe	469 (52.5)	175 (55.0)	134 (55.6)	7 (58.3)	20 (52.6)	82 (42.5)	55 (47.4)	30 (60.0)	19 (43.2)	132 (44.0)	90 (47.9)
Sore Throat (0 = Absent, 1, 2 or 3=Present)											
Absent	157 (17.6)	43 (13.5)	28 (11.6)	2 (16.7)	6 (15.8)	47 (24.4)	35 (30.2)	7 (14.0)	7 (15.9)	70 (23.3)	47 (25.0)
Present	737 (82.4)	275 (86.5)	213 (88.4)	10 (83.3)	32 (84.2)	146 (75.6)	81 (69.8)	43 (86.0)	37 (84.1)	230 (76.7)	141 (75.0)
Cough (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	149 (16.7)	69 (21.7)	56 (23.2)	5 (41.7)	15 (39.5)	21 (10.9)	20 (17.2)	4 (8.0)	10 (22.7)	28 (9.3)	48 (25.5)
Severe	745 (83.3)	249 (78.3)	185 (76.8)	7 (58.3)	23 (60.5)	172 (89.1)	96 (82.8)	46 (92.0)	34 (77.3)	272 (90.7)	140 (74.5)
Cough (0 = Absent, 1, 2 or 3=Present)											
Absent	22 (2.5)	18 (5.7)	11 (4.6)	1 (8.3)	4 (10.5)	3 (1.6)	6 (5.2)	2 (4.0)	1 (2.3)	5 (1.7)	13 (6.9)
Present	872 (97.5)	300 (94.3)	230 (95.4)	11 (91.7)	34 (89.5)	190 (98.4)	110 (94.8)	48 (96.0)	43 (97.7)	295 (98.3)	175 (93.1)
Myalgia (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	81 (9.1)	48 (15.1)	22 (9.1)	4 (33.3)	8 (21.1)	30 (15.5)	40 (34.5)	4 (8.0)	10 (22.7)	109 (36.3)	43 (22.9)
Severe	813 (90.9)	270 (84.9)	219 (90.9)	8 (66.7)	30 (78.9)	163 (84.5)	76 (65.5)	46 (92.0)	34 (77.3)	191 (63.7)	145 (77.1)
Myalgia (0 = Absent, 1, 2 or 3=Present)											
Absent	21 (2.3)	14 (4.4)	2 (0.8)	1 (8.3)	2 (5.3)	6 (3.1)	18 (15.5)	0 (0.0)	3 (6.8)	46 (15.3)	14 (7.4)
Present	873 (97.7)	304 (95.6)	239 (99.2)	11 (91.7)	36 (94.7)	187 (96.9)	98 (84.5)	50 (100.0)	41 (93.2)	254 (84.7)	174 (92.6)
Fatigue (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	67 (7.5)	46 (14.5)	20 (8.3)	3 (25.0)	6 (15.8)	14 (7.3)	17 (14.7)	4 (8.0)	4 (9.1)	37 (12.3)	16 (8.5)
Severe	827 (92.5)	272 (85.5)	221 (91.7)	9 (75.0)	32 (84.2)	179 (92.7)	99 (85.3)	46 (92.0)	40 (90.9)	263 (87.7)	172 (91.5)
Fatigue (0 = Absent, 1, 2 or 3=Present)											
Absent	10 (1.1)	7 (2.2)	2 (0.8)	0 (0.0)	2 (5.3)	4 (2.1)	3 (2.6)	0 (0.0)	2 (4.5)	9 (3.0)	0 (0.0)
Present	884 (98.9)	311 (97.8)	239 (99.2)	12 (100.0)	36 (94.7)	189 (97.9)	113 (97.4)	50 (100.0)	42 (95.5)	291 (97.0)	188 (100.0)
Headache (0 or 1 = Moderate, 2 or 3=Severe)											

Moderate	228 (25.5)	88 (27.7)	65 (27.0)	5 (41.7)	4 (10.5)	58 (30.1)	52 (44.8)	16 (32.0)	17 (38.6)	130 (43.3)	45 (23.9)
Severe	666 (74.5)	230 (72.3)	176 (73.0)	7 (58.3)	34 (89.5)	135 (69.9)	64 (55.2)	34 (68.0)	27 (61.4)	170 (56.7)	143 (76.1)
Headache (0 = Absent, 1, 2 or 3=Present)											
Absent	71 (7.9)	28 (8.8)	21 (8.7)	1 (8.3)	2 (5.3)	24 (12.4)	24 (20.7)	5 (10.0)	5 (11.4)	58 (19.3)	10 (5.3)
Present	823 (92.1)	290 (91.2)	220 (91.3)	11 (91.7)	36 (94.7)	169 (87.6)	92 (79.3)	45 (90.0)	39 (88.6)	242 (80.7)	178 (94.7)
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	127 (14.2)	49 (15.4)	48 (19.9)	6 (50.0)	3 (7.9)	28 (14.5)	38 (32.8)	6 (12.0)	10 (22.7)	59 (19.7)	24 (12.8)
Severe	767 (85.8)	269 (84.6)	193 (80.1)	6 (50.0)	35 (92.1)	165 (85.5)	78 (67.2)	44 (88.0)	34 (77.3)	241 (80.3)	164 (87.2)
Chills/Sweats (0 = Absent, 1, 2 or 3=Present)											
Absent	19 (2.1)	12 (3.8)	7 (2.9)	0 (0.0)	0 (0.0)	6 (3.1)	17 (14.7)	3 (6.0)	1 (2.3)	17 (5.7)	1 (0.5)
Present	875 (97.9)	306 (96.2)	234 (97.1)	12 (100.0)	38 (100.0)	187 (96.9)	99 (85.3)	47 (94.0)	43 (97.7)	283 (94.3)	187 (99.5)
Body Temperature											
Mean (SD) °F	101.08 (0.95)	101.41 (0.98)	100.84 (0.90)	101.28 (1.44)	101.30 (0.85)	100.95 (0.96)	100.71 (0.99)	100.65 (1.16)	100.56 (0.96)	100.77 (0.97)	100.77 (0.93)
Physician Reported Signs: Ears											
Normal	771 (86.2)	298 (93.7)	210 (87.1)	9 (75.0)	29 (76.3)	181 (93.8)	104 (89.7)	43 (86.0)	42 (95.5)	289 (96.3)	187 (99.5)
Abnormal	123 (13.8)	20 (6.3)	31 (12.9)	3 (25.0)	9 (23.7)	12 (6.2)	12 (10.3)	7 (14.0)	2 (4.5)	11 (3.7)	1 (0.5)
Physician Reported Signs: Nose											
Normal	554 (62.0)	201 (63.2)	155 (64.3)	11 (91.7)	28 (73.7)	130 (67.4)	67 (57.8)	19 (38.0)	17 (38.6)	224 (74.7)	133 (70.7)
Abnormal	340 (38.0)	117 (36.8)	86 (35.7)	1 (8.3)	10 (26.3)	63 (32.6)	49 (42.2)	31 (62.0)	27 (61.4)	76 (25.3)	55 (29.3)
Physician Reported Signs: Throat											
Normal	405 (45.3)	161 (50.6)	104 (43.2)	10 (83.3)	16 (42.1)	93 (48.2)	46 (39.7)	8 (16.0)	14 (31.8)	200 (66.7)	115 (61.2)
Abnormal	489 (54.7)	157 (49.4)	137 (56.8)	2 (16.7)	22 (57.9)	100 (51.8)	70 (60.3)	42 (84.0)	30 (68.2)	100 (33.3)	73 (38.8)
Physician Reported Signs: Lymph node											
Normal	759 (84.9)	275 (86.5)	206 (85.5)	11 (91.7)	31 (81.6)	173 (89.6)	104 (89.7)	43 (86.0)	40 (90.9)	292 (97.3)	170 (90.4)
Abnormal	135 (15.1)	43 (13.5)	35 (14.5)	1 (8.3)	7 (18.4)	20 (10.4)	12 (10.3)	7 (14.0)	4 (9.1)	8 (2.7)	18 (9.6)
Medical History Question: Asthma											
No	850 (95.1)	317 (99.7)	236 (97.9)	12 (100.0)	37 (97.4)	102 (52.8)	115 (99.1)	22 (44.0)	43 (97.7)	294 (98.0)	186 (98.9)
Yes	44 (4.9)	1 (0.3)	5 (2.1)	0 (0.0)	1 (2.6)	91 (47.2)	1 (0.9)	28 (56.0)	1 (2.3)	6 (2.0)	2 (1.1)
Medical History Question: COPD											
No	891 (99.7)	318 (100.0)	241 (100.0)	0 (0.0)	38 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Yes	3 (0.3)	0 (0.0)	0 (0.0)	12 (100.0)	0 (0.0)	193 (100.0)	116 (100.0)	50 (100.0)	44 (100.0)	300 (100.0)	188 (100.0)
Medical History Question: Any Allergies or Atopies											
No	735 (82.2)	299 (94.0)	218 (90.5)	11 (91.7)	37 (97.4)	167 (86.5)	109 (94.0)	49 (98.0)	44 (100.0)	292 (97.3)	185 (98.4)
Yes	159 (17.8)	19 (6.0)	23 (9.5)	1 (8.3)	1 (2.6)	26 (13.5)	7 (6.0)	1 (2.0)	0 (0.0)	8 (2.7)	3 (1.6)
Medical History Question: Taking Medication for Asthma or COPD											
No	850 (95.1)	317 (99.7)	237 (98.3)	12 (100.0)	37 (97.4)	86 (44.6)	108 (93.1)	16 (32.0)	43 (97.7)	268 (89.3)	178 (94.7)
Yes	44 (4.9)	1 (0.3)	4 (1.7)	0 (0.0)	1 (2.6)	107 (55.4)	8 (6.9)	34 (68.0)	1 (2.3)	32 (10.7)	10 (5.3)
Medical History Question: Taking Medication for Diabetes											
No	874 (97.8)	318 (100.0)	241 (100.0)	12 (100.0)	38 (100.0)	177 (91.7)	106 (91.4)	48 (96.0)	43 (97.7)	283 (94.3)	185 (98.4)
Yes	20 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	16 (8.3)	10 (8.6)	2 (4.0)	1 (2.3)	17 (5.7)	3 (1.6)
Medical History Question: Any Current Prescriptions											

No	753 (84.2)	284 (89.3)	223 (92.5)	7 (58.3)	34 (89.5)	30 (15.5)	43 (37.1)	5 (10.0)	21 (47.7)	112 (37.3)	164 (87.2)
Yes	141 (15.8)	34 (10.7)	18 (7.5)	5 (41.7)	4 (10.5)	163 (84.5)	73 (62.9)	45 (90.0)	23 (52.3)	188 (62.7)	24 (12.8)

SM Table 2.3 ILI Population outcomes and predictors stratified by study

Study ID	M76001	WV15670	WV15671	WV15707	WV15730	WV15812	WV15819	WV15872	WV15876	WV15978	WV16277
Study size	1234	468	397	26	58	292	163	96	96	452	402
Age mean(sd)	37.79 (13.35)	37.78 (11.47)	32.72 (10.49)	71.65 (5.40)	35.16 (10.93)	53.74 (16.71)	73.50 (6.39)	48.64 (18.05)	73.25 (6.00)	72.10 (5.71)	36.94 (12.82)
Sex											
Female	693 (56.2)	234 (50.0)	210 (52.9)	12 (46.2)	28 (48.3)	173 (59.2)	89 (54.6)	45 (46.9)	67 (69.8)	248 (54.9)	207 (51.5)
Male	541 (43.8)	234 (50.0)	187 (47.1)	14 (53.8)	30 (51.7)	119 (40.8)	74 (45.4)	51 (53.1)	29 (30.2)	204 (45.1)	195 (48.5)
Tamiflu Treatment											
Placebo	415 (33.6)	229 (48.9)	197 (49.6)	9 (34.6)	27 (46.6)	147 (50.3)	90 (55.2)	50 (52.1)	42 (43.8)	226 (50.0)	198 (49.3)
75 mg	819 (66.4)	239 (51.1)	200 (50.4)	17 (65.4)	31 (53.4)	145 (49.7)	73 (44.8)	46 (47.9)	54 (56.2)	226 (50.0)	204 (50.7)
Outcome: Hospitalization, Sepsis, or Pneumonia											
Yes	25 (2.0)	4 (0.9)	3 (0.8)	3 (11.5)	0 (0.0)	16 (5.5)	10 (6.1)	6 (6.2)	6 (6.2)	11 (2.4)	7 (1.7)
No	1209 (98.0)	464 (99.1)	394 (99.2)	23 (88.5)	58 (100.0)	276 (94.5)	153 (93.9)	90 (93.8)	90 (93.8)	441 (97.6)	395 (98.3)
Outcome: Complications requiring an antibiotic											
Yes	104 (8.4)	6 (1.3)	5 (1.3)	1 (3.8)	0 (0.0)	35 (12.0)	11 (6.7)	3 (3.1)	10 (10.4)	19 (4.2)	15 (3.7)
No	1130 (91.6)	462 (98.7)	392 (98.7)	25 (96.2)	58 (100.0)	257 (88.0)	152 (93.3)	93 (96.9)	86 (89.6)	433 (95.8)	387 (96.3)
Outcome: Complications requiring follow up treatment											
Yes	249 (20.2)	17 (3.6)	15 (3.8)	3 (11.5)	2 (3.4)	79 (27.1)	32 (19.6)	35 (36.5)	28 (29.2)	85 (18.8)	43 (10.7)
No	985 (79.8)	451 (96.4)	382 (96.2)	23 (88.5)	56 (96.6)	213 (72.9)	131 (80.4)	61 (63.5)	68 (70.8)	367 (81.2)	359 (89.3)
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	512 (41.5)	253 (54.1)	174 (43.8)	12 (46.2)	32 (55.2)	124 (42.5)	80 (49.1)	41 (42.7)	34 (35.4)	228 (50.4)	189 (47.0)
Severe	722 (58.5)	215 (45.9)	223 (56.2)	14 (53.8)	26 (44.8)	168 (57.5)	83 (50.9)	55 (57.3)	62 (64.6)	224 (49.6)	213 (53.0)
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present)											
Absent	118 (9.6)	81 (17.3)	37 (9.3)	6 (23.1)	11 (19.0)	40 (13.7)	39 (23.9)	9 (9.4)	12 (12.5)	77 (17.0)	63 (15.7)
Present	1116 (90.4)	387 (82.7)	360 (90.7)	20 (76.9)	47 (81.0)	252 (86.3)	124 (76.1)	87 (90.6)	84 (87.5)	375 (83.0)	339 (84.3)
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	561 (45.5)	197 (42.1)	170 (42.8)	14 (53.8)	26 (44.8)	163 (55.8)	88 (54.0)	33 (34.4)	45 (46.9)	249 (55.1)	202 (50.2)
Severe	673 (54.5)	271 (57.9)	227 (57.2)	12 (46.2)	32 (55.2)	129 (44.2)	75 (46.0)	63 (65.6)	51 (53.1)	203 (44.9)	200 (49.8)
Sore Throat (0 = Absent, 1, 2 or 3=Present)											
Absent	203 (16.5)	68 (14.5)	49 (12.3)	5 (19.2)	8 (13.8)	66 (22.6)	52 (31.9)	14 (14.6)	14 (14.6)	100 (22.1)	91 (22.6)
Present	1031 (83.5)	400 (85.5)	348 (87.7)	21 (80.8)	50 (86.2)	226 (77.4)	111 (68.1)	82 (85.4)	82 (85.4)	352 (77.9)	311 (77.4)
Cough (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	292 (23.7)	142 (30.3)	138 (34.8)	9 (34.6)	30 (51.7)	47 (16.1)	39 (23.9)	13 (13.5)	28 (29.2)	75 (16.6)	145 (36.1)
Severe	942 (76.3)	326 (69.7)	259 (65.2)	17 (65.4)	28 (48.3)	245 (83.9)	124 (76.1)	83 (86.5)	68 (70.8)	377 (83.4)	257 (63.9)
Cough (0 = Absent, 1, 2 or 3=Present)											

Absent	78 (6.3)	57 (12.2)	36 (9.1)	1 (3.8)	12 (20.7)	10 (3.4)	11 (6.7)	3 (3.1)	6 (6.2)	21 (4.6)	53 (13.2)
Present	1156 (93.7)	411 (87.8)	361 (90.9)	25 (96.2)	46 (79.3)	282 (96.6)	152 (93.3)	93 (96.9)	90 (93.8)	431 (95.4)	349 (86.8)
Myalgia (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	110 (8.9)	76 (16.2)	41 (10.3)	11 (42.3)	9 (15.5)	49 (16.8)	56 (34.4)	17 (17.7)	26 (27.1)	160 (35.4)	89 (22.1)
Severe	1124 (91.1)	392 (83.8)	356 (89.7)	15 (57.7)	49 (84.5)	243 (83.2)	107 (65.6)	79 (82.3)	70 (72.9)	292 (64.6)	313 (77.9)
Myalgia (0 = Absent, 1, 2 or 3=Present)											
Absent	25 (2.0)	20 (4.3)	3 (0.8)	6 (23.1)	2 (3.4)	9 (3.1)	26 (16.0)	4 (4.2)	8 (8.3)	67 (14.8)	22 (5.5)
Present	1209 (98.0)	448 (95.7)	394 (99.2)	20 (76.9)	56 (96.6)	283 (96.9)	137 (84.0)	92 (95.8)	88 (91.7)	385 (85.2)	380 (94.5)
Fatigue (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	91 (7.4)	67 (14.3)	29 (7.3)	6 (23.1)	7 (12.1)	28 (9.6)	29 (17.8)	13 (13.5)	17 (17.7)	55 (12.2)	28 (7.0)
Severe	1143 (92.6)	401 (85.7)	368 (92.7)	20 (76.9)	51 (87.9)	264 (90.4)	134 (82.2)	83 (86.5)	79 (82.3)	397 (87.8)	374 (93.0)
Fatigue (0 = Absent, 1, 2 or 3=Present)											
Absent	12 (1.0)	12 (2.6)	2 (0.5)	1 (3.8)	2 (3.4)	5 (1.7)	9 (5.5)	1 (1.0)	4 (4.2)	12 (2.7)	2 (0.5)
Present	1222 (99.0)	456 (97.4)	395 (99.5)	25 (96.2)	56 (96.6)	287 (98.3)	154 (94.5)	95 (99.0)	92 (95.8)	440 (97.3)	400 (99.5)
Headache (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	310 (25.1)	127 (27.1)	112 (28.2)	13 (50.0)	11 (19.0)	97 (33.2)	71 (43.6)	33 (34.4)	37 (38.5)	202 (44.7)	111 (27.6)
Severe	924 (74.9)	341 (72.9)	285 (71.8)	13 (50.0)	47 (81.0)	195 (66.8)	92 (56.4)	63 (65.6)	59 (61.5)	250 (55.3)	291 (72.4)
Headache (0 = Absent, 1, 2 or 3=Present)											
Absent	105 (8.5)	37 (7.9)	38 (9.6)	4 (15.4)	3 (5.2)	37 (12.7)	36 (22.1)	12 (12.5)	14 (14.6)	81 (17.9)	33 (8.2)
Present	1129 (91.5)	431 (92.1)	359 (90.4)	22 (84.6)	55 (94.8)	255 (87.3)	127 (77.9)	84 (87.5)	82 (85.4)	371 (82.1)	369 (91.8)
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe)											
Moderate	203 (16.5)	90 (19.2)	88 (22.2)	12 (46.2)	10 (17.2)	56 (19.2)	55 (33.7)	18 (18.8)	24 (25.0)	89 (19.7)	54 (13.4)
Severe	1031 (83.5)	378 (80.8)	309 (77.8)	14 (53.8)	48 (82.8)	236 (80.8)	108 (66.3)	78 (81.2)	72 (75.0)	363 (80.3)	348 (86.6)
Chills/Sweats (0 = Absent, 1, 2 or 3=Present)											
Absent	42 (3.4)	25 (5.3)	17 (4.3)	1 (3.8)	0 (0.0)	13 (4.5)	25 (15.3)	7 (7.3)	3 (3.1)	23 (5.1)	8 (2.0)
Present	1192 (96.6)	443 (94.7)	380 (95.7)	25 (96.2)	58 (100.0)	279 (95.5)	138 (84.7)	89 (92.7)	93 (96.9)	429 (94.9)	394 (98.0)
Body Temperature											
Mean (SD) °F	100.97 (0.94)	101.36 (0.95)	100.69 (0.91)	100.80 (1.25)	101.27 (0.91)	100.84 (0.97)	100.56 (0.96)	100.45 (1.03)	100.21 (0.99)	100.58 (1.00)	100.69 (0.94)
Physician Reported Signs: Ears											
Normal	1063 (86.1)	436 (93.2)	337 (84.9)	22 (84.6)	47 (81.0)	275 (94.2)	147 (90.2)	86 (89.6)	93 (96.9)	436 (96.5)	398 (99.0)
Abnormal	171 (13.9)	32 (6.8)	60 (15.1)	4 (15.4)	11 (19.0)	17 (5.8)	16 (9.8)	10 (10.4)	3 (3.1)	16 (3.5)	4 (1.0)
Physician Reported Signs: Nose											
Normal	757 (61.3)	297 (63.5)	256 (64.5)	19 (73.1)	42 (72.4)	194 (66.4)	92 (56.4)	30 (31.2)	31 (32.3)	328 (72.6)	281 (69.9)
Abnormal	477 (38.7)	171 (36.5)	141 (35.5)	7 (26.9)	16 (27.6)	98 (33.6)	71 (43.6)	66 (68.8)	65 (67.7)	124 (27.4)	121 (30.1)
Physician Reported Signs: Throat											
Normal	550 (44.6)	223 (47.6)	170 (42.8)	16 (61.5)	24 (41.4)	141 (48.3)	64 (39.3)	15 (15.6)	24 (25.0)	289 (63.9)	261 (64.9)
Abnormal	684 (55.4)	245 (52.4)	227 (57.2)	10 (38.5)	34 (58.6)	151 (51.7)	99 (60.7)	81 (84.4)	72 (75.0)	163 (36.1)	141 (35.1)
Physician Reported Signs: Lymph node											
Normal	1038 (84.1)	403 (86.1)	329 (82.9)	22 (84.6)	46 (79.3)	260 (89.0)	151 (92.6)	85 (88.5)	92 (95.8)	441 (97.6)	363 (90.3)
Abnormal	196 (15.9)	65 (13.9)	68 (17.1)	4 (15.4)	12 (20.7)	32 (11.0)	12 (7.4)	11 (11.5)	4 (4.2)	11 (2.4)	39 (9.7)
Medical History Question: Asthma											

No	1168 (94.7)	466 (99.6)	391 (98.5)	26 (100.0)	57 (98.3)	163 (55.8)	161 (98.8)	42 (43.8)	95 (99.0)	441 (97.6)	393 (97.8)
Yes	66 (5.3)	2 (0.4)	6 (1.5)	0 (0.0)	1 (1.7)	129 (44.2)	2 (1.2)	54 (56.2)	1 (1.0)	11 (2.4)	9 (2.2)
Medical History Question: COPD											
No	1228 (99.5)	468 (100.0)	397 (100.0)	0 (0.0)	58 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Yes	6 (0.5)	0 (0.0)	0 (0.0)	26 (100.0)	0 (0.0)	292 (100.0)	163 (100.0)	96 (100.0)	96 (100.0)	452 (100.0)	402 (100.0)
Medical History Question: Any Allergies or Atopies											
No	1007 (81.6)	441 (94.2)	353 (88.9)	25 (96.2)	57 (98.3)	255 (87.3)	154 (94.5)	94 (97.9)	95 (99.0)	439 (97.1)	395 (98.3)
Yes	227 (18.4)	27 (5.8)	44 (11.1)	1 (3.8)	1 (1.7)	37 (12.7)	9 (5.5)	2 (2.1)	1 (1.0)	13 (2.9)	7 (1.7)
Medical History Question: Taking Medication for Asthma or COPD											
No	1171 (94.9)	464 (99.1)	393 (99.0)	26 (100.0)	56 (96.6)	135 (46.2)	147 (90.2)	27 (28.1)	92 (95.8)	402 (88.9)	381 (94.8)
Yes	63 (5.1)	4 (0.9)	4 (1.0)	0 (0.0)	2 (3.4)	157 (53.8)	16 (9.8)	69 (71.9)	4 (4.2)	50 (11.1)	21 (5.2)
Medical History Question: Taking Medication for Diabetes											
No	1206 (97.7)	466 (99.6)	397 (100.0)	25 (96.2)	58 (100.0)	265 (90.8)	149 (91.4)	90 (93.8)	93 (96.9)	410 (90.7)	395 (98.3)
Yes	28 (2.3)	2 (0.4)	0 (0.0)	1 (3.8)	0 (0.0)	27 (9.2)	14 (8.6)	6 (6.2)	3 (3.1)	42 (9.3)	7 (1.7)
Medical History Question: Any Current Prescriptions											
No	1031 (83.5)	415 (88.7)	372 (93.7)	11 (42.3)	52 (89.7)	40 (13.7)	59 (36.2)	8 (8.3)	39 (40.6)	159 (35.2)	340 (84.6)
Yes		203 (16.5)	53 (11.3)	25 (6.3)	15 (57.7)	6 (10.3)	252 (86.3)	104 (63.8)	88 (91.7)	57 (59.4)	293 (64.8)

SM Table 3.4 Results for FLU C-AB bivariate logistic regression for all predictors.

Variable [Reference level if categorical]	Odds Ratio	95% CI Lower bound	95% CI Upper bound	P-value
AGE	1.01	1.00	1.02	0.13
SEX [Male]	1.59	1.01	2.56	0.05
Tamiflu Treatment: [Placebo]	1.04	0.66	1.63	0.86
Body Temperature	0.88	0.69	1.11	0.30
Physician Reported Signs: Ears [Normal]	1.20	0.55	2.34	0.61
Physician Reported Signs: Nose [Normal]	1.17	0.73	1.84	0.51
Physician Reported Signs: Throat [Normal]	1.59	1.01	2.54	0.05
Physician Reported Signs: Lymph node [Normal]	1.13	0.56	2.09	0.71
Medical History Question: Asthma [No]	2.27	1.14	4.17	0.01
Medical History Question: COPD [No]	1.17	0.73	1.83	0.51
Medical History Question: Any Allergies or Atopies [No]	1.53	0.77	2.79	0.19
Medical History Question: Taking Medication for Asthma or COPD [No]	2.00	1.06	3.54	0.02
Medical History Question: Taking Medication for Diabetes [No]	1.23	0.29	3.45	0.73
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.74	1.09	2.85	0.02
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present) [Absent]	1.22	0.63	2.66	0.58
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.05	0.67	1.65	0.84
Sore Throat (0 = Absent, 1, 2 or 3=Present) [Absent]	0.73	0.44	1.28	0.25
Cough (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.27	0.71	2.50	0.45
Cough (0 = Absent, 1, 2 or 3=Present) [Absent]	1.37	0.41	8.44	0.67
Myalgia (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	2.10	1.03	5.07	0.06
Myalgia (0 = Absent, 1, 2 or 3=Present) [Absent]	1.04	0.42	3.48	0.94
Fatigue (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.36	0.63	3.54	0.48
Fatigue (0 = Absent, 1, 2 or 3=Present) [Absent]	NA	NA	NA	0.98
Headache (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.22	0.74	2.07	0.45
Headache (0 = Absent, 1, 2 or 3=Present) [Absent]	1.09	0.55	2.50	0.81
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.02	0.58	1.92	0.95
Chills/Sweats (0 = Absent, 1, 2 or 3=Present) [Moderate]	0.54	0.23	1.57	0.19
Medical History Question: Any Current Prescriptions [No]	1.68	1.06	2.65	0.03

SM Table 2.5 Results for ILI C-AB bivariate logistic regression for all predictors.

Variable [Reference level if categorical]	Odds Ratio	95% CI Lower bound	95% CI Upper bound	P-value
AGE	1.00	0.99	1.01	0.98
SEX [Male]	1.85	1.28	2.72	0.00
Tamiflu Treatment: [Placebo]	1.08	0.76	1.54	0.66
Body Temperature	1.02	0.85	1.21	0.85
Physician Reported Signs: Ears [Normal]	1.25	0.69	2.11	0.43
Physician Reported Signs: Nose [Normal]	1.20	0.84	1.71	0.31
Physician Reported Signs: Throat [Normal]	1.35	0.95	1.94	0.10
Physician Reported Signs: Lymph node [Normal]	1.33	0.79	2.13	0.25
Medical History Question: Asthma [No]	2.06	1.19	3.39	0.01
Medical History Question: COPD [No]	1.16	0.82	1.65	0.40
Medical History Question: Any Allergies or Atopies [No]	1.42	0.82	2.31	0.18
Medical History Question: Taking Medication for Asthma or COPD [No]	2.17	1.36	3.35	0.00
Medical History Question: Taking Medication for Diabetes [No]	1.29	0.50	2.78	0.55
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.18	0.83	1.69	0.37
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present) [Absent]	0.98	0.60	1.69	0.94
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.00	0.71	1.42	0.99
Sore Throat (0 = Absent, 1, 2 or 3=Present) [Absent]	0.69	0.46	1.06	0.08
Cough (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.96	0.65	1.45	0.84
Cough (0 = Absent, 1, 2 or 3=Present) [Absent]	0.65	0.39	1.19	0.13
Myalgia (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.42	0.87	2.43	0.18
Myalgia (0 = Absent, 1, 2 or 3=Present) [Absent]	1.46	0.65	4.17	0.42
Fatigue (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.74	0.90	3.91	0.14
Fatigue (0 = Absent, 1, 2 or 3=Present) [Absent]	2.29	0.49	40.72	0.42
Headache (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.97	0.67	1.42	0.85
Headache (0 = Absent, 1, 2 or 3=Present) [Absent]	0.85	0.51	1.51	0.56
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.06	0.69	1.71	0.80
Chills/Sweats (0 = Absent, 1, 2 or 3=Present) [Moderate]	0.76	0.38	1.72	0.46

Medical History Question: Any Current Prescriptions [No]	1.43	0.99	2.04	0.05

SM Table 2.6 Results for FLU C-S bivariate logistic regression for all predictors.

Variable [Reference level if categorical]	Odds Ratio	95% CI Lower bound	95% CI Upper bound	P-value
AGE	1.03	1.01	1.05	0.00
SEX [Male]	1.62	0.79	3.53	0.20
Tamiflu Treatment: [Placebo]	1.94	0.94	4.13	0.08
Body Temperature	0.99	0.68	1.42	0.97
Physician Reported Signs: Ears [Normal]	1.40	0.41	3.65	0.53
Physician Reported Signs: Nose [Normal]	1.16	0.54	2.38	0.69
Physician Reported Signs: Throat [Normal]	1.20	0.59	2.48	0.62
Physician Reported Signs: Lymph node [Normal]	1.05	0.31	2.71	0.93
Medical History Question: Asthma [No]	2.40	0.80	5.87	0.08
Medical History Question: COPD [No]	2.32	1.14	4.87	0.02
Medical History Question: Any Allergies or Atopies [No]	1.26	0.37	3.27	0.67
Medical History Question: Taking Medication for Asthma or COPD [No]	1.35	0.39	3.50	0.58
Medical History Question: Taking Medication for Diabetes [No]	2.20	0.35	7.58	0.29
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.76	0.37	1.56	0.46
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present) [Absent]	0.63	0.27	1.72	0.32
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.74	0.84	3.78	0.14
Sore Throat (0 = Absent, 1, 2 or 3=Present) [Absent]	0.97	0.42	2.62	0.94
Cough (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.51	0.59	5.14	0.44
Cough (0 = Absent, 1, 2 or 3=Present) [Absent]	1.04	0.22	18.65	0.97
Myalgia (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.85	0.65	7.77	0.31
Myalgia (0 = Absent, 1, 2 or 3=Present) [Absent]	0.79	0.23	4.94	0.75
Fatigue (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.73	0.28	2.48	0.56
Fatigue (0 = Absent, 1, 2 or 3=Present) [Absent]	NA	NA	NA	0.99
Headache (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.78	0.38	1.69	0.50
Headache (0 = Absent, 1, 2 or 3=Present) [Absent]	0.81	0.31	2.78	0.70
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.47	0.57	5.00	0.48

Chills/Sweats (0 = Absent, 1, 2 or 3=Present) [Moderate]	1.12	0.23	20.04	0.91
Medical History Question: Any Current Prescriptions [No]	2.54	1.24	5.22	0.01

SM Table 2.7 Results for ILI C-S bivariate logistic regression for all predictors.

Variable [Reference level if categorical]	Odds Ratio	95% CI Lower bound	95% CI Upper bound	P-value
AGE	1.02	1.01	1.04	0.00
SEX [Male]	1.06	0.64	1.78	0.83
Tamiflu Treatment: [Placebo]	1.40	0.84	2.33	0.20
Body Temperature	1.13	0.88	1.45	0.32
Physician Reported Signs: Ears [Normal]	0.67	0.20	1.65	0.44
Physician Reported Signs: Nose [Normal]	1.01	0.59	1.69	0.97
Physician Reported Signs: Throat [Normal]	1.25	0.75	2.10	0.40
Physician Reported Signs: Lymph node [Normal]	0.95	0.39	1.97	0.90
Medical History Question: Asthma [No]	1.91	0.83	3.86	0.09
Medical History Question: COPD [No]	3.01	1.77	5.26	0.00
Medical History Question: Any Allergies or Atopies [No]	0.96	0.37	2.08	0.92
Medical History Question: Taking Medication for Asthma or COPD [No]	2.84	1.52	5.05	0.00
Medical History Question: Taking Medication for Diabetes [No]	1.92	0.57	4.79	0.22
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.75	0.45	1.25	0.27
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present) [Absent]	0.70	0.37	1.42	0.29
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.94	0.57	1.57	0.82
Sore Throat (0 = Absent, 1, 2 or 3=Present) [Absent]	0.75	0.42	1.43	0.36
Cough (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.29	0.72	2.50	0.42
Cough (0 = Absent, 1, 2 or 3=Present) [Absent]	0.96	0.42	2.78	0.93
Myalgia (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.00	0.54	2.04	1.00
Myalgia (0 = Absent, 1, 2 or 3=Present) [Absent]	0.80	0.32	2.67	0.67
Fatigue (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.58	0.64	5.24	0.38
Fatigue (0 = Absent, 1, 2 or 3=Present) [Absent]	NA	NA	NA	0.98
Headache (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.92	0.54	1.62	0.77
Headache (0 = Absent, 1, 2 or 3=Present) [Absent]	0.59	0.31	1.26	0.14

Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.71	0.41	1.33	0.27
Chills/Sweats (0 = Absent, 1, 2 or 3=Present) [Moderate]	0.70	0.28	2.34	0.50
Medical History Question: Any Current Prescriptions [No]	2.28	1.37	3.80	0.00

SM Table 2.8 Results FLU-FT bivariate logistic regression for all predictors.

Variable [Reference level if categorical]	Odds Ratio	95% CI Lower bound	95% CI Upper bound	P-value
AGE	1.02	1.01	1.03	0.00
SEX [Male]	1.19	0.91	1.55	0.21
Tamiflu Treatment: [Placebo]	1.31	1.01	1.70	0.04
Body Temperature	0.94	0.82	1.07	0.35
Physician Reported Signs: Ears [Normal]	0.78	0.47	1.23	0.31
Physician Reported Signs: Nose [Normal]	1.25	0.95	1.63	0.11
Physician Reported Signs: Throat [Normal]	1.27	0.98	1.66	0.07
Physician Reported Signs: Lymph node [Normal]	0.85	0.55	1.26	0.43
Medical History Question: Asthma [No]	1.73	1.11	2.63	0.01
Medical History Question: COPD [No]	1.71	1.31	2.22	0.00
Medical History Question: Any Allergies or Atopies [No]	1.25	0.82	1.85	0.28
Medical History Question: Taking Medication for Asthma or COPD [No]	2.01	1.37	2.90	0.00
Medical History Question: Taking Medication for Diabetes [No]	1.63	0.81	3.05	0.15
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.43	1.09	1.87	0.01
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present) [Absent]	0.94	0.65	1.39	0.73
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.15	0.89	1.50	0.29
Sore Throat (0 = Absent, 1, 2 or 3=Present) [Absent]	0.65	0.48	0.89	0.01
Cough (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.68	1.15	2.51	0.01
Cough (0 = Absent, 1, 2 or 3=Present) [Absent]	1.13	0.56	2.61	0.75
Myalgia (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.51	1.04	2.28	0.04
Myalgia (0 = Absent, 1, 2 or 3=Present) [Absent]	1.31	0.72	2.65	0.41
Fatigue (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.97	1.18	3.54	0.02
Fatigue (0 = Absent, 1, 2 or 3=Present) [Absent]	NA	NA	NA	0.96
Headache (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.29	0.96	1.75	0.09

Headache (0 = Absent, 1, 2 or 3=Present) [Absent]	1.03	0.68	1.61	0.89
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.45	1.01	2.14	0.05
Chills/Sweats (0 = Absent, 1, 2 or 3=Present) [Moderate]	0.74	0.40	1.48	0.37
Medical History Question: Any Current Prescriptions [No]	1.98	1.51	2.60	0.00

SM Table 3.9 Results ILI-FT bivariate logistic regression for all predictors.

Variable [Reference level if categorical]	Odds Ratio	95% CI Lower bound	95% CI Upper bound	P-value
AGE	1.01	1.01	1.02	0.00
SEX [Male]	1.30	1.05	1.61	0.02
Tamiflu Treatment: [Placebo]	1.26	1.02	1.55	0.04
Body Temperature	0.97	0.87	1.08	0.59
Physician Reported Signs: Ears [Normal]	0.80	0.53	1.16	0.25
Physician Reported Signs: Nose [Normal]	1.28	1.03	1.59	0.02
Physician Reported Signs: Throat [Normal]	1.27	1.03	1.57	0.03
Physician Reported Signs: Lymph node [Normal]	0.88	0.62	1.22	0.45
Medical History Question: Asthma [No]	2.20	1.56	3.07	0.00
Medical History Question: COPD [No]	1.86	1.50	2.30	0.00
Medical History Question: Any Allergies or Atopies [No]	1.16	0.82	1.61	0.40
Medical History Question: Taking Medication for Asthma or COPD [No]	2.36	1.76	3.13	0.00
Medical History Question: Taking Medication for Diabetes [No]	1.12	0.62	1.89	0.69
Nasal Symptoms (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.18	0.96	1.47	0.12
Nasal Symptoms (0 = Absent, 1, 2 or 3=Present) [Absent]	0.94	0.69	1.28	0.67
Sore Throat (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	0.93	0.75	1.15	0.48
Sore Throat (0 = Absent, 1, 2 or 3=Present) [Absent]	0.64	0.50	0.82	0.00
Cough (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.28	1.00	1.66	0.06
Cough (0 = Absent, 1, 2 or 3=Present) [Absent]	0.83	0.58	1.22	0.32
Myalgia (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.15	0.87	1.55	0.32
Myalgia (0 = Absent, 1, 2 or 3=Present) [Absent]	1.26	0.78	2.15	0.38
Fatigue (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.98	1.30	3.17	0.00
Fatigue (0 = Absent, 1, 2 or 3=Present) [Absent]	3.87	1.19	23.83	0.06

Headache (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.09	0.86	1.37	0.49
Headache (0 = Absent, 1, 2 or 3=Present) [Absent]	0.88	0.64	1.25	0.47
Chills/Sweats (0 or 1 = Moderate, 2 or 3=Severe) [Moderate]	1.20	0.91	1.60	0.21
Chills/Sweats (0 = Absent, 1, 2 or 3=Present) [Moderate]	0.89	0.56	1.48	0.64
Medical History Question: Any Current Prescriptions [No]	1.95	1.57	2.43	0.00

SM Table 2.10 Final Model for PCR and ILI population. Outcome is hospitalization, sepsis, and pneumonia (C-S).

FLU C-S Final Model	
Variable	**OR (95% CI)**
Age:	
(per year)	1.03 (1.01, 1.05)
Asthma (Yes/No):	
Yes	2.85 (0.93, 7.15)
Sore Throat (Mild/Severe):	
Severe	1.88 (0.91, 4.10)
Tamiflu Rx (Placebo/75mg)	
Placebo	1.87 (0.91, 4.02)
ILI C-S Final Model	
Variable	**OR (95% CI)**
COPD (Yes/No):	
Yes	2.54 (1.45, 4.55)
Asthma or COPD RX: (Yes/No)	
Yes	1.95 (1.01, 3.58)
Tamiflu Rx (Placebo/75mg)	
Placebo	1.28 (0.76, 2.14)

SM Table 2.11 Model performance in test and train data from FLU and ILI patients for the serious outcomes (C-S).

FLU C-S-Model		
Model (data)	FLU C-S (train)	FLU C-S (test)
AUC (95%CI)	0.69 (0.59,0.79)	0.77 (0.67, 0.87)
Cut-off probability	0.017	0.017
Accuracy (95%CI)	0.62 (0.60, 0.64)	0.60 (0.56, 0.64)
Sensitivity (95%CI)	0.74 (0.55, 0.88)	0.35 (0.28, 0.44)
Specificity (95%CI)	0.62 (0.59, 0.64)	0.76 (0.73, 0.79)
PPV (95%CI)	0.035 (0.032, 0.087)	0.25 (0.20, 0.32)
NPV (95%CI)	0.992 (0.982, 0.993)	0.83 (0.80, 0.86)
Likelihood (+) (95%CI)	1.97 (1.58, 2.44)	1.95 (1.49, 2.53)
Likelihood (-) (95%CI)	0.41 (0.22, 0.75)	0.36 (0.15, 0.87)
DOR (95%CI)	4.765 (2.11, 10.72)	5.28 (1.72, 16.21)
HL GOF	X^2= 11.4, df= 8, p = 0.18	X^2= 9.38, df= 8, p = 0.31
Delong's AUC Test	D=-0.256, df=1496.6, p=0.79	
ILI C-S-Model		

Model (data)	ILI C-S (train)	ILI C-S (test)
AUC (95%CI)	0.66 (0.59, 0.73)	0.69 (0.59, 0.79)
Cut-off probability	0.028	0.028
Accuracy (95%CI)	0.58 (0.57, 0.60)	0.74 (0.71, 0.76)
Sensitivity (95%CI)	0.68 (0.55, 0.80)	0.56 (0.37, 0.74)
Specificity (95%CI)	0.58 (0.56, 0.60)	0.74 (0.71, 0.77)
PPV (95%CI)	0.03 (0.02, 0.05)	0.05 (0.3, 0.09)
NPV (95%CI)	0.987 (0.980, 0.992)	0.98 (0.97, 0.99)
Likelihood (+) (95%CI)	1.66 (1.40, 1.98)	2.23 (1.60, 3.10)
Likelihood (-) (95%CI)	0.53 (0.36, 0.77)	0.58 (0.38, 0.87)
DOR (95%CI)	3.14 (1.81, 5.44)	3.84 (1.84, 8.01)
H-L GOF	X^2= 0.609, df= 2, p = 0.73	X^2= 0.045, df= 2, p = 0.97
Delong's AUC Test	D= 0.49, df= 2202.1 , p = 0.62	

SM Table 2.12 Final Model for PCR and ILI population. Outcome is complication requiring an antibiotic (AB).

FLU C-AB Final Model	
Variable	**OR (95% CI)**
Age:	
(per year)	1.01 (1.001, 1.02)
Sex (Male/Female)	
Female	1.52 (0.95, 2.46)
Throat Physical (Normal/Abnormal)	
Abnormal	1.63 (1.03, 2.63)
Asthma (Yes/No):	
Yes	1.99 (0.99, 3.69)
Nasal Symptoms (Mild/Severe):	
Severe	1.68 (1.05, 2.77)
Myalgia (Mild/Severe):	
Severe	2.03 (0.97, 4.97)
Tamiflu Rx (Placebo/75mg)	
Placebo	1.05 (0.66, 1.66)
ILI C-AB Final Model	
Variable	**OR (95% CI)**
Sore Throat (Yes/No):	
Yes	0.67 (0.45, 1.04)
Sex (Male/Female)	
Female	1.81 (1.25, 2.67)
Throat Physical (Normal/Abnormal)	
Abnormal	1.63 (0.98, 2.04)
Asthma or COPD RX (Yes/No):	
Yes	2.09 (1.30, 3.24)
Cough (Yes/No):	
Yes	0.62 (0.36, 1.14)
Fatigue (Mild/Severe):	
Severe	1.66 (0.84, 3.76)
Tamiflu Rx (Placebo/75mg)	
Placebo	1.11 (0.78, 1.58)

SM Table 2.13 Model performance in test and train data from FLU and ILI patients for complications requiring an antibiotic (C-AB)

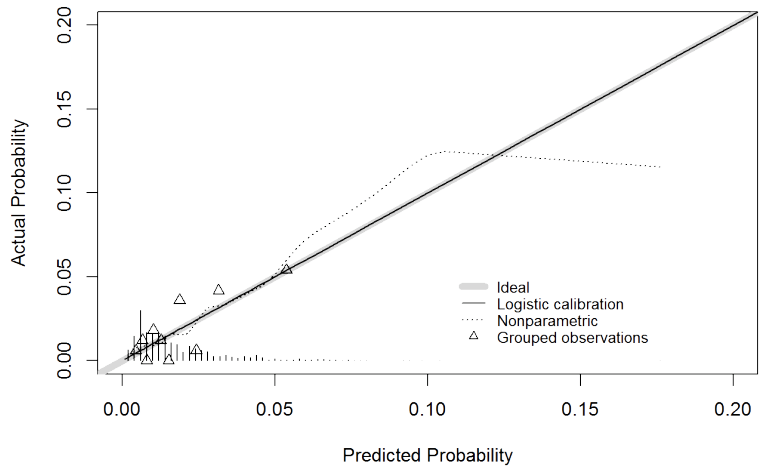
FLU C-AB Model		
Model (data)	FLU C-AB (train)	FLU C-AB (test)
Cut-off probability	0.047	0.047
AUC (95%CI)	0.657 (0.59,0.72)	0.64 (0.55, 0.73)
Accuracy (95%CI)	0.61 (0.59, 0.64)	0.48 (0.45, 0.52)
Sensitivity (95%CI)	0.63 (0.52, 0.74)	0.78 (0.63, 0.89)
Specificity (95%CI)	0.61 (0.59, 0.64)	0.46 (0.43, 0.50)
PPV (95%CI)	0.077 (0.070, 0.12)	0.08 (0.05, 0.11)
NPV (95%CI)	0.971 (0.954, 0.974)	0.97 (0.94, 0.98)
Likelihood (+) (95%CI)	1.66 (1.39, 1.99)	1.47 (1.24, 1.75)
Likelihood (-) (95%CI)	0.58 (0.43, 0.78)	0.45 (0.25, 0.81)
DOR (95%CI)	2.84 (1.78, 4.43)	3.23 (1.52, 6.87)
H-L GOF	X^2= 15.3, df= 8, p = 0.053	X^2= 15.82, df= 8, p = 0.045
Delong's AUC Test	D=-0.261, df=1526.8, p=0.794	
ILI C-AB Model		
Model (data)	ILI C-AB (train)	ILI C-AB (test)
AUC (95%CI)	0.642 (0.59, 0.69)	0.59 (0.53, 0.66)
Cut-off probability	0.048	0.048
Accuracy (95%CI)	0.59 (0.57, 0.60)	0.17 (0.15, 0.20)
Sensitivity (95%CI)	0.65 (0.56, 0.73)	0.96 (0.89, 0.99)
Specificity (95%CI)	0.58 (0.56, 0.60)	0.12 (0.10, 0.14)
PPV (95%CI)	0.07 (0.06, 0.09)	0.07 (0.05, 0.09)
NPV (95%CI)	0.96 (0.95, 0.97)	0.97 (0.93, 0.99)
Likelihood (+) (95%CI)	1.58 (1.38, 1.80)	1.09 (1.03, 1.14)
Likelihood (-) (95%CI)	0.59 (0.46, 0.75)	0.32 (0.10, 0.99)
DOR (95%CI)	2.66 (1.84, 3.84)	3.38 (1.05, 10.89)
H-L GOF	X^2 = 11.84, df = 8, p = 0.15	X^2 = 13.94, df = 7, p = 0.052
Delong's AUC Test	D = -1.09, df = 2316.6, p = 0.27	

SM Table 2.14 Final Model for PCR and ILI population. Outcome is complication requiring further treatment (C-FT).

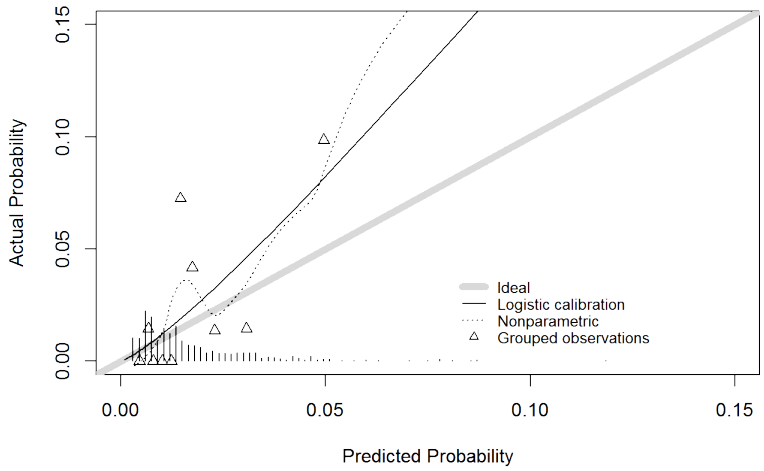
FLU C-FT Model	
Variable	**OR (95% CI)**
Age:	
(per year)	1.02 (1.015, 1.03)
Throat Physical (Normal/Abnormal)	
Abnormal	1.42 (1.03, 2.63)
Asthma (Yes/No):	
Yes	1.67 (1.05, 2.58)
Nasal Symptoms (Mild/Severe):	
Severe	1.43 (1.08, 1.90)
Sore Throat (Yes/No):	
Yes	0.59 (0.43, 0.82)
Cough (Mild/Severe):	
Severe	1.35 (0.91, 2.05)
Myalgia (Mild/Severe):	
Severe	1.62 (1.07, 2.54)
Fatigue (Mild/Severe):	
Severe	1.55 (0.90, 2.86)
Chills Sweats (Mild/Severe):	
Severe	1.33 (0.90, 2.02)
Tamiflu Rx (Placebo/75mg)	
Placebo	1.29 (0.98, 1.69)
ILI C-FT Model	
Variable	**OR (95% CI)**
Age:	
(per year)	1.01 (1.002, 1.017)
Sex (Male/Female)	
Female	1.20 (0.96, 1.50)
Asthma (Yes/No):	
Yes	1.49 (0.933, 2.37)
Nasal Symptoms (Mild/Severe):	
Severe	1.19 (0.95, 1.49)
Sore Throat (Yes/No):	
Yes	0.66 (0.51, 0.86)
COPD (Yes/No):	
Yes	1.27 (0.96, 1.68)
Fatigue (Mild/Severe):	
Severe	2.01 (1.31, 3.23)
Asthma or COPD Rx (Yes/No):	
Yes	1.54 (1.02, 2.28)
Tamiflu Rx (Placebo/75mg)	
Placebo	1.25 (1.009, 1.56)

SM Table 2.15 Model performance in test and train data from FLU and ILI patients for complications requiring further treatment (C-FT)

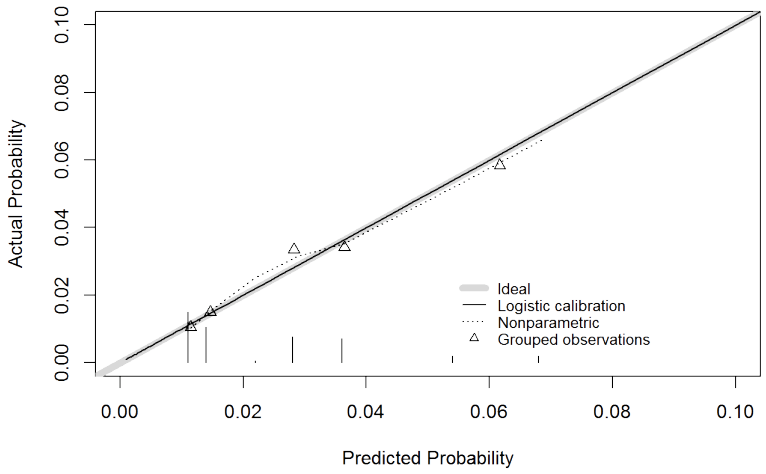
FLU C-FT Model		
Model (data)	FLU-FT (train)	FLU-FT (test)
AUC (95%CI)	0.66 (0.63, 0.70)	0.65 (0.60, 0.71)
Cut-off probability	0.168	0.168
Accuracy (95%CI)	0.65 (0.62, 0.67)	0.61 (0.57, 0.65)
Sensitivity (95%CI)	0.58 (0.52, 0.64)	0.57 (0.48, 0.66)
Specificity (95%CI)	0.66 (0.63, 0.68)	0.62 (0.58, 0.66)
PPV (95%CI)	0.24 (0.22, 0.29)	0.23 (0.19, 0.29)
NPV (95%CI)	0.89 (0.86, 0.90)	0.87 (0.84, 0.90)
Likelihood (+) (95%CI)	1.74 (1.53, 1.97)	1.52 (1.26, 1.83)
Likelihood (-) (95%CI)	0.62 (0.53, 0.72)	0.68 (0.55, 0.84)
DOR (95%CI)	2.78 (2.13, 3.64)	2.23 (1.50, 3.31)
H-L GOF	X^2= 10.18, df= 8, p = 0.21	X^2= 7.85, df= 8, p = 0.44
Delong's AUC Test	D= -0.253 , df= 1372.7, p= 0.799	
ILI C-FT Model		
Model (data)	ILI-FT (train)	ILI-FT (test)
AUC (95%CI)	0.63 (0.60, 0.66)	0.63 (0.59, 0.68)
Cut-off probability	0.156	0.156
Accuracy (95%CI)	0.62 (0.60, 0.64)	0.56 (0.53, 0.59)
Sensitivity (95%CI)	0.56 (0.51, 0.61)	0.61 (0.53, 0.68)
Specificity (95%CI)	0.63 (0.61, 0.65)	0.55 (0.52, 0.58)
PPV (95%CI)	0.22 (0.19, 0.25)	0.21 (0.18, 0.25)
NPV (95%CI)	0.88 (0.87, 0.90)	0.87 (0.84, 0.90)
Likelihood (+) (95%CI)	1.55 (1.40, 1.72)	1.37 (1.20, 1.58)
Likelihood (-) (95%CI)	0.68 (0.60, 0.76)	0.69 (0.57, 0.84)
DOR (95%CI)	2.27 (1.83, 2.82)	1.97 (1.43, 2.73)
H-L GOF	X^2 =5.61, df = 8, p = 0.68	X^2 = 11.27, df = 8, p = 0.18
Delong's AUC Test	D = -0.0117, df = 2177.4, p = 0.99	



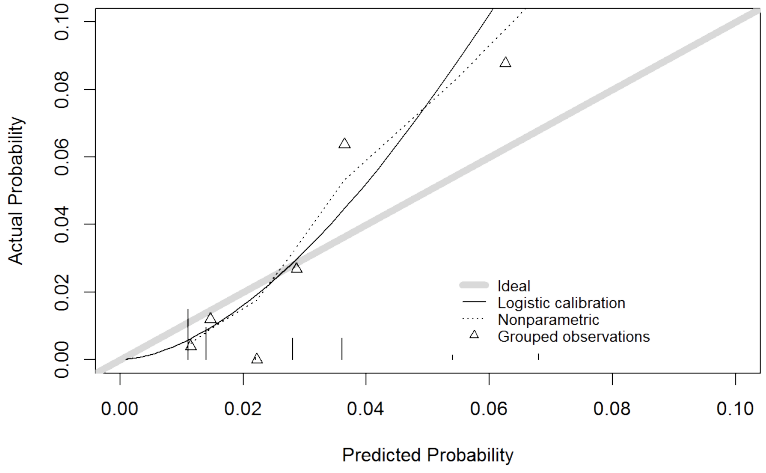
SM Figure 2.1 Observed versus expected for FLU C-S model in the training data.



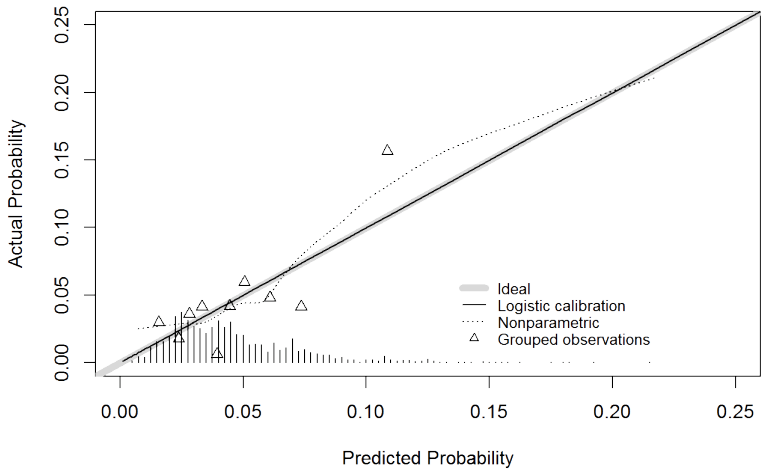
SM Figure 2.2 Observed versus expected for FLU C-S model in the test data.



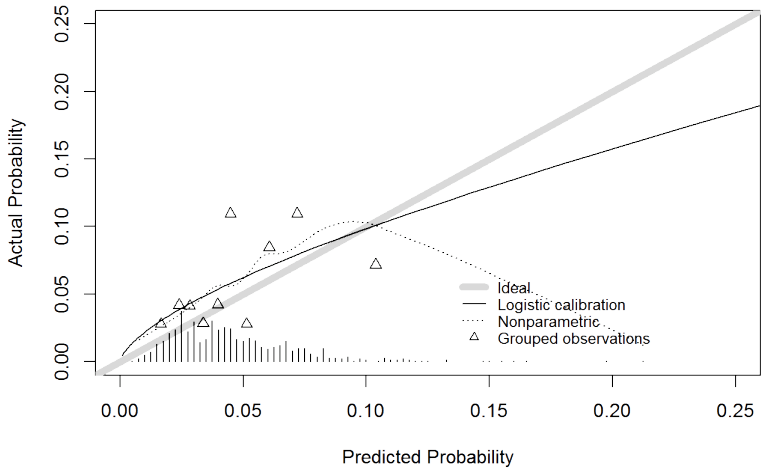
SM Figure 2.3 Observed versus expected for ILI C-S model in the training data.



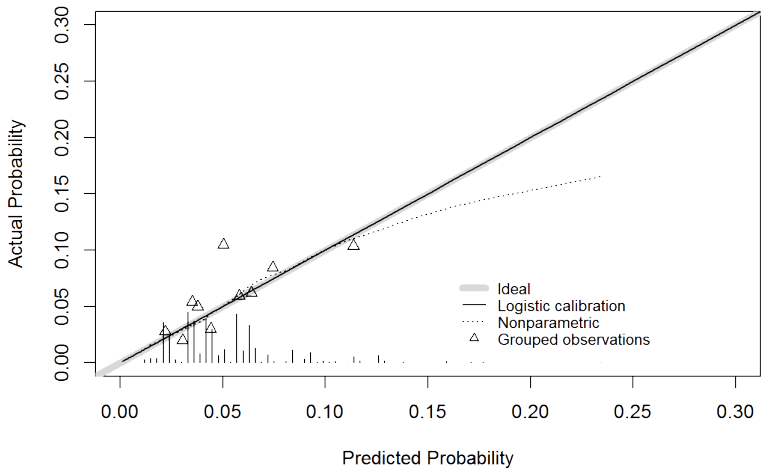
SM Figure 2.4 Observed versus expected for ILI C-S model in the test data.



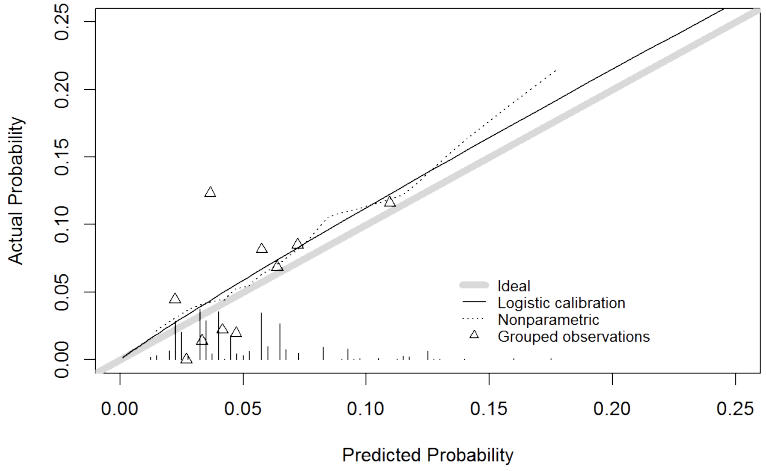
SM Figure 2.5 Observed versus expected for FLU C-AB model in the training data.



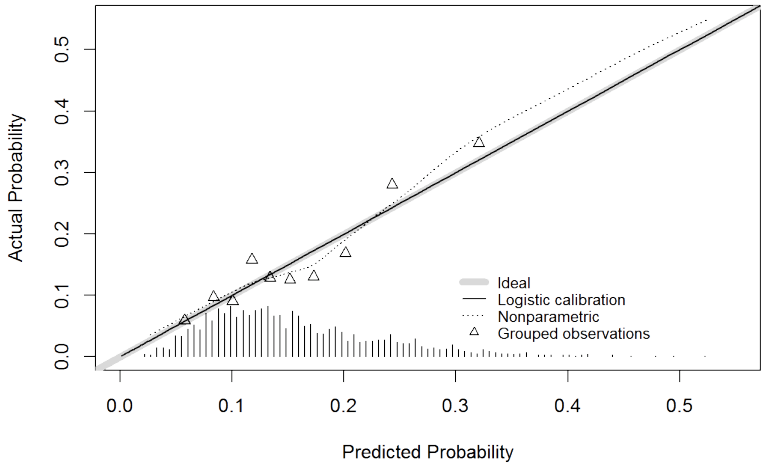
SM Figure 2.6 Observed versus expected for FLU C-AB model in the test data.



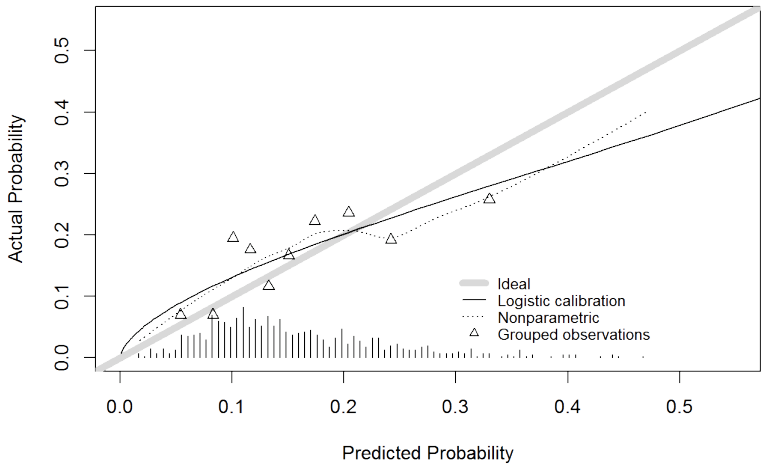
SM Figure 2.7 Observed versus expected for ILI C-AB model in the training data.



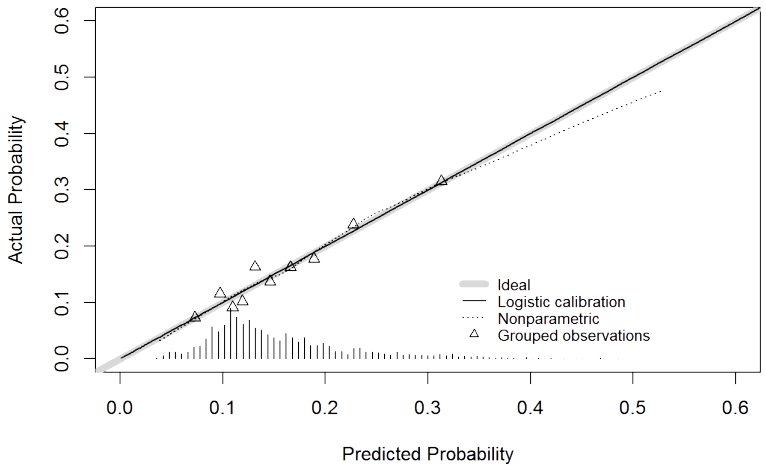
SM Figure 2.8 Observed versus expected for ILI C-AB model in the test data.



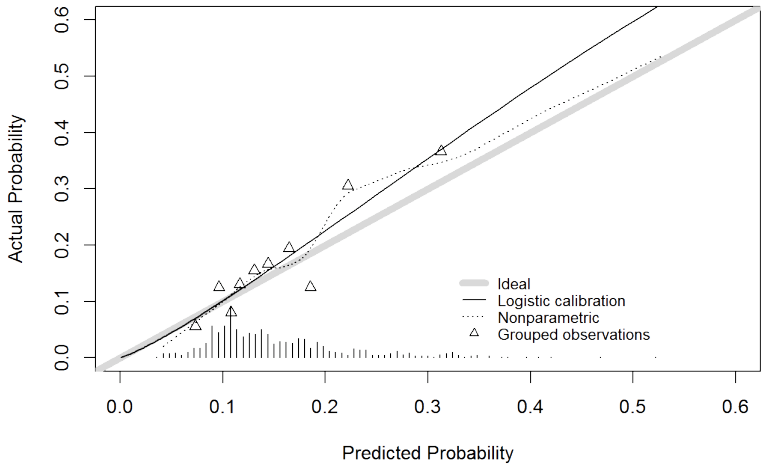
SM Figure 2.9 Observed versus expected FLU-FT model in the training data.



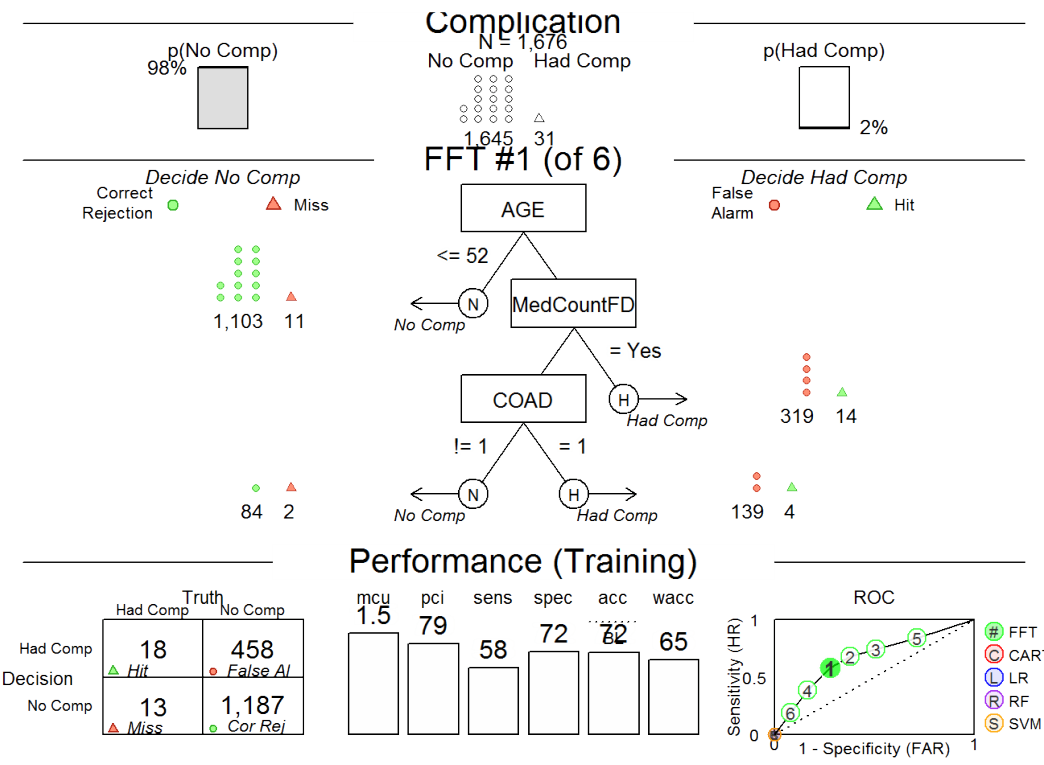
SM Figure 2.10 Observed versus expected FLU-FT model in the test data.



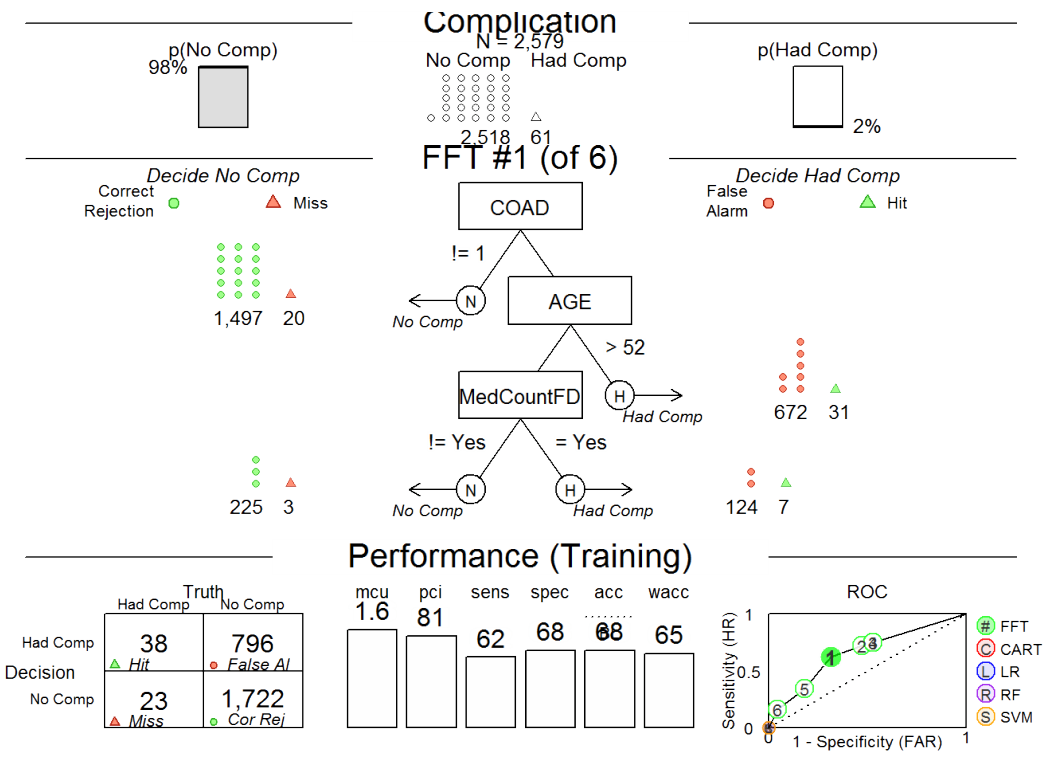
SM Figure 2.11 Observed versus expected for ILI-FT model in the training data.



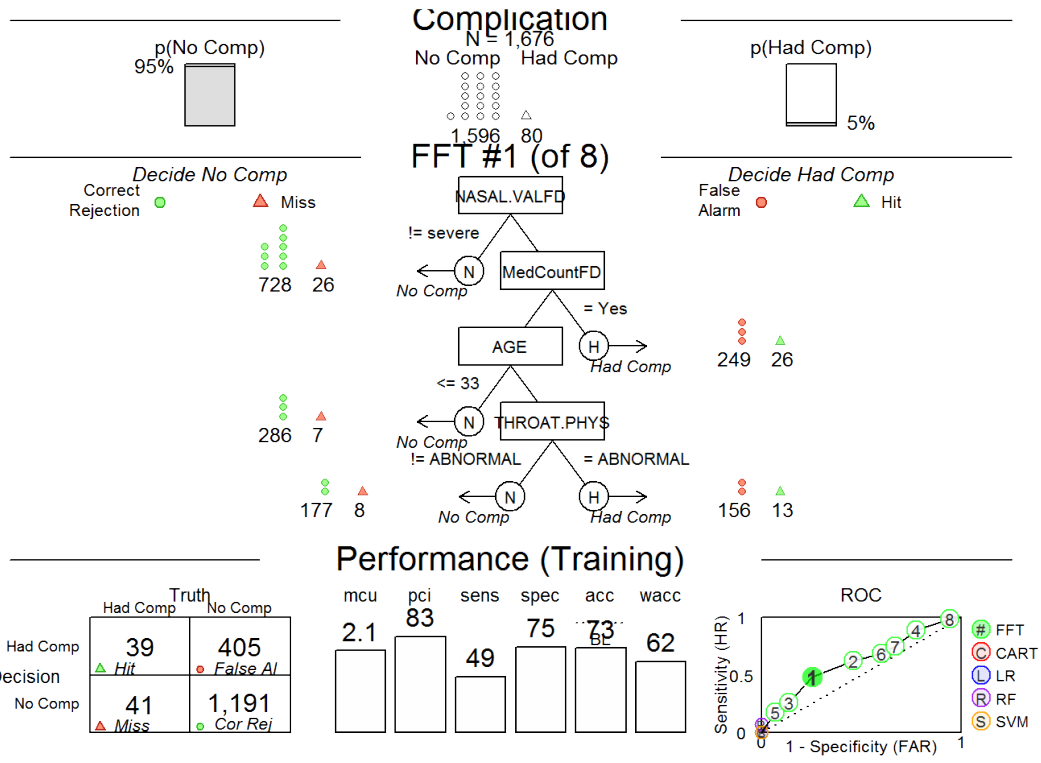
SM Figure 2.12 Observed versus expected for ILI-FT model in the test data.



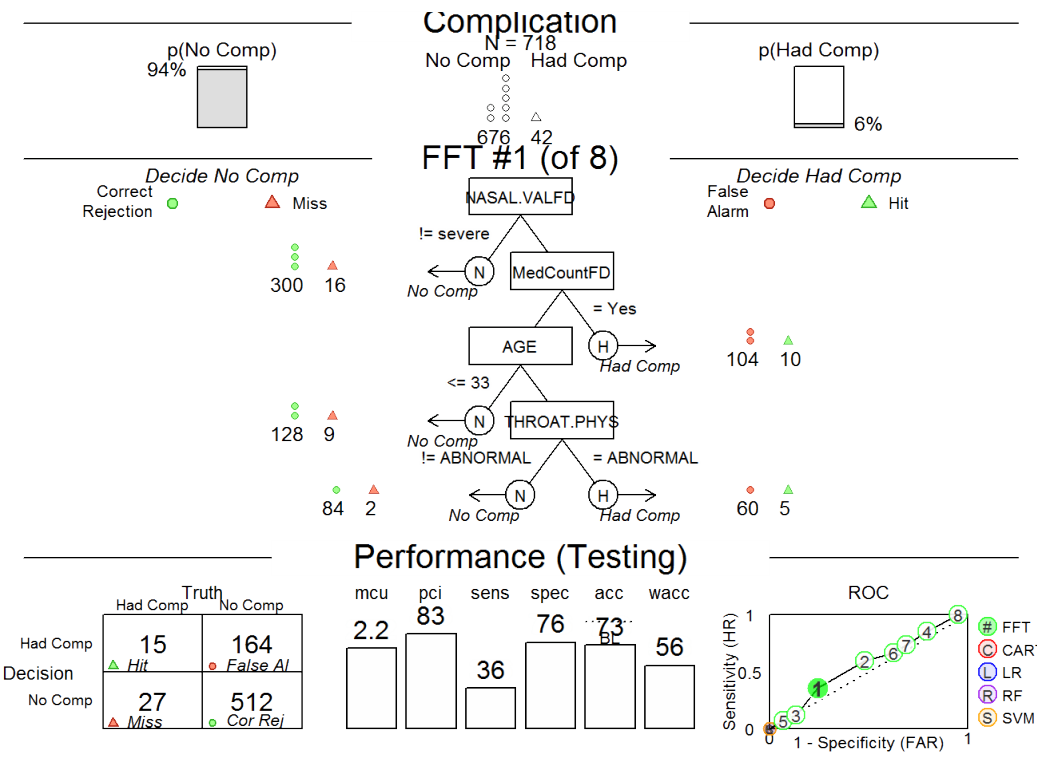
SM Figure 2.13 C-S-Tree developed in the training data to predict the serious complications.



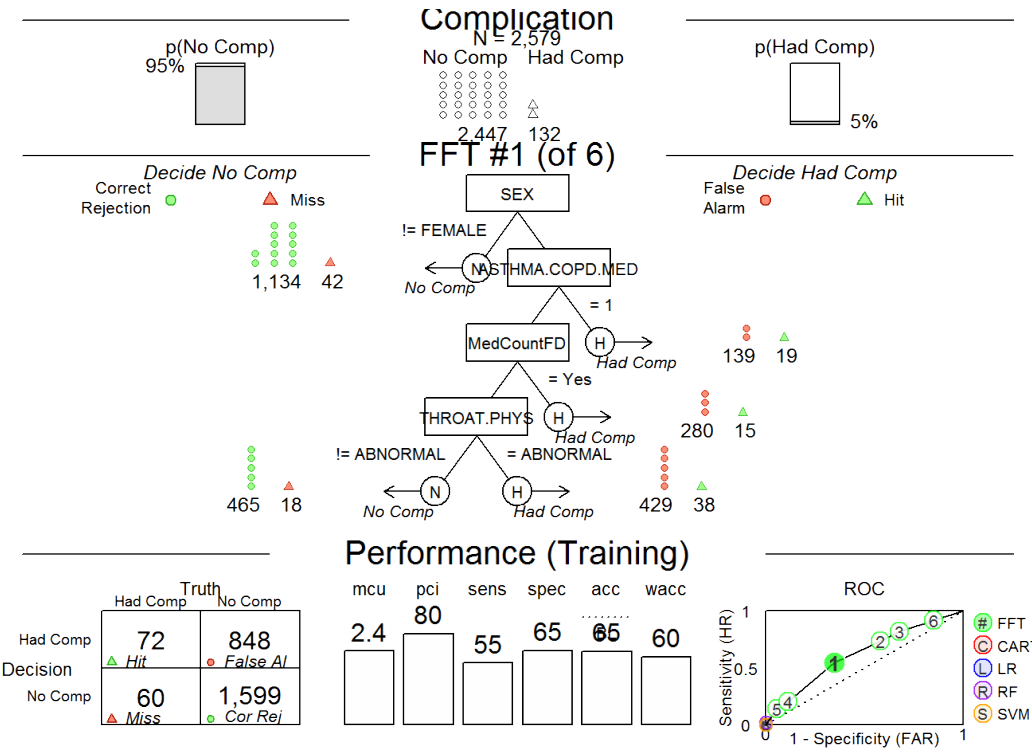
SM Figure 2.14 ILI C-S tree developed in the training data to predict serious complications.



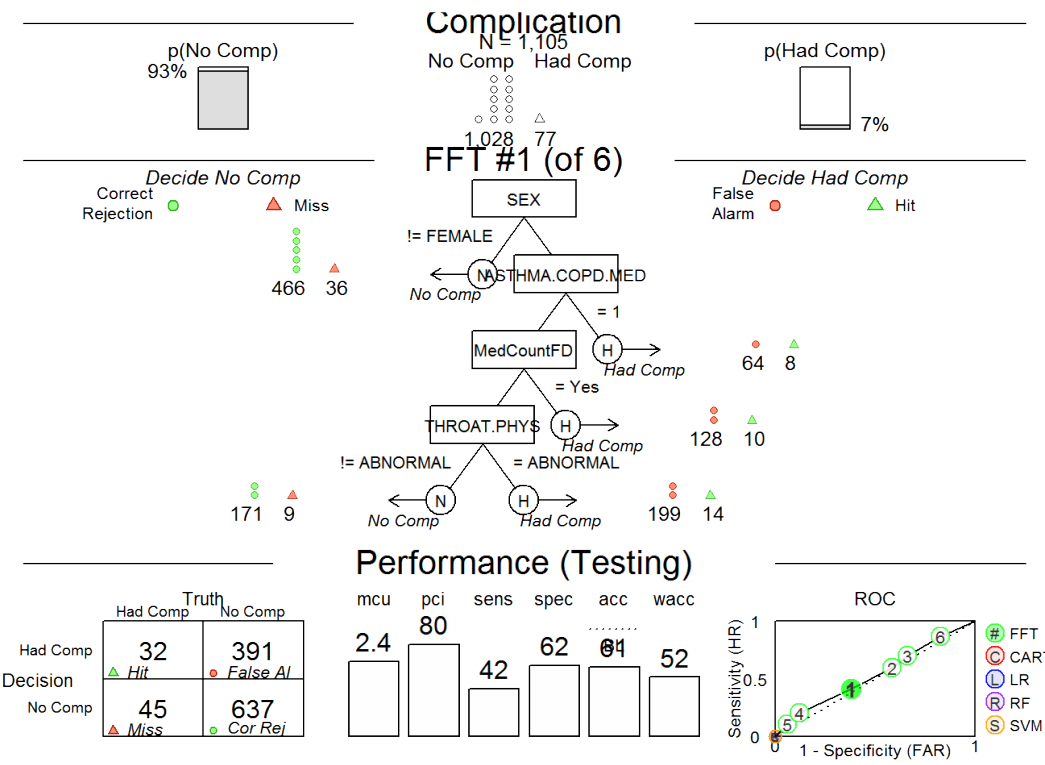
SM Figure 2.15 FLU C-AB tree developed in training data to predict complications that require antibiotics.



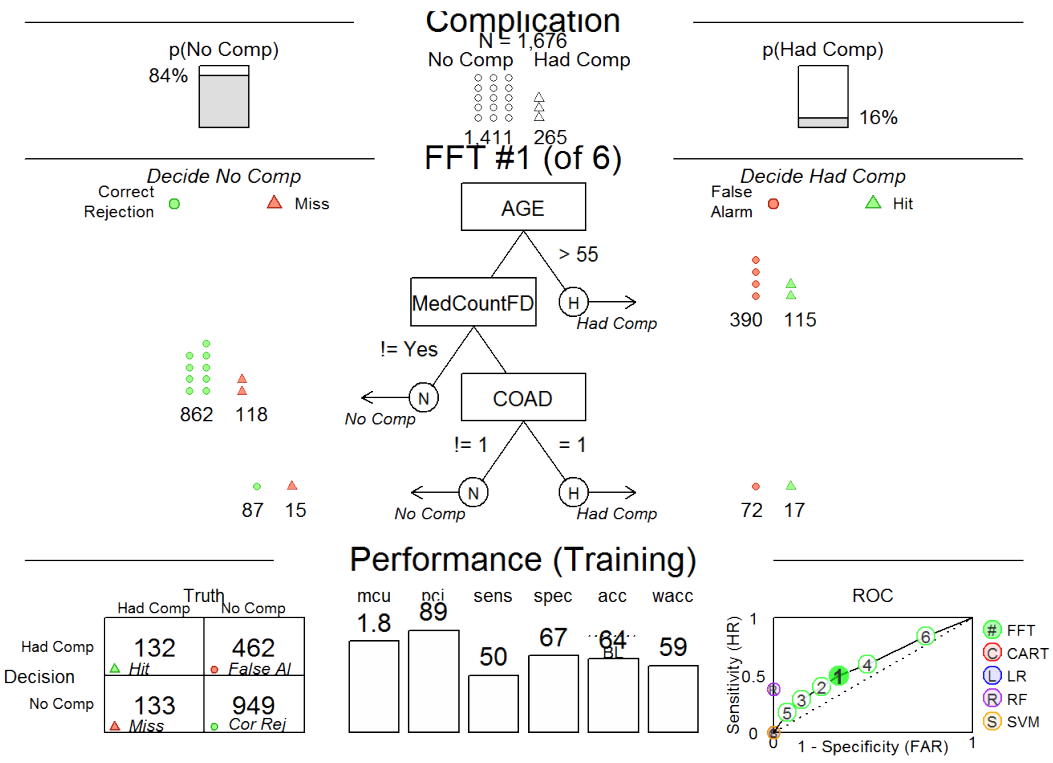
SM Figure 2.16 FLU C-AB tree applied to the test data to predict complications that require antibiotics.



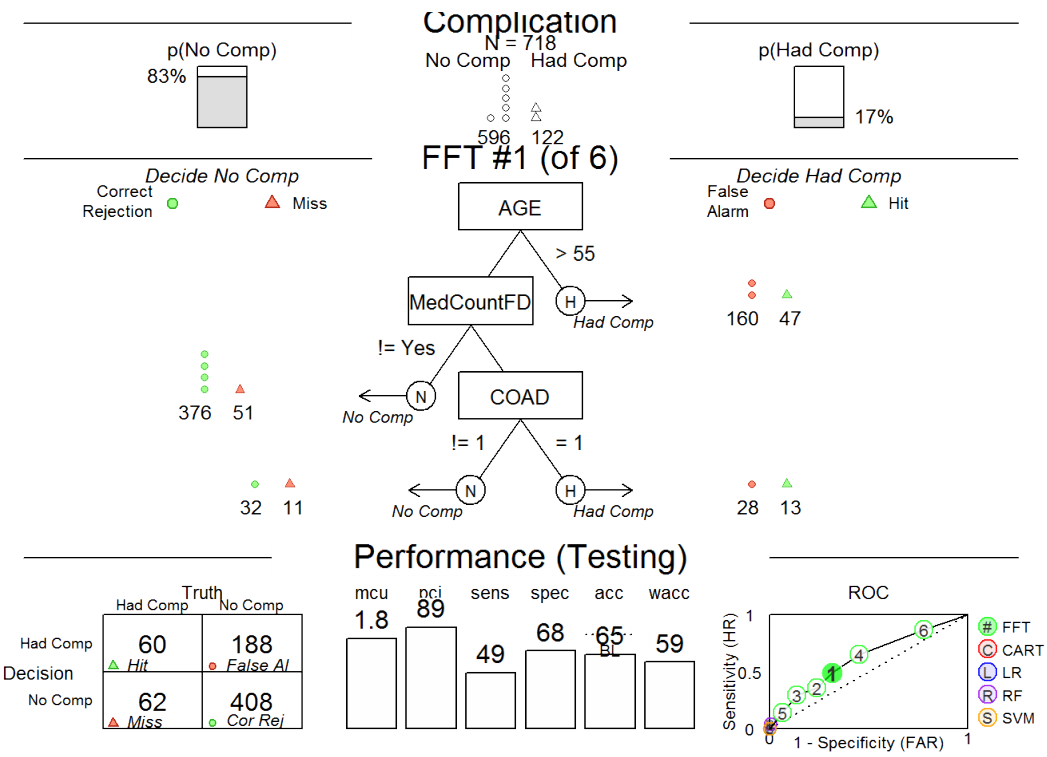
SM Figure 2.17 ILI C-AB tree developed in training data to predict complications that require antibiotics.



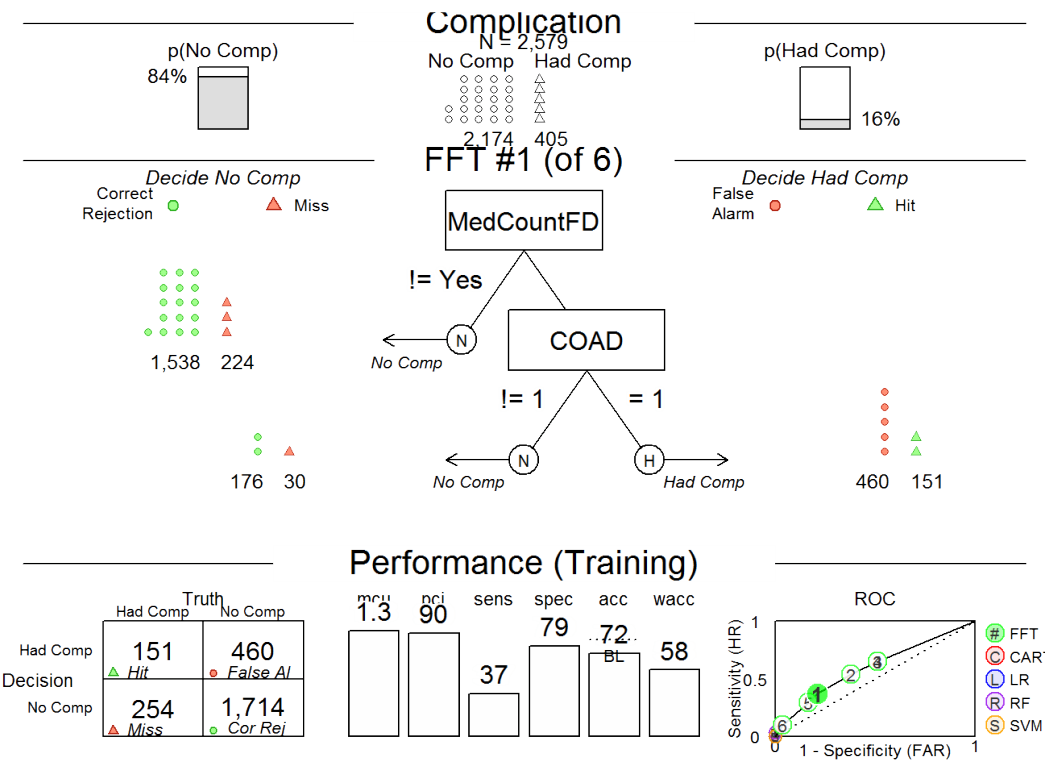
SM Figure 2.18 ILI C-AB tree applied to the test data to predict complications that require antibiotics.



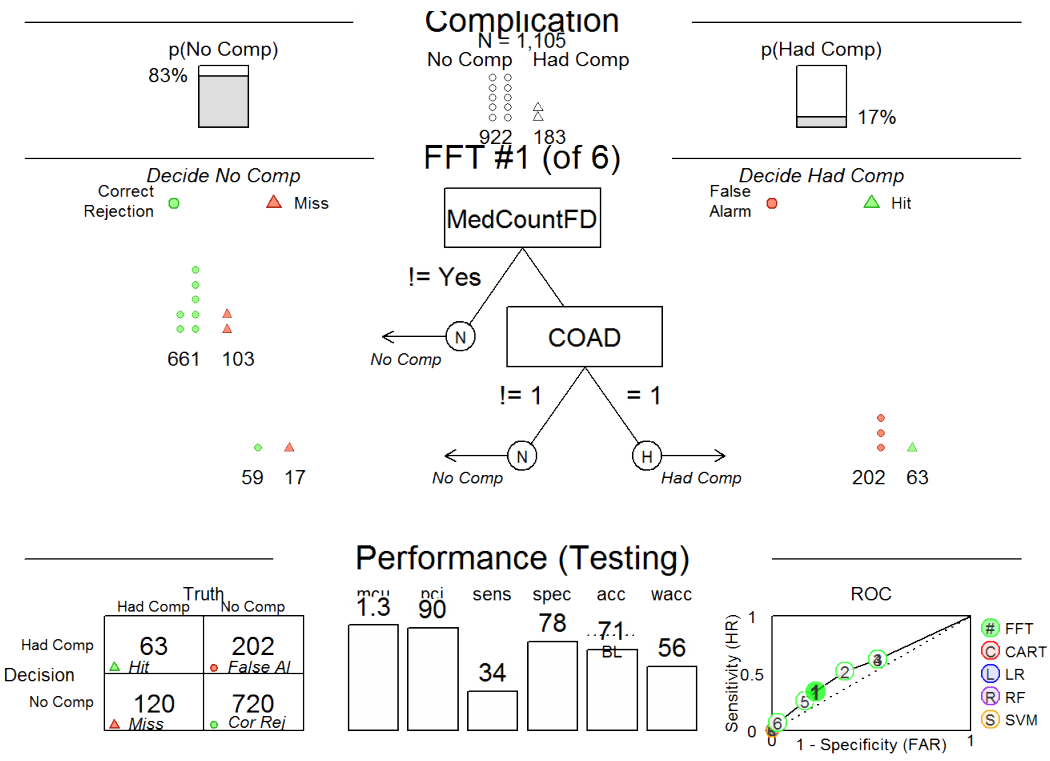
SM Figure 2.19 FLU-FT tree developed in the training data to predict complications that require further treatment.



SM Figure 2.20 FLU-FT tree applied to the test data to predict complications that require further treatment.



SM Figure 2.21 ILI-FT tree developed in the training data to predict complications that require further treatment.

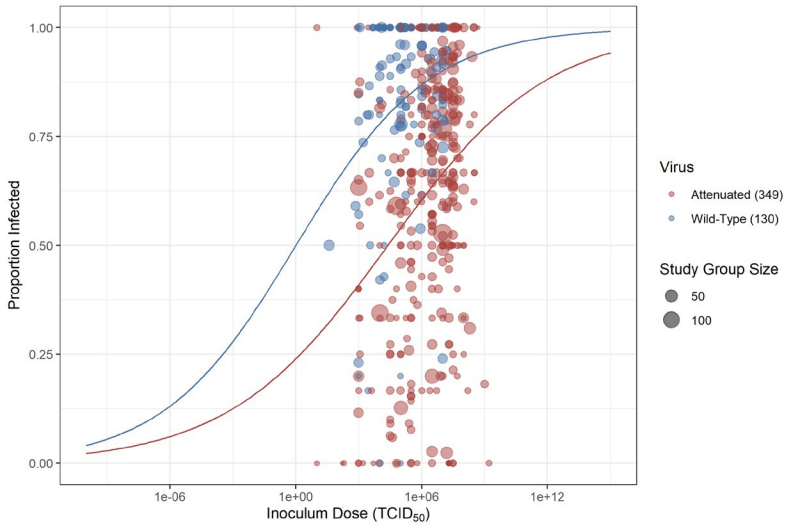


SM Figure 2.22 ILI-FT tree applied to the test data to predict complications that require further treatment.

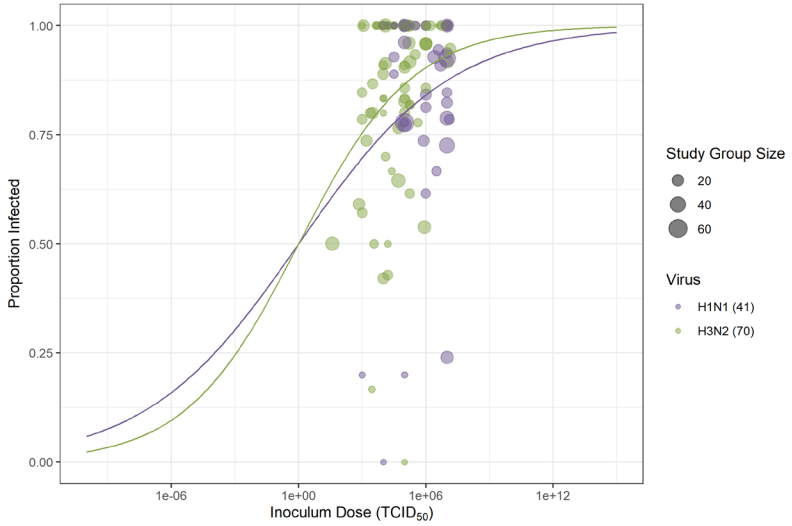
APPENDIX B

CHAPTER 3 SUPPLEMENTARY MATERIAL

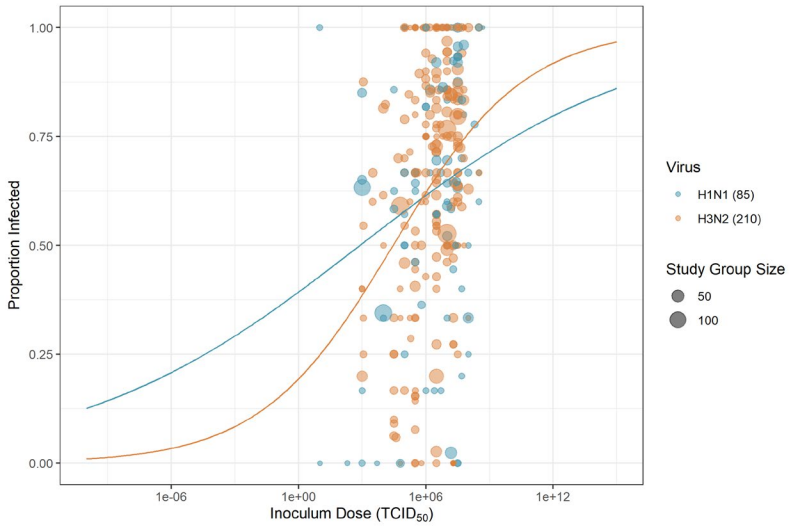
THE IMPACT OF INOCULUM DOSE ON INFECTION AND IMMUNITY OUTCOMES FOR INFLUENZA VIRUS



SM Figure 3.14: Impact of Inoculum Dose on proportion infected stratified by wild-type and attenuated. Weighted fit using approximate beta Poison function minimizing sum of square residuals SSR

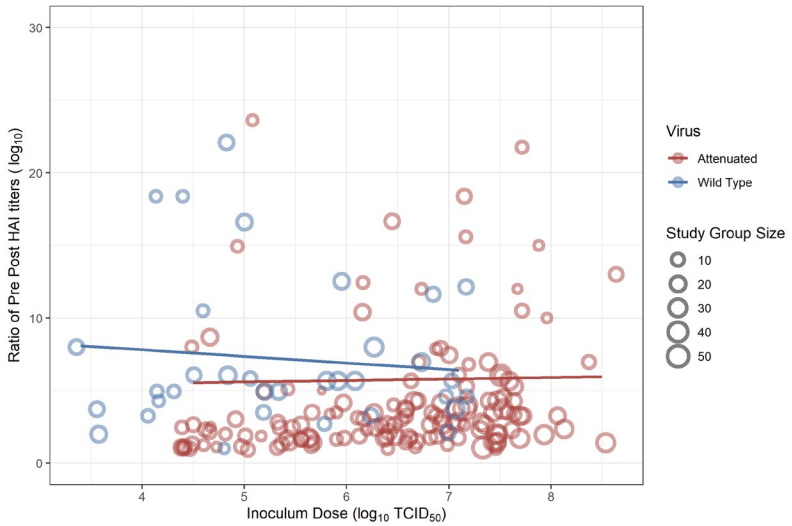


SM Figure 3.15: Impact of Inoculum Dose on proportion infected wild-type stratified by subtype. Weighted fit using approximate beta Poison function minimizing sum of square residuals SSR

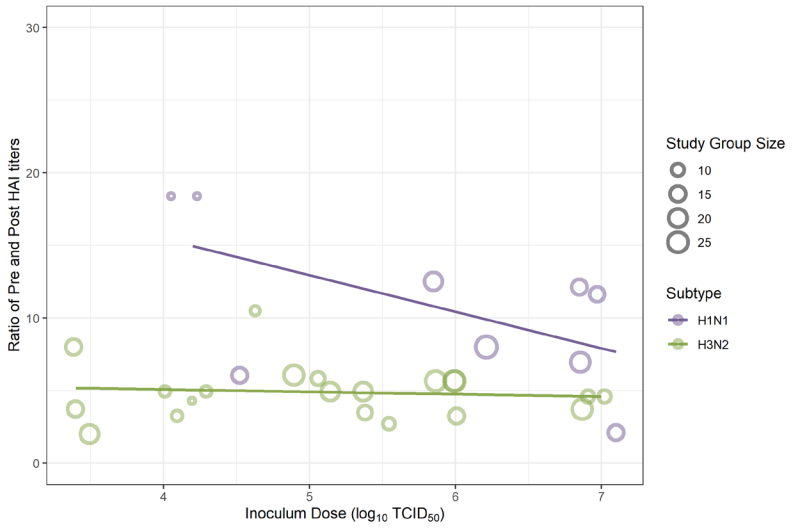


SM Figure 3.16: Impact of Inoculum Dose on proportion infected wild-type stratified by subtype. Weighted fit using approximate beta Poison function minimizing sum of square residuals SSR.

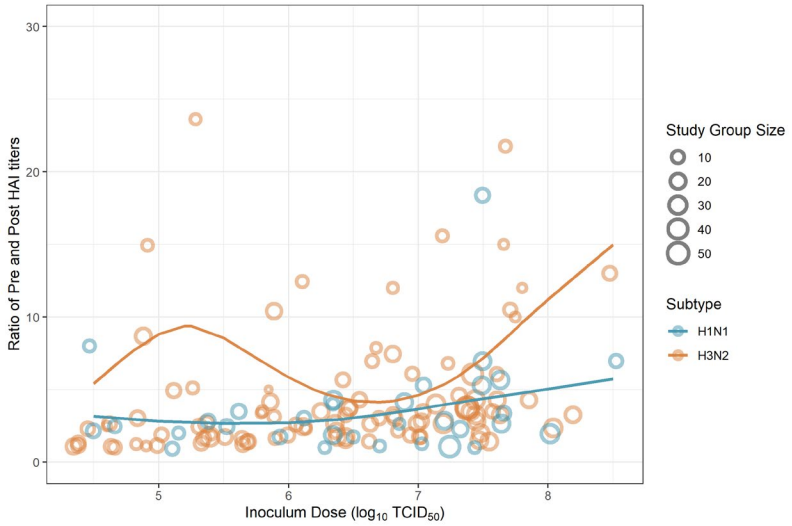
Immune ratio



SM Figure 3.17: Impact of Inoculum Dose on proportion Systemic Weighted. Stratified by wild type vs attenuated



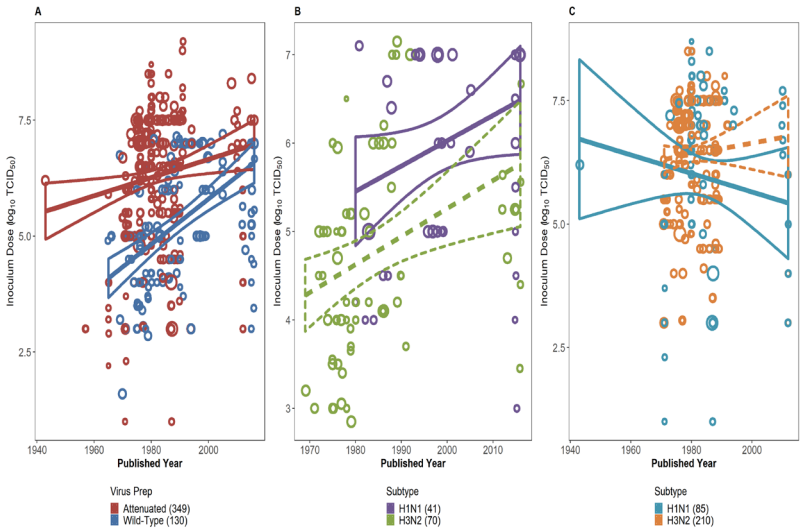
SM Figure 3.18: Impact of Inoculum Dose on proportion of patients with 4-fold or significant increase in HAI. Wild type virus stratified by subtype



SM Figure 3.19: Impact of Inoculum Dose on proportion of patients with 4-fold or significant increase in HAI. Attenuated virus stratified by subtype

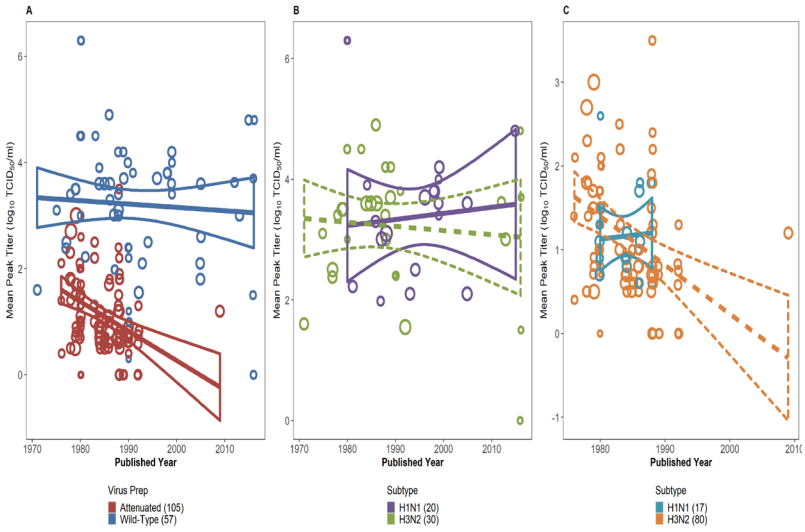
Changes over time

Dose



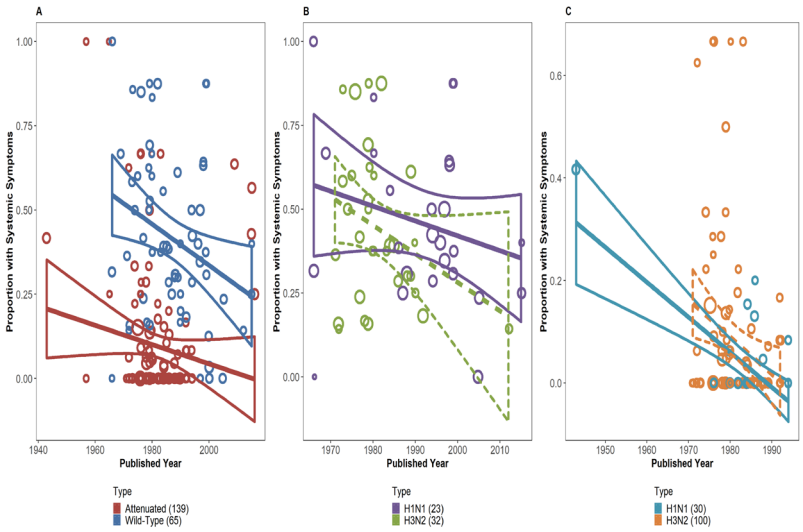
SM Figure 3.20: Change in Dose over Time A: Stratified by Virus Prep, B: Wild-type Stratified by Virus Subtype, C: Attenuated Stratified by Virus Subtype

Mean peak titer



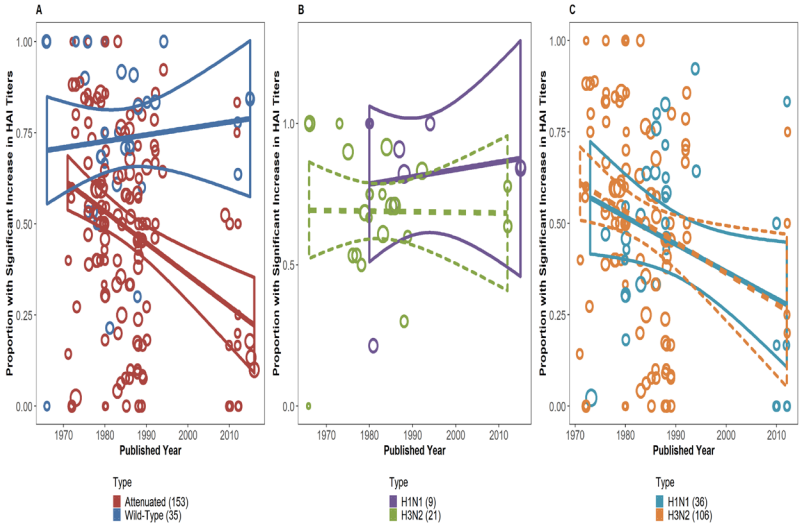
SM Figure 3.21: Proportion Systemic over Time A: Stratified by Virus Prep, B: Wild-type Stratified by Virus Subtype, C: Attenuated Stratified by Virus Subtype

Proportion Systemic



SM Figure 3.22: Proportion Systemic over Time A: Stratified by Virus Prep, B: Wild-type Stratified by Virus Subtype, C: Attenuated Stratified by Virus Subtype

Immune response



SM Figure 3.23: Proportion with significant increase in HAI titers over Time A: Stratified by Virus Prep, B: Wild-type Stratified by Virus Subtype, C: Attenuated Stratified by Virus Subtype

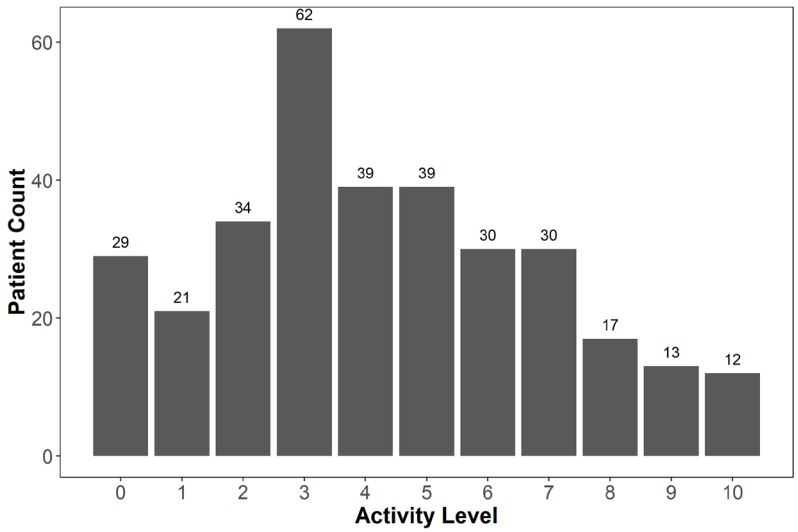
APPENDIX C

CHAPTER 4 SUPPLEMENTARY MATERIAL

VIRULENCE-MEDIATED INFECTIOUSNESS AND ACTIVITY TRADE-OFFS AND THEIR IMPACT ON TRANSMISSION POTENTIAL OF PATIENTS INFECTED WITH INFLUENZA

Histogram of reported activity levels

Reported activity levels ranging from 0 to 10 with a median of 4 for those patients with a lab diagnosis of influenza (SM Figure 4.1)

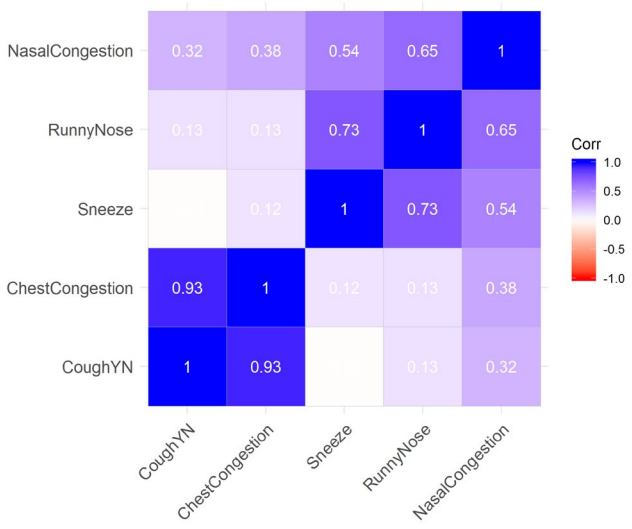


SM Figure 4.1: Histogram of reported activity levels for patients with a lab diagnosis of influenza.

Correlation of symptoms reported in the main text

Infectiousness symptom correlation

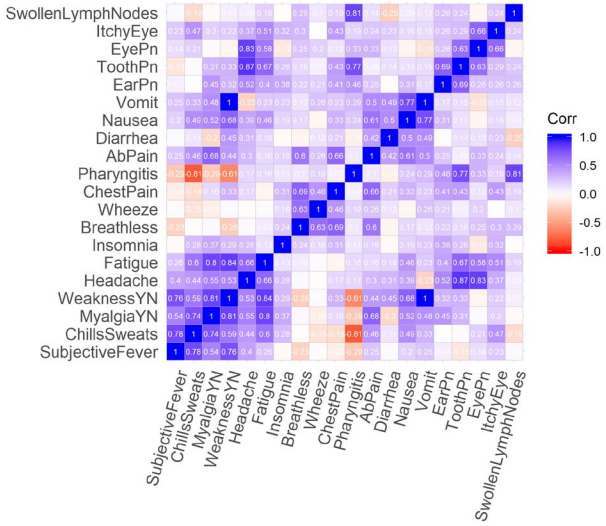
Cough and chest congestion had a Yule correlation coefficient greater than 0.9 (SM Figure 4.2).



SM Figure 4.2: Correlation of infectiousness symptoms for patients with a lab diagnosis of influenza.

Morbidity symptom correlation

Vomiting and weakness had a Yule correlation coefficient greater than 0.9 (SM Figure 3).



SM Figure 4.3: Correlation of morbidity symptoms for patients with a lab diagnosis of influenza.

Sensitivity Analyses

Correlation Cut off of 0.75 vs. 0.9

Summary of differences

The overall conclusions and the infectiousness score did not change at all. The morbidity score changed with 7 symptoms being excluded. This new morbidity score included Abdominal Pain, Breathlessness, Chest pain, Diarrhea, Ear Pain, Headache, Itchy Eyes, Myalgia, Nausea, Sleeplessness, Subjective Fever, Swollen Lymph Nodes, and Wheezing. The new morbidity score had a possible range of 0 to 13.

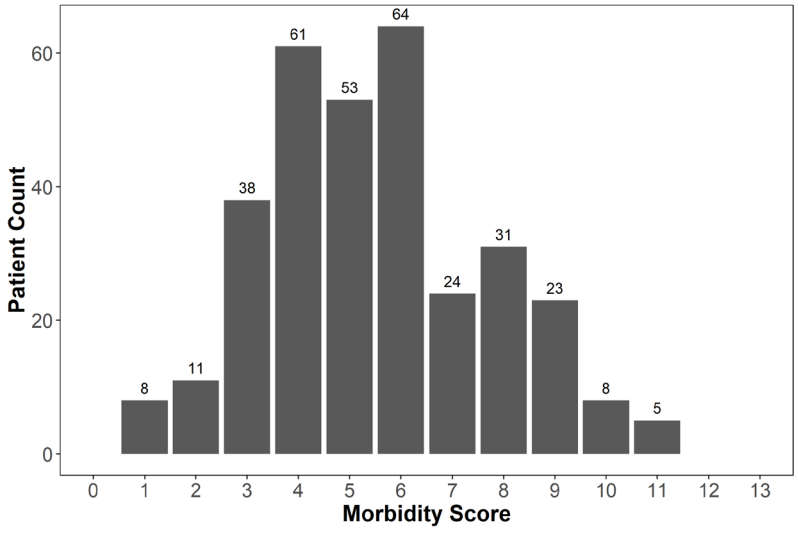
The distribution is similar in that is centered but there is a difference in the minimum and maximum score (1 compared to 2 and 11 compared to 17 respectively) (SM Figure 4). The observed

relationship the morbidity score had between activity and infectiousness score are unchanged (SM Figure 4.5 and 4.6).

Calculating new morbidity score

The morbidity score did change. When the cut off of 0.75 was applied, seven symptoms were dropped. Starting with the highest correlations first: Weakness/Vomit (Q=1) keep vomit, Tooth pain/Headache (Q=.87) keep Headache, Headache/Eye pain (Q=.83) keep Headache, swollen lymph nodes/SoreThroat (Q=.81) keep SwollenLympnodes, Fatigue/Myalgia (Q=.80) keep BodyAches, SubjectiveFever/ChillsSweats (Q=.78) keep SubjectiveFever, Vomit/Nausea (Q=.77) keep Nausea. The new morbidity score includes Abdominal Pain, Breathlessness, Chest pain, Diarrhea, Ear Pain, Headache, Itchy Eyes, Myalgia, Nausea, Sleeplessness, Subjective Fever, Swollen Lymph Nodes, and Wheezing. The new morbidity score ranges from 0 to 13.

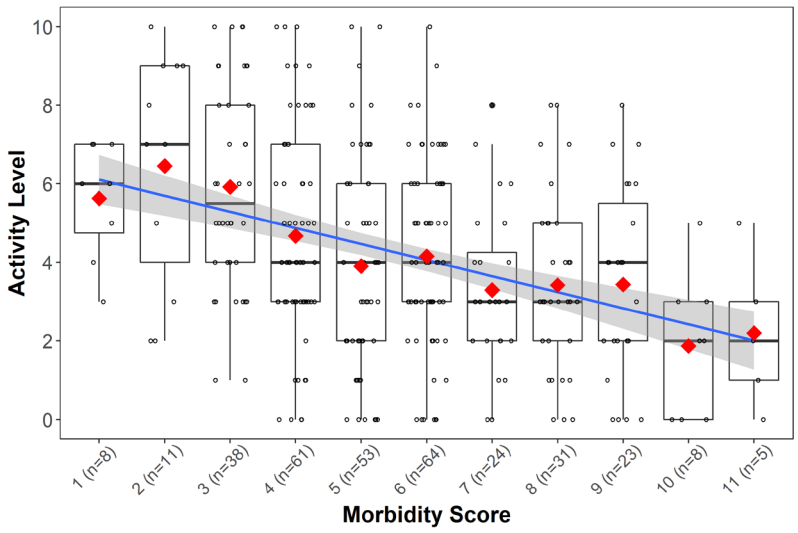
The mean morbidity score when 0.75 was used as the cut off was 5.51, and no patients had a morbidity score of 0, 12, or 13 (SM Figure 4.4). The distribution is still as expected since all the patients felt ill enough to seek medical care, but none were sick enough to require urgent care or hospitalization.



SM Figure 4.4: Distribution of the morbidity score.

Impact of morbidity score on activity

Analysis of the association between the new morbidity score and the patient's self-reported activity level suggests that higher morbidity score is associated with a reduced activity. Spearman's rank correlation indicates a negative relationship r = -0.33 (95% CI: -0.42, -0.23) and the Cochran-Mantel-Haenszel trend test is statistically significant ($\chi^2 = 36.78$, $df = 1$, $p < 0.01$) (SM Figure 4.5). The observed pattern is consistent and clear, with a reduction of 67% in mean activity going from the lowest to the highest morbidity score.

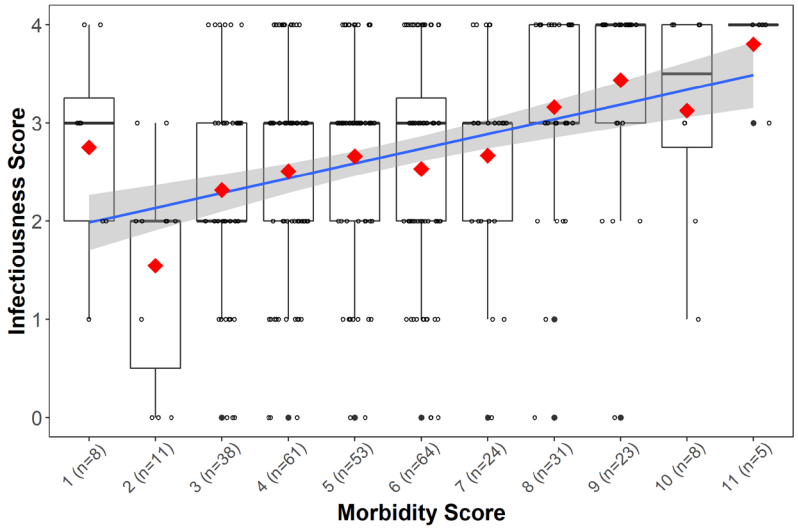


SM Figure 4.5: Activity level for each level of the morbidity score. Red diamonds indicate the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

Impact of morbidity score on infectiousness score

Analysis of the relationship between the morbidity and infectiousness scores show a positive correlation. Spearman's rank correlation indicates a positive relationship $r = 0.28$ (95% CI: 0.18, 0.38) and the Cochran-Mantel-Haenszel trend test is statistically significant ($\chi^2 = 25.52$, $df = 1$, $p < 0.01$) (SM Figure 6). Apart from the activity levels for low morbidity score values (with small

sample sizes), the pattern is consistent and clear, with an increase of 33% in the infectiousness score going from the lowest to the highest morbidity score.



SM Figure 4.6: Infectiousness score for each level of the morbidity score. Red diamonds indicate the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

Analysis using all patients diagnosed with influenza

Summary of differences

The overall conclusions remain the same when the empirically diagnosis patients are included. From here on the population used to generate the results in the main text will be referenced to as "lab diagnosis" and the population to generate the results below will be referenced to as "any diagnosis"

There were no meaningful differences in the univariate analysis (SM Table 4.2). Among patients with any diagnosis the most predictive multi-variate model was different then the model selected using lab diagnosis and included chest congestion, headache, sleeplessness, subjective fever, vomiting, and weakness (SM Table 4.2). Both models included 6 symptoms, and 5 of the symptoms are in both (headache, sleeplessness, subjective fever, vomiting, and weakness). For

patients with any diagnosis chills/sweats was included while chest congestion was included for the patients with a lab diagnosis.

Both of the scores were different for the any diagnosis patients compared to the lab diagnosis patients. The infectiousness score for the any diagnosis patients included all of the possible symptoms (SM Figure 7, 9), compared to the lab diagnosed patients where cough was removed. Among the morbidity symptoms for patients with any diagnosis none were had a correlation greater than 0.9. Compared to the patients with a lab diagnosis were the morbidity score excluded weakness. Based on these results two new scores were calculated for the patients with any diagnosis. The infectiousness score had a possible range of 0 to 5, and the morbidity score had a possible range of 0 to 20.

Using the new scores we examined the relationships of the scores between each other and activity levels. We again found that the infectiousness score had a weak association with reported activity, while the morbidity score showed a clear correlation with both the reported activity level and infectiousness score (SM Figures 4.11 - 4.13).

Description of the population

Influenza diagnosis for our population is determined using three different methods; a rapid antigen test, a PCR test, or by a physician giving an empirical diagnosis. In the main text, we considered any person who was diagnosed by either a rapid antigen or PCR test as having influenza. Here we repeat the analyses completed in the main text with the addition of patients with a diagnosis of influenza empirically based on symptoms. Patients with an empirical diagnosis are generally defined as having influenza-like illness (ILI). In total there are 716 patients with any diagnosis of influenza. These Patients reported activity levels ranging from 0 to 10 with a mean of 4.46. All of

the patients had symptoms of disease with only 16% reporting 10 or fewer. The most common symptom is weakness, and the least common symptom is vomiting (SM Table 4.1).

SM Table 4.1: Out of the 716 patients included the table shows the number of patients who reported having the following symptoms and the corresponding percentage.

	Overall
n	716
Abdominal Pain = Yes (%)	91 (12.7)
Breathlessness = Yes (%)	287 (40.1)
Chest Congestion = Yes (%)	398 (55.6)
Chest Pain = Yes (%)	224 (31.3)
Chills/Sweats = Yes (%)	589 (82.3)
Cough = Yes (%)	646 (90.2)
Diarrhea = Yes (%)	98 (13.7)
Ear Pain = Yes (%)	158 (22.1)
Eye Pain = Yes (%)	112 (15.6)
Fatigue = Yes (%)	653 (91.2)
Headache = Yes (%)	604 (84.4)
Itchy Eyes = Yes (%)	179 (25.0)
Myalgia = Yes (%)	637 (89.0)
Nasal Congestion = Yes (%)	550 (76.8)
Nausea = Yes (%)	254 (35.5)
Runny Nose = Yes (%)	511 (71.4)
Sleeplessness = Yes (%)	409 (57.1)

Sneeze = Yes (%)	388 (54.2)
Sore Throat = Yes (%)	598 (83.5)
Subjective Fever = Yes (%)	493 (68.9)
Swollen Lymph Nodes = Yes (%)	308 (43.0)
Tooth Pain = Yes (%)	163 (22.8)
Vomiting = Yes (%)	79 (11.0)
Weakness = Yes (%)	667 (93.2)
Wheezing = Yes (%)	217 (30.3)

Univariate and Subset Selection

We explored the univariate correlations between activity level and each symptom. All of the symptoms that were statistically significantly related to activity showed a negative correlation with activity level (SM Table 4.2). Based on cross-validated variable selection we found that a model that included chills/sweats, subjective fever, headache, weakness, sleeplessness, and vomiting creates the most predictive model (SM Table 4.2).

SM Table 4.2: Results of the univariate and multivariate linear regression of symptoms and activity. The coefficients are the estimated effect on activity when the symptom is present. The multivariate model was selected with a sequential forward floating selection, minimizing the root mean square error on test data through a 5-fold cross validation (20 times repeated). 95%CI = The 95% confidence interval for the coefficient.

Dependent: Activity				
Level		Mean (sd)	Coefficient (univariable)	Coefficient (multivariable)
Abdominal Pain	No	4.6 (2.6)	-	-
	Yes	3.8 (2.7)	-0.79 (-1.37 to -0.21, p=0.008)	-
Breathlessness	No	4.6 (2.7)	-	-

	Yes	4.2 (2.6)	-0.37 (-0.77 to 0.02, p=0.066)	-
Chest Congestion	No	4.7 (2.7)	-	-
	Yes	4.2 (2.5)	-0.49 (-0.88 to -0.10, p=0.013)	-
Chest Pain	No	4.6 (2.6)	-	-
	Yes	4.1 (2.8)	-0.45 (-0.87 to -0.03, p=0.035)	-
Chills/Sweats	No	6.2 (2.6)	-	-
	Yes	4.1 (2.5)	-2.07 (-2.55 to -1.58, p<0.001)	-1.27 (-1.77 to -0.77, p<0.001)
Cough	No	4.8 (2.8)	-	-
	Yes	4.4 (2.6)	-0.43 (-1.08 to 0.22, p=0.196)	-
Diarrhea	No	4.6 (2.7)	-	-
	Yes	3.7 (2.5)	-0.82 (-1.38 to -0.26, p=0.004)	-
Ear Pain	No	4.5 (2.6)	-	-
	Yes	4.2 (2.6)	-0.35 (-0.82 to 0.12, p=0.143)	-
Eye Pain	No	4.4 (2.6)	-	-
	Yes	4.5 (2.6)	0.04 (-0.49 to 0.58, p=0.876)	-
Fatigue	No	5.5 (2.6)	-	-
	Yes	4.4 (2.6)	-1.19 (-1.87 to -0.51, p=0.001)	-
Headache	No	5.6 (2.6)	-	-
	Yes	4.2 (2.6)	-1.31 (-1.84 to -0.79, p<0.001)	-0.89 (-1.38 to -0.40, p<0.001)
Sleeplessness	No	5.0 (2.7)	-	-
	Yes	4.1 (2.5)	-0.94 (-1.32 to -0.55, p<0.001)	-0.68 (-1.04 to -0.32, p<0.001)
Itchy Eyes	No	4.5 (2.7)	-	-
	Yes	4.4 (2.5)	-0.05 (-0.50 to 0.40, p=0.832)	-
Myalgia	No	5.5 (2.7)	-	-
	Yes	4.3 (2.6)	-1.15 (-1.77 to -0.54, p<0.001)	-
Nasal Congestion	No	4.8 (2.6)	-	-
	Yes	4.4 (2.7)	-0.39 (-0.85 to 0.07, p=0.098)	-
Nausea	No	4.8 (2.7)	-	-
	Yes	3.8 (2.5)	-0.97 (-1.37 to -0.58, p<0.001)	-
Sore Throat	No	4.5 (2.7)	-	-
	Yes	4.4 (2.6)	-0.07 (-0.60 to 0.45, p=0.782)	-

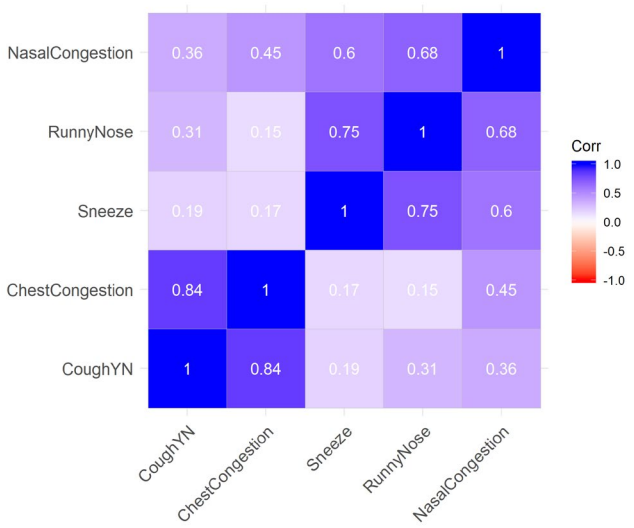
Runny Nose	No	4.6 (2.7)	-	-
	Yes	4.4 (2.6)	-0.15 (-0.58 to 0.27, p=0.479)	-
Sneeze	No	4.6 (2.7)	-	-
	Yes	4.4 (2.6)	-0.22 (-0.61 to 0.17, p=0.273)	-
Subjective Fever	No	5.6 (2.5)	-	-
	Yes	3.9 (2.6)	-1.64 (-2.04 to -1.24, p<0.001)	-0.94 (-1.35 to -0.53, p<0.001)
Swollen Lymph Nodes	No	4.5 (2.6)	-	-
	Yes	4.4 (2.6)	-0.09 (-0.48 to 0.30, p=0.643)	-
Tooth Pain	No	4.5 (2.6)	-	-
	Yes	4.2 (2.7)	-0.34 (-0.81 to 0.12, p=0.145)	-
Vomiting	No	4.6 (2.6)	-	-
	Yes	3.1 (2.3)	-1.56 (-2.17 to -0.96, p<0.001)	-1.27 (-1.83 to -0.71, p<0.001)
Weakness	No	6.3 (2.5)	-	-
	Yes	4.3 (2.6)	-1.99 (-2.74 to -1.23, p<0.001)	-0.94 (-1.66 to -0.22, p=0.010)
Wheezing	No	4.7 (2.7)	-	-
	Yes	4.0 (2.5)	-0.69 (-1.11 to -0.27, p=0.001)	-

Computation of Transmission and Morbidity Scores

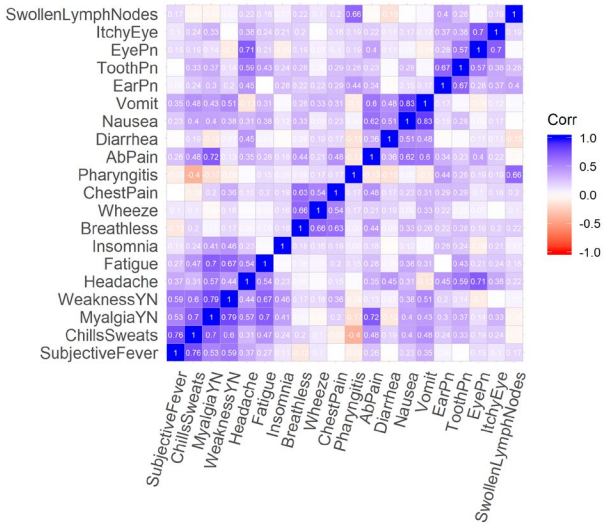
We used the same symptom classification presented in the main text.

None of the symptoms related to infectiousness were correlated with each other at a level of greater than 0.9 (SM Figure 4.7). This result differs from that in the main text where were cough was excluded. A new infectiousness score was calculated for this population ranging from 0 to 5.

Among the morbidity symptoms none had a correlation greater than 0.9 (SM Figure 8). This result differs from the analysis in the main text where vomiting was retained, and weakness was excluded. A new morbidity score was calculated for this population ranging from 0 to 20.



SM Figure 4.7: Correlation of infectiousness symptoms for patients with any diagnosis of influenza



SM Figure 4.8: Correlation of morbidity symptoms for patients with any diagnosis of influenza

The median infectiousness score is 4, and only 13 patients have an infectiousness score of 0 (SM Figure 9). Only 23% of patients have a score of 3 or less (SM Figure 4.9).

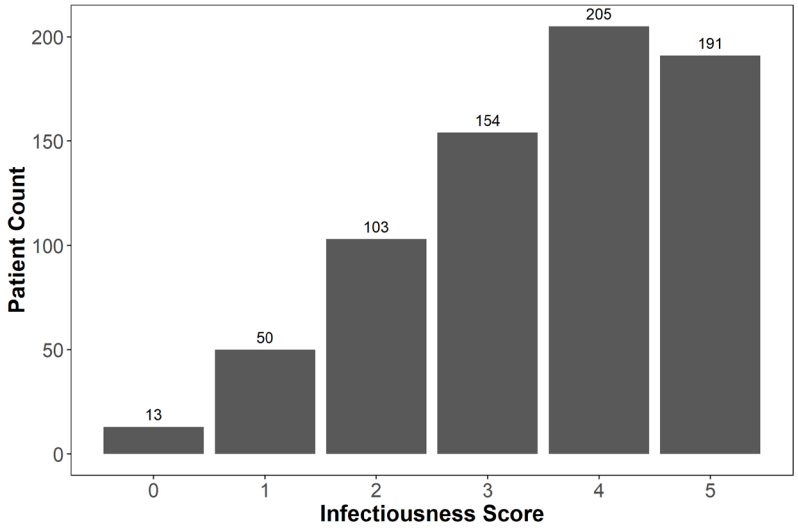
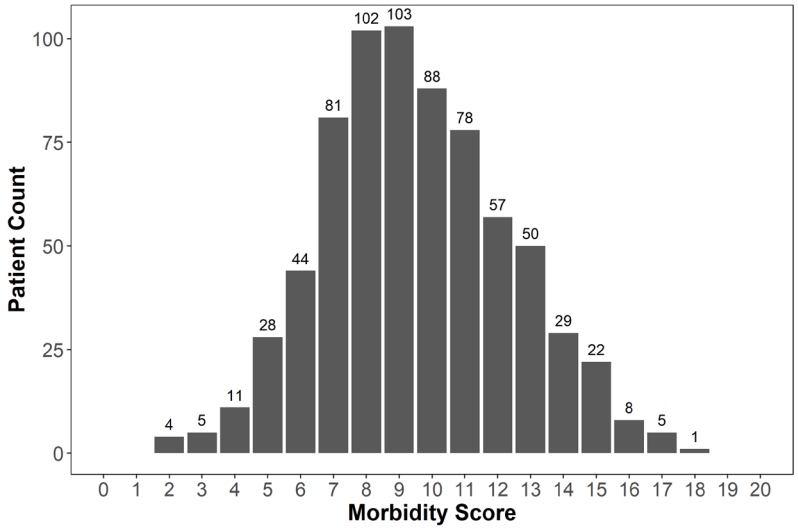


Figure 4.9: Distribution of the infectiousness score.

The median morbidity score is 9, and no patients have a morbidity score of 0, 1, 19, 20 (SM Figure 4.10). Such a centered distribution is expected since all the patients felt ill enough to seek medical care, but none were sick enough to require urgent care or hospitalization.

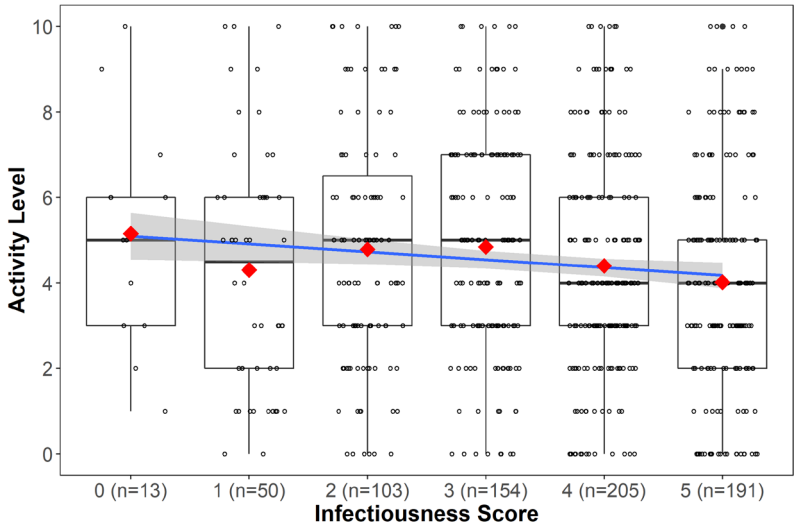


SM Figure 4.10: Distribution of the morbidity score.

Impact of infectiousness score on activity

Analysis of the impact of the infectiousness score on activity suggests that the value of this score has a negative correlation with the activity level. Spearman's rank correlation is r = -0.09 (95%

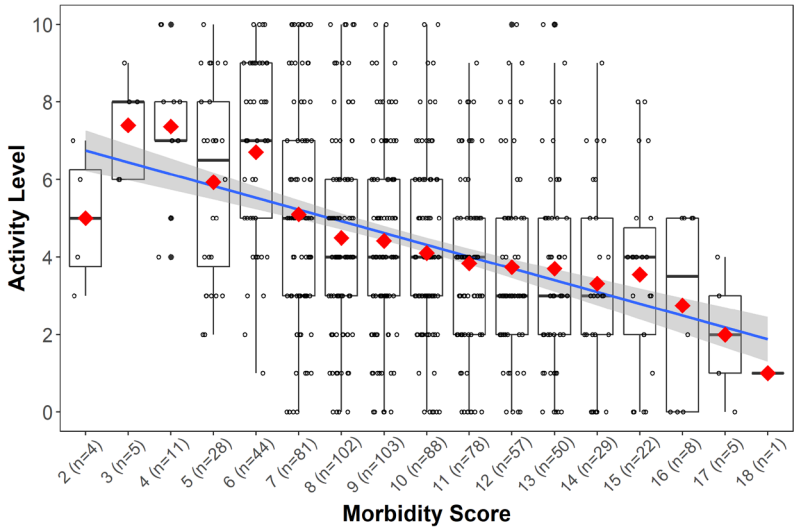
CI: -0.17, -0.02) and the Cochran-Mantel-Haenszel trend test is statistically significant ($\chi^2 = 5.94$, $df = 1$, $p = 0.01$) (SM Figure 4.11). This is different from the main analysis were we did not observe a clear relationship between activity and the infectiousness score.



SM Figure 4.11: Activity level for each level of the infectiousness score. The red diamond is the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

Impact of morbidity score on activity

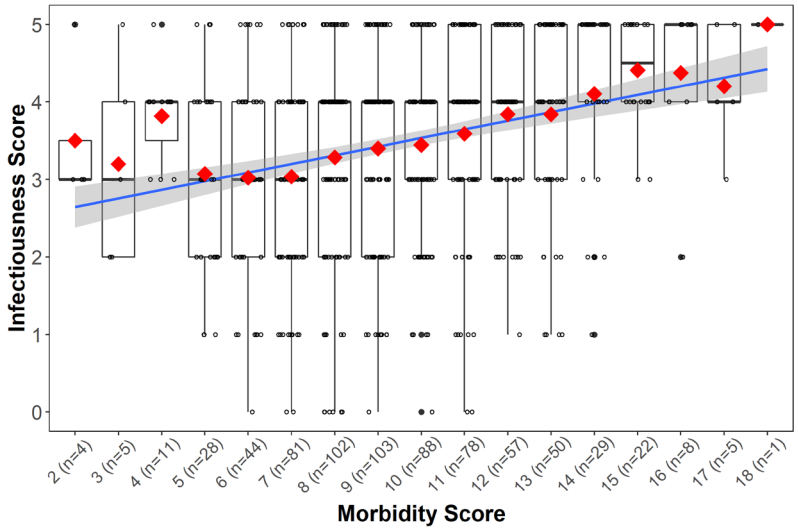
Analysis of the impact of the morbidity score on activity suggests that the value of this score is correlated with the activity level of a patient, with higher morbidity correlating with reduced activity. Spearman's rank correlation indicates a negative relationship r = -0.32 (95% CI: -0.38, -0.25) and the Cochran-Mantel-Haenszel trend test is statistically significant ($\chi^2 = 76.04$, $df = 1$, $p < 0.01$) (SM Figure 4.12). There is a reduction of 80% in mean activity going from the lowest to the highest morbidity score.



SM Figure 4.12: Activity level for each level of the morbidity score. The red diamond is the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

Impact of morbidity score on infectiousness score

Analysis of the relationship between the morbidity and infectiousness scores show a positive correlation. Spearman's rank correlation indicates a positive relationship ($r = 0.26$ (95% CI: 0.19, 0.32)) and the Cochran-Mantel-Haenszel trend test is statistically significant ($\chi^2 = 41.66$, $df = 1$, $p < 0.01$) (SM Figure 4.13). Apart from the values activity levels for low morbidity score (with small sample sizes), the pattern is consistent and clear, with an increase of 67% in the infectiousness score going from the lowest to the highest morbidity score.



SM Figure 4.13: Infectiousness score for each level of the morbidity score. The red diamond is the mean. The solid blue line is the linear regression fit. The shaded area is the 95% confidence interval for the linear regression.

APPENDIX D

SUPPLEMENTARY MATERIAL FOR CHAPTER 5

ASSOCIATIONS BETWEEN RELATIVE VIRAL LOAD AT DIAGNOSIS AND INFLUENZA A INFECTION SEVERITY AND RECOVERY

Univariate results

Pre-visit Questionnaire Table

The pre-visit questionnaire collected data on patient-reported activity level, symptom severity, and symptom evolution time. Activity levels in the 24 hours prior to the survey are reported on a Likert scale from 0 - 10, with 0 being bedridden, and 10 being normal levels of activity. Patient's symptom severity for cough, weakness, and body aches were each recorded as none, mild, moderate, or severe. Additionally, patients were asked to assess their symptom evolution time ("How long it took to feel this bad"), providing an estimate of perceived disease acuteness. Patients also reported the presence/absence of 27 additional symptoms (SM Table 5.1). The redacted previsit survey is available in the SM.

SM Table 5.1: Pre-visit questionnaire data.

	level	Overall
n		123
Patient sex (%)	F	69 (56.1)
	M	54 (43.9)
Patient age (mean (SD))		20.02 (1.50)
Activity level (mean (SD))		4.22 (2.59)
Days since onset (%)	0-1	53 (43.1)
	1-2	60 (48.8)
	3+	10 (8.1)
Intensity of aches and pains (%)	None	16 (13.0)
	Mild	36 (29.3)
	Moderate	48 (39.0)

	Severe	23 (18.7)
Intensity of cough (%)	None	3 (2.4)
	Mild	18 (14.6)
	Moderate	72 (58.5)
	Severe	30 (24.4)
Intensity of weakness (%)	None	8 (6.5)
	Mild	40 (32.5)
	Moderate	54 (43.9)
	Severe	21 (17.1)
Abdominal pain (%)	Present	18 (14.6)
	Not reported	105 (85.4)
Cough (%)	Present	118 (95.9)
	Not reported	5 (4.1)
Chest congestion (%)	Present	79 (64.2)
	Not reported	44 (35.8)
Chest pain (%)	Present	44 (35.8)
	Not reported	79 (64.2)
Chills sweats (%)	Present	112 (91.1)
	Not reported	11 (8.9)
Diarrhea (%)	Present	10 (8.1)
	Not reported	113 (91.9)
Ear pain (%)	Present	27 (22.0)
	Not reported	96 (78.0)

Eye pain (%)	Present	20 (16.3)
	Not reported	103 (83.7)
Fatigue (%)	Present	116 (94.3)
	Not reported	7 (5.7)
Subjective fever (%)	Present	92 (74.8)
	Not reported	31 (25.2)
Hearing loss (%)	Present	5 (4.1)
	Not reported	118 (95.9)
Headache (%)	Present	100 (81.3)
	Not reported	23 (18.7)
Insomnia (%)	Present	64 (52.0)
	Not reported	59 (48.0)
Itchy eye (%)	Present	25 (20.3)
	Not reported	98 (79.7)
Nasal congestion (%)	Present	97 (78.9)
	Not reported	26 (21.1)
Nausea (%)	Present	48 (39.0)
	Not reported	75 (61.0)
Myalgia (%)	Present	107 (87.0)
	Not reported	16 (13.0)
Runny nose (%)	Present	90 (73.2)
	Not reported	33 (26.8)
Sore throat (%)	Present	103 (83.7)

	Not reported	20 (16.3)
Shortness of breath (%)	Present	55 (44.7)
	Not reported	68 (55.3)
Sneeze (%)	Present	65 (52.8)
	Not reported	58 (47.2)
Swollen lymph nodes (%)	Present	52 (42.3)
	Not reported	71 (57.7)
Tooth pain (%)	Present	26 (21.1)
	Not reported	97 (78.9)
Vomiting (%)	Present	15 (12.2)
	Not reported	108 (87.8)
Vision change (%)	Present	1 (0.8)
	Not reported	122 (99.2)
Weakness (%)	Present	115 (93.5)
	Not reported	8 (6.5)
Wheezing (%)	Present	50 (40.7)
	Not reported	73 (59.3)

Visit Data Table

During the visit, physicians assessed the presence or absence of 29 signs and symptoms, as well as the duration of symptoms, but no qualitative assessment was made. Unlike the patient surveys, responses were not required in the EHR. As a result, we classified symptoms as present or not present. Each patient's body temperature was also measured during the visit (SM Table 5.2). The redacted electronic health record template is available in the SM.

SM Table 5.2: Data captured from the electronic health record of the patient's clinical visit.

	level	Overall
n		136
Patient sex (%)	F	75 (55.1)
	M	61 (44.9)
Patient age (mean (SD))		20.01 (1.47)
Patient temperature (mean (SD))		99.14 (1.28)
Days since onset of symptoms (mean (SD))		2.52 (1.24)
Abdominal pain (%)	Present	4 (2.9)
	Not reported	132 (97.1)
Cough (%)	Present	131 (96.3)
	Not reported	5 (3.7)
Chest congestion (%)	Present	34 (25.0)
	Not reported	102 (75.0)
Chest pain (%)	Present	13 (9.6)
	Not reported	123 (90.4)
Chills (%)	Present	121 (89.0)
	Not reported	15 (11.0)
Diarrhea (%)	Present	7 (5.1)
	Not reported	129 (94.9)
Ear Pain (%)	Present	8 (5.9)
	Not reported	128 (94.1)
Eye pain (%)	Present	63 (46.3)

	Not reported	73 (53.7)
Vomiting (%)	Present	9 (6.6)
	Not reported	127 (93.4)
Eye irritation (%)	Present	5 (3.7)
	Not reported	131 (96.3)
Face pain (%)	Present	5 (3.7)
	Not reported	131 (96.3)
Fatigue (%)	Present	117 (86.0)
	Not reported	19 (14.0)
Fever (%)	Present	119 (87.5)
	Not reported	17 (12.5)
Headache (%)	Present	117 (86.0)
	Not reported	19 (14.0)
Joint pain (%)	Present	80 (58.8)
	Not reported	56 (41.2)
Myalgia (%)	Present	109 (80.1)
	Not reported	27 (19.9)
Nasal discharge (%)	Present	127 (93.4)
	Not reported	9 (6.6)
Nasal congestion (%)	Present	129 (94.9)
	Not reported	7 (5.1)
Nausea (%)	Present	34 (25.0)
	Not reported	102 (75.0)

Post-nasal drip (%)	Present	37 (27.2)
	Not reported	99 (72.8)
Sinus pressure (%)	Present	27 (19.9)
	Not reported	109 (80.1)
Shortness of breath (%)	Present	17 (12.5)
	Not reported	119 (87.5)
Sore throat (%)	Present	125 (91.9)
	Not reported	11 (8.1)
Sneezing (%)	Present	17 (12.5)
	Not reported	119 (87.5)
Sputum (%)	Present	17 (12.5)
	Not reported	119 (87.5)
Substernal burning (%)	Present	63 (46.3)
	Not reported	73 (53.7)
Swollen lymph nodes (%)	Present	14 (10.3)
	Not reported	122 (89.7)
Voice loss (%)	Present	4 (2.9)
	Not reported	132 (97.1)
Wheezing (%)	Present	17 (12.5)
	Not reported	119 (87.5)

Post-visit Questionnaire Table

The post-visit survey was emailed to patients five days after their visit (SM Table 5.3). The patient had 24 hours to respond and then the link expired. The outcomes included symptom resolution and disease impact on school and work. The redacted post-visit survey is available in the SM.

SM Table 5.3: Patient outcomes as reported on the follow-up questionnaire.

	level	Overall
n		115
Patient sex (%)	F	64 (55.7)
	M	51 (44.3)
Patient age (mean (SD))		19.97 (1.44)
Days of work/class missed (%)	0	17 (14.8)
	1	27 (23.5)
	2	35 (30.4)
	3	25 (21.7)
	4	7 (6.1)
	5	4 (3.5)
Recovery from cough in 5 days (%)	I did not have a cough	6 (5.2)
	No improvement	8 (7.0)
	Improved somewhat	56 (48.7)
	Improved dramatically	45 (39.1)
Days fever was present after visit (mean (SD))		1.59 (1.21)

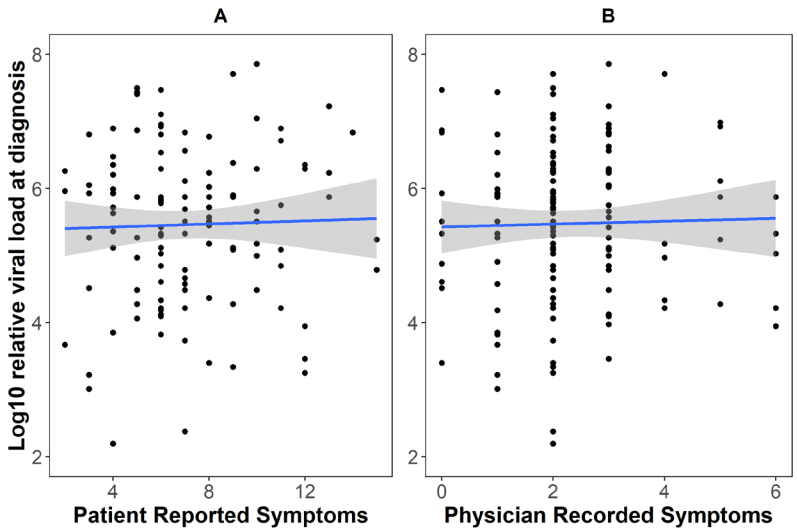
Symptom Score Sensitivity Analysis

Starting with the symptoms in the total symptom scores, we removed all symptoms, which were at least 95% yes or no responses. We then analyzed the remaining symptoms for pairwise

correlation, so redundant symptoms could be removed from the scores. We calculated Yule's Q value for each pair of symptoms within the two total symptom scores [1,2]. For symptom pairs with a Q-value greater than 0.9, the symptom with the least amount of variation in the responses was removed. Pairs of symptoms were compared iteratively, starting with the pair with the highest absolute correlation; no distinction was made between pairs with the same absolute correlation. When no pairs had an absolute Yule's Q value greater than 0.9, the remaining symptoms were summed to create a second reduced symptom score for each patient.

Results of reduced symptom score

There was no apparent relationship between RVL and the physician or patient reported reduced symptom scores (SM Figure 5.1). The linear regression for the physician score did not show any significant trends ($\beta = 0.02$ (95% CI: -0.13, 0.17), $p = 0.77$). Similarly, there was no apparent relationship between the patient reported symptom score and RVL ($\beta = 0.01$ (95% CI: -0.06, 0.08), $p = 0.75$).



SM Figure 5.1: A: Relationship between the log10 relative viral load at diagnosis of the patients and the calculated reduced symptom scores, using symptoms reported by the patient. B:

Relationship between the log10 relative viral load at diagnosis of the patients and the calculated reduced symptom scores, using symptoms reported by the physician

References

1. Yule GU. An introduction to the theory of statistics. C. Griffin, limited, 1919.

2. Warrens MJ. On association coefficients for 2× 2 tables and properties that do not depend on the marginal distributions. Psychometrika **2008**; 73:777.