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# Statin influence on influenza mortality

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Thesis

# STATIN INFLUENCE ON INFLUENZA MORTALITY

by

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**STATIN INFLUENCE ON INFLUENZA MORTALITY**

**MICHAEL SIMONS**

ABSTRACT

**Background**

Seasonal influenza is a virus which causes significant morbidity and mortality annually. The virus has the capability of mutating frequently and is able to avoid prior immune system defenses from previous infection or vaccination. This frequent variation of the virus and its prevalence pose significant public health threat.

Statins are a class of medications traditionally used for atherosclerotic heart disease and elevated cholesterol. Though these medications have been very effective in managing these disease states, there is evidence that the drugs may also have multiple effects, including a proposed anti-inflammatory effect and effect on virus production.

**Literature Review Findings**

Previous research has shown that statins may have an effect on inflammation. Influenza can lead to a systemic inflammatory response and statins have been proposed as a potential treatment in these situations. There have been studies assessing this, however, there is a gap in the research. Particularly, there are not randomized control trials which compare a large, diverse cohort of patients who were on statins and how these patients would be managed while critically ill with influenza. The exact mechanism of this potential reduction in mortality is currently unknown.

**Proposed Project**

The goal of the proposed double blinded randomized control study is to determine the effect of continuing statin therapy for patients admitted to the hospital. The study will aim to include an equal number of men and women and will include a total of 7188 patients over three years in Boston and New York City hospitals. The study will include patients previously on a statin and diagnosed by PCR with influenza. The data will then be compared based upon CFR at 15- and 30-day mortality, while also assessing IL-6 levels in serum. This will be able to further elucidate the mechanism of potential reduction in mortality.

**Conclusions**

The conclusions of this study will be to determine reduction in mortality from influenza for patients that are taking a statin. This will also be useful in predicting outcomes of patients being admitted with influenza. The study will also be able to further elucidate the mechanism of the potential reduction in mortality through assessment of inflammatory markers, which could be informative for future studies.

**Significance**

The public health implications of this study would be significant in reducing influenza mortality around the world, and allow for better access to treatment worldwide. This could serve as an important tool in management of future pandemics and epidemics caused my influenza.

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## LIST OF ABBREVIATIONS

ACC ..... American College of Cardiology

AHA ..... American Heart Association

BMI ..... Body Mass Index

BU ..... Boston University

CDC ..... Centers for Disease Control and Prevention

COPD ..... Chronic Obstructive Pulmonary Disease

cRNA ..... Complement Ribonucleic Acid

CRPD ..... Clinical Practice Research Datalink

DPH ..... Department of Public Health

ICD ..... International Statistical Classification of Diseases and Related Health Problems

IL-6 ..... Interleukin-6

LDL ..... Low-density Lipoprotein

mRNA ..... Messenger Ribonucleic Acid

M1 ..... Matrix Protein 1

M2 ..... Matrix Protein 2

NA ..... Neuraminidase

NYC ..... New York City

PCR ..... Polymerase Chain Reaction

PI ..... Principle Investigator

PRISMA ..... Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT ..... Randomized Control Trial

RNA ..... Ribonucleic Acid

TNF-α..... Tumor Necrosis Factor alpha

UK..... United Kingdom

US ..... United States

vRNA ..... Viral Ribonucleic Acid

WHO..... World Health Organization

# INTRODUCTION

## Background

Seasonal influenza remains a major issue in the world, through the genetic changes in the virus and the various circulating strains. According to CDC data, the 2019-2020 influenza season saw an estimate of 38 million cases, 400,000 hospitalizations and 22,000 deaths.[1] The CDC also recognizes that this is lower than average. This constant genetic variation can lead to the emergence of new strains that are not recognized by pre-existing immunity in the human population. The resulting variability leads to possible outbreaks of the virus in any given year. Although influenza vaccines exist, they must be re-formulated each year to match the current and most common circulating strains.[1] Due to the necessity to start manufacture of the vaccines with a long lead time prior to the influenza season, the final vaccine formulation sometimes no longer matches the viral strains circulating during peak influenza season. Along with suboptimal vaccine uptake by the general population, these factors can result in many individuals more susceptible to infection.[1] The virus can lead to constellation of symptoms, but also can cause significant mortality. Symptoms are not often gradual, and may include; fevers, sore throat, cough, myalgias, fatigue and gastrointestinal symptoms. Individuals at highest risk of developing mortality are those at extremes of age; older than 65 or younger than two years old.[2] Other risk factors for developing severe disease include lung disease, heart disease and diabetes.[2] The mortality is often associated with an overactive immune response, resulting in a cytokine storm which can cause organ failure.[2] Due to the possibility of outbreaks, and the resulting mortality, it is important to utilize vaccines and

treatments to decrease morbidity and mortality, as well as the burden on the health system. Antivirals, such as oseltamivir, and vaccines exist for treatment and prevention of influenza, and supportive care is administered in more severe cases, but there is still room for improvement in morbidity and mortality reduction.

One such proposed improvement in the treatment of influenza is statin therapy.[3] This has been noted in hospitalized patients through multiple studies. This class of medication is normally utilized for hypercholesterolemia and other patients who are at risk of cardiovascular disease due to other comorbidities, such as diabetes.[4] This medication has also been shown to have pleiotropic effects which could decrease mortality from influenza and sepsis.[3] There are multiple proposed mechanisms of reduction in mortality, including improving endothelial function, antiviral activity and immunomodulation leading to a reduction in pro-inflammatory cytokines.[5-6] Some studies have shown a reduction in influenza mortality for hospitalized patients who are on a statin when compared to hospitalized patients who were not taking a statin.[7] If a reduction in influenza mortality could be the result of statin use, this could help providers better predict mortality and possibly reduce mortality of diagnosed patients. This could then help with major outbreaks and hospital capacity in a possible flu epidemic, and with the medication being cheap and available, this treatment could be useful globally.[8]

**Statement of the Problem**

Pandemic influenza is still a real risk to society, especially given the high capacity of this virus to mutate and for new viral strains to emerge due to circulation and selective pressure in humans and animals. As previously stated, in the US, this is a virus which can

cause excess of 20,000 deaths annually, particularly to those that fall into high risk groups, such as those with underlying conditions of heart disease, lung disease, diabetes and patients of an older age.[1] The risk of a possible influenza epidemic or pandemic could be better mitigated by having more effective treatments, in addition to vaccines which are not always 100% effective or widely administered. Although existing antivirals work, the medications are much more efficacious earlier in the disease. The use of a widely available, cheap medication could be beneficial in alleviating the problem of flu, or possibly other viral outbreaks which lead to death by an overactive immune response.[8] Though there are some data suggesting that drugs which suppress an excessive immune response to infections can reduce mortality, there is a gap in how best to administer the medication, and randomized controlled clinical trials are needed to demonstrate that statins can reduce mortality in patients with infections associated with an excessive immune response characterized by a "cytokine storm," like influenza does.[2]

**Hypothesis**

Patients who were previously on a moderate or high intensity statin and are continued with this medication during hospital admission due to influenza will have a decreased mortality rate due to reduction in inflammatory cytokines.

**Objectives and Specific Aims**

The goal of this study is to establish a better understanding of the effect of statins on influenza mortality. This will be useful in better predicting patient outcomes, and possibly improving these outcomes. This will be examined through studying both mortality rate, and examining the proposed mechanism of action of statins on influenza

virus and cytokine levels during infection in infected patients (and/or animal or in vitro models or all human subjects). In order to test the hypothesis, the following will be measured and compared:

- 30- and 90-day mortality rate of influenza in patients who are taking a prescribed statin compared with a similar patient cohort who are not taking a statin
- Viral titers in the patient cohorts describe above
- Pro-inflammatory cytokine levels at various time points during the study (in the patient cohorts describe above

## REVIEW OF THE LITERATURE

### Overview

In order to delve further into the topic of how statin therapies affect morbidity and mortality of influenza, or other pathogens, it is first important to understand the background of statins. Statins are prescribed in a number of conditions including; known cardiovascular disease due to atherosclerosis, such as stroke or myocardial infarction, LDL>190mg/dL, individuals 40-75 years old with diabetes, or individuals 40-75 years old with elevated LDL and other risk factors leading to an increased risk of developing cardiovascular disease from plaques.[4] The main purpose of prescribing these medications is to both lower cholesterol, and decrease the risk of developing more advanced cardiovascular disease. Statins work as HMG-CoA reductase inhibitors, this is the rate limiting enzyme in the mevalonic acid synthesis pathway.[3] Inhibition of this enzyme leads to a decrease in mevalonic acid, which leads to a downstream reduction of cholesterol synthesis within the liver and an increase in LDL clearance from an increase in LDL receptors as illustrated in figure 1.[3]

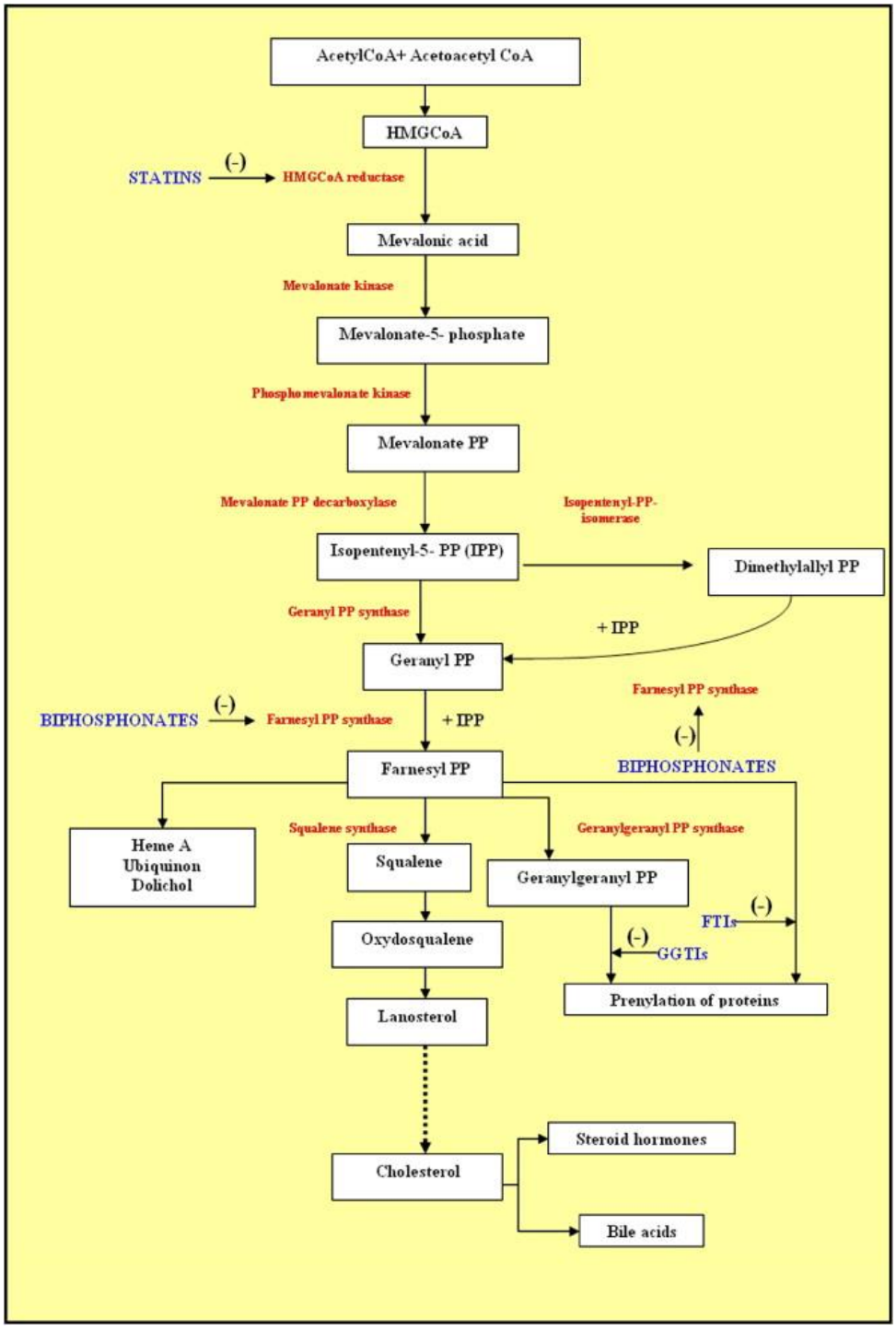


**Figure 1: Mevalonic acid synthesis pathway imported from Buhaesco et al[3]**

**The mevalonic acid synthesis pathway and where statins interfere.**

These effects, in turn, lead to a decrease in the amount of LDL in the blood. This mechanism is the same for all statins, but doses and strength vary based on what an individual patient is able to tolerate.[9] This is important because LDL is not soluble in human plasma, and is able to bind to the arterial wall due to opposing charges of the apoB lipoprotein and the proteoglycan on the vessel. Once retained within the vessel, there is further accumulation of lipoprotein through macrophage uptake, further LDL modification and eventually further endothelial dysfunction. This cycle leads to plaque development and can occur more readily with the presence of more LDL.[10] LDL cholesterol has a large benefit on reduction of morbidity and mortality, with the lower the level of LDL obtained, the lower the risk of cardiovascular events measured within five years.[11] Like all medications, there are multiple reported side effects of statins. The most common side effect, is muscle pain. This has been partially attributed to a nocebo effect, meaning that the side effect was reported even if individual patients were on a placebo, due to negative associations with the medication.[12] There have also been serious adverse effects associated with statin use, but these are very rare. The major adverse effects include; increase in blood sugar leading to possible increase risk of type 2 diabetes - though this is difficult to assess because of the fact that diabetic patients often also take a statin, hepatotoxicity, statin-associated muscle symptoms such as myalgias or weakness, and increased risk of hemorrhagic stroke, particularly in patients with a previous history of a hemorrhagic stroke.[13] Other rarer adverse effects are peripheral neuropathy, rhabdomyolysis and autoimmune diseases.[13] Lastly, there is a possible adverse effect of confusion or memory loss, but these symptoms cease after the individuals stop taking the

medication.[12] The risk of these is small, and are outweighed by the benefit of using statin therapy.[13] There is also a worry that LDL could be lowered too much, but this is still safe as there has not yet been sufficient data to suggest adverse events related to low LDL.[13]

Although statins are prescribed for lowering LDL cholesterol, the medications also have pleiotropic effects, including improving endothelial cell function.[3] The reason for the variety of additional effects of statins is that the mevalonic pathway is involved in isoprenoid production. Inhibition of isoprenoid leads to improved endothelial function, and the statin mechanism also leads to diminished cytokine activity. These effects are because the isoprenoid pathway has byproducts which have proliferative effects within the body. These are the non-sterol isoprenoids; farnesylpyrophosphate and geranylgeranylpyrophosphate.[3] Decreased production of these leads to a decrease in inflammation.

In order to better understand how the mechanisms of how statins affect viruses such as influenza, it is first important to examine the virus. Influenza virus is an enveloped RNA virus with a helical nucleocapsid and a segmented genome composed of 8 single-stranded, negative-sense RNA molecules. The envelope is lined by the viral matrix protein (M1) and 3 important viral proteins are inserted into the envelope, hemagglutinin (HA, as a trimer), neuraminidase (NA, as a tetramer), and the M2 protein.[14]

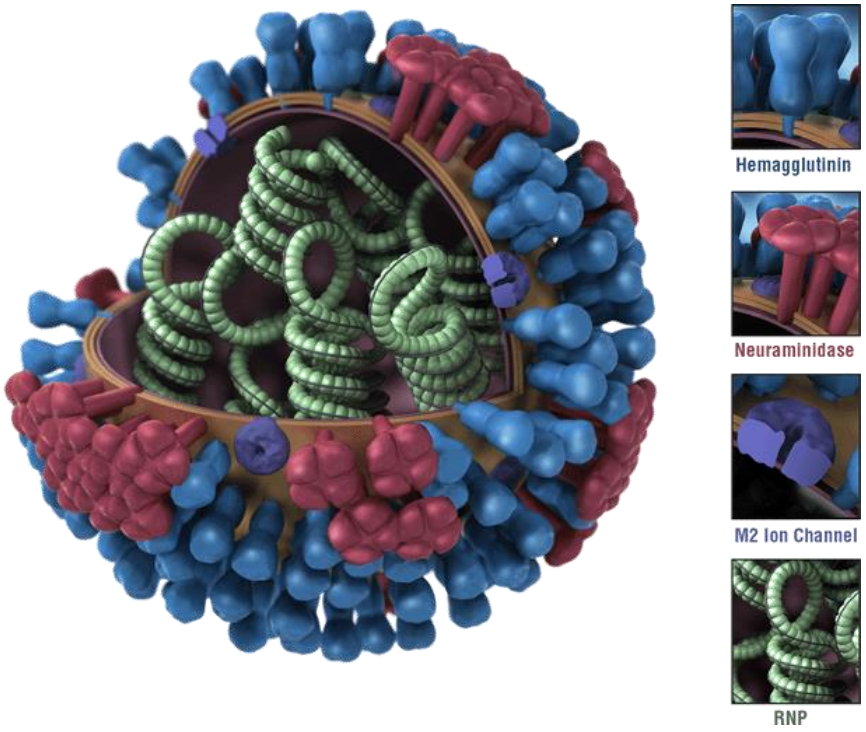


**Figure 2: Influenza Virus structure**

**This is an influenza virus structure. The key hemagglutinin and neuraminidase are labeled, as well as other major structures.[3] Imported from CDC.**

There are four types of influenza virus, A, B, C, and D. Both influenza A and B viruses cause seasonal outbreaks of disease, but only influenza A virus has been shown to cause pandemics due to its ability to undergo antigenic shift or reassortment. Influenza A is classified as subtypes based on the viral hemagglutinin and neuraminidase proteins. The viral hemagglutinin serves as the viral attachment protein and neuraminidase is required for release of progeny virions from infected cells. Both of these proteins elicit neutralizing antibodies in the infected host.[14,15] There are 18 subtypes of hemagglutinin and 11 of neuraminidase, but despite the potential for many different potential subtypes, only two that infect humans are currently circulating, H1N1 and H3N2.[15]

Influenza viruses are transmitted easily via respiratory droplets. The virus targets ciliated airway epithelial cells, and type II alveolar epithelial cells by attaching to sialic acid via the viral hemagglutinin.[16] The virus then enters the cell via endocytosis and is trafficked to an endosome where acidification of the endosome causes a conformational change in the HA promoting fusion of the viral envelop with the endosomal membrane. The viral M2 protein in the viral envelope forms a proton channel which promotes acidification inside the envelope leading to release of the viral nucleocapsid-gene segments into the cell cytoplasm.[15] The viral RNAs then enter the cell nucleus through transporters. Once within the nucleus, the viral genome segments are transcribed by the viral RNA dependent RNA polymerase which uses host cell mRNA as a primer for viral mRNA synthesis and thereby "steals" the methylated cap region of the host cell mRNA.[15] This is illustrated in figure 3.

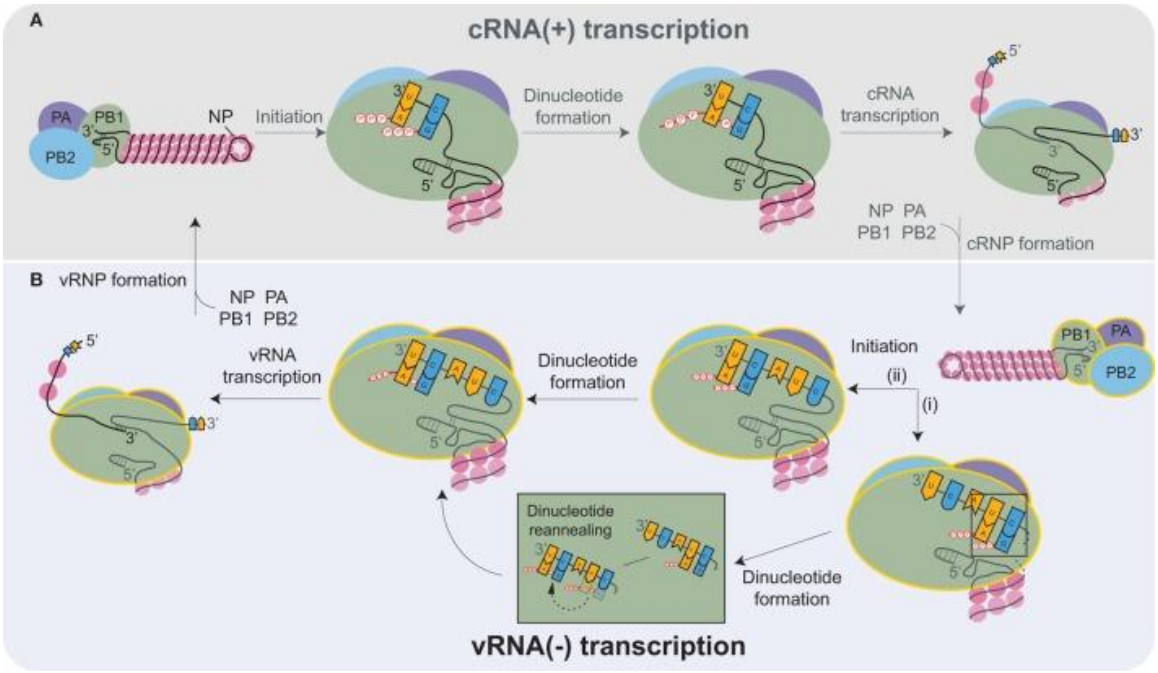


**Figure 3: Viral RNA synthesis imported from Dou, et al.[15]**

**This figure demonstrates viral RNA transcription once the virus is within the cell.**

These mRNAs are then translated by host cell ribosomes in the cytoplasm and outcompete translation of host cell mRNA's lacking the 5' CAP.[15] Viral proteins, including polymerase and nucleoproteins, are trafficked to the nucleus where the positive-sense viral RNA gene segments are used as templates for synthesis of the negative-sense genome segments by the viral polymerase complex, and assembly of the nucleocapsids occurs. The viral hemagglutinin, neuraminidase and M1 and M2 proteins, synthesized in the cytoplasm, are processed in the ER and the Golgi apparatus and trafficked to the plasma membrane, where M2, HA and NA are inserted into the membrane and M1 protein lines the inner surface.[15] The viral nucleocapsids are transported from the nucleus to the M1-lined areas of the plasma membrane where virions acquire their envelope as they bud off from the cell. Release of progeny virions requires the action of neuraminidase, which hydrolyzes sialic acid, releasing bound HA at the cell surface.[15]

This virus infects most susceptible individuals in the U.S. between the months of December and February.[1] Annually, according to Somes et al., 2018, it is estimated that the virus infects about 1 in 5 vaccinated individuals, and 1 in 10 that are unvaccinated.[17] This RNA virus has a high mutation rate since the viral RNA-dependent RNA polymerase does not have a proofreading function, resulting in a population of viruses with extensive genetic variability subject to selective pressure within the host.[15] Genetic change resulting from point mutations due to errors in transcription is referred to as genetic drift. Influenza A viruses can also undergo another type of genetic variation,

antigenic shift, which is caused by reassortment of genome segments from 2 different viral strains co-infecting the same cell. These mechanisms of genetic change contribute to the genetic variability observed within influenza virus populations, i.e. quasispecies, and contributes to immune evasion when infecting a host with no pre-existing immunity.[1] According to the CDC, the most common symptoms of flu are fever/chills, cough, sore throat, fatigue, muscle and body aches, headaches, a runny or stuffy nose, and, more common in children, sometimes diarrhea or vomiting. Though this may sound similar to a common cold, the symptoms are more abrupt in onset, and fevers, chills, headaches and body aches are more commonly associated with the flu.[2]

Although influenza often resolves within a few days to two weeks, severe complications can result from the infection. These include pneumonia, bacterial co- or super-infection, which could also lead to a pneumonia, myocarditis, encephalitis or rhabdomyolysis.[2] The virus can also exacerbate existing medical issues like asthma or heart failure. The complication which often leads to death in influenza patients is the overactive immune response, which is more commonly seen in older individuals, or individuals with underlying conditions such as diabetes or asthma.[2] Infection is also more severe in individuals less than two years old and pregnant women.[2]

Existing treatments for influenza are antivirals, which can shorten duration and/or severity of symptoms. Currently, the antiviral medications in use are neuraminidase inhibitors, such as oseltamivir and Baloxavir marboxil (Xofluza) which inhibits the cap-dependent endonuclease activity of the viral polymerase complex that mediates transcription of viral mRNAs in the nucleus of the infected cell, and therefore blocks

synthesis of viral proteins required for replication.[18,19] Unfortunately, resistance to a previous drug targeting the M2 protein became so prevalent that the drug is no longer recommended for use.[19] According to the CDC, patients who require hospitalization receive supportive care, with fluid resuscitation and oxygen as needed, on top of antivirals.[20]

The ability of influenza viruses to undergo antigenic drift and antigenic shift enhance the emergence of strains that can evade the immune response or are resistant to anti-viral drugs under those selective pressures, making it a continuing risk in causing epidemics and pandemics.[1] Although there are available treatments, it is important for providers to better understand risk factors of individuals diagnosed with influenza, and to continue to investigate possible treatments that may further reduce morbidity and mortality. Statins are an example of other treatments under investigation for treating patients with influenza. There are a few possible reasons that this class of drug may reduce mortality in these patients. Statins, as previously stated, have pleiotropic effects.[3,5,6] First, the drug may have antiviral effects. One study has shown that in vitro, the drug interferes with the Rho/Rho kinase pathway, and through this inhibition the virus was not able to proliferate. The study states that an influenza infected cell line had enhanced activation of RhoA. GTPAse in this pathway, and when atorvastatin or rosuvastatin were introduced, viral production was blocked.[6] Also in vitro, statins have been shown to decrease the levels of inflammatory cytokines, such as TNF-α or IL-6, which would be important in reduction of mortality.[5]

Lastly, in vitro, H1N1 viral load was able to be reduced by statins, and endocytosis of the virus was able to be inhibited by the interference of the RhoA pathway and Rab proteins.[21]

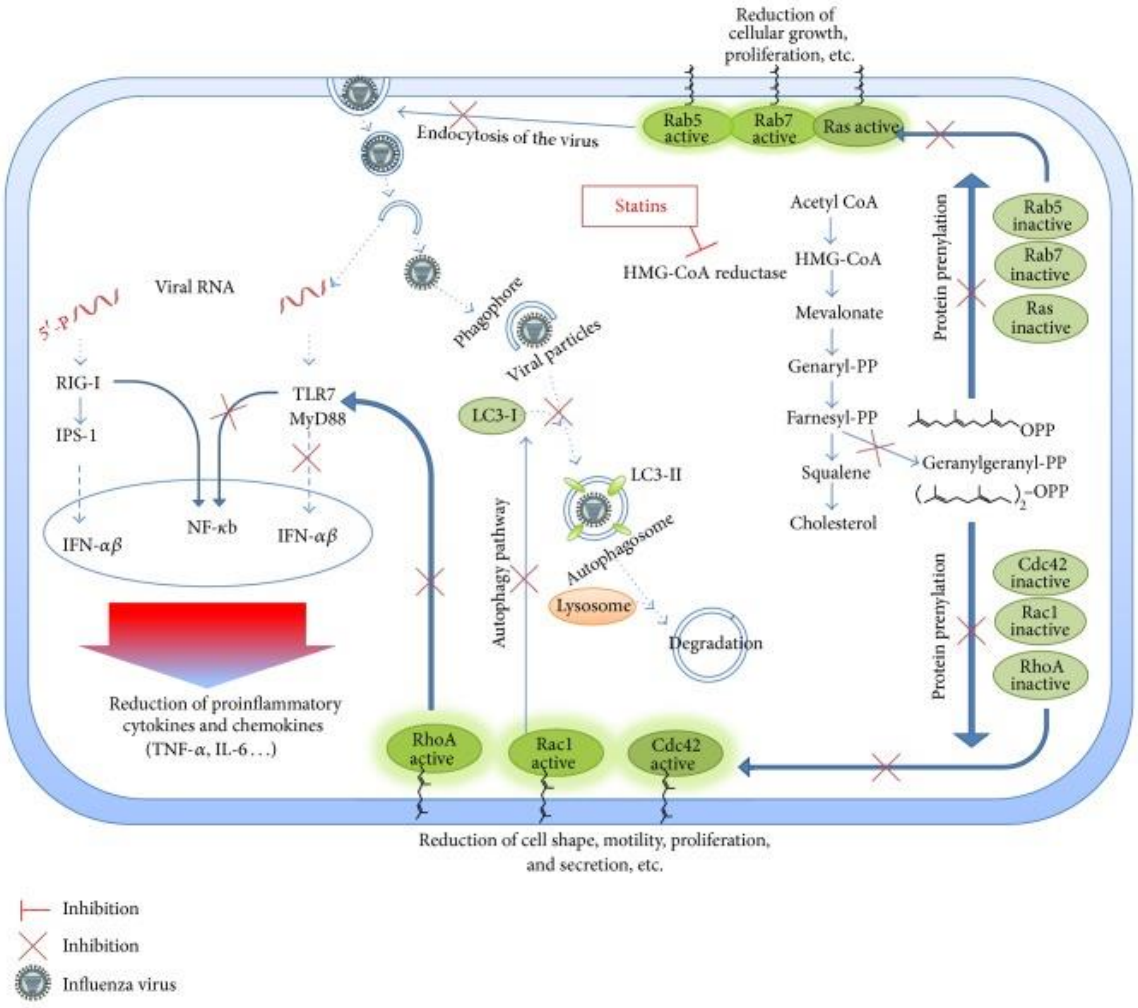


**Figure 4: Potential mechanism of statin in reduction of influenza expression**

**This image demonstrates proposed mechanisms from Mehrbod et al., 2014, on how statin may be reducing influenza mortality in vitro[21]**

In mice models, a Glück et al., 2013, compared the effect of statins on 2 influenza virus strains, H5N1 and pH1N1, found that when the mice were given simvastatin, the mice were not protected from infection.[22] This study referenced possible decrease in cytokines in the mice but did not test this. The strains were chosen due to the potential drug resistance in these strains. In the study, the mice were inoculated intranasally with 20 microliters of virus.[22] Statins were administered either orally or by intraperitoneal route at 20mg/kg, and were delivered daily, beginning at three days prior to viral inoculation until two weeks following infection. The resulting mortality, lung injury and change in weight between placebo and statin treated group were not statistically significantly different.[22]

Statins have been shown to be immunomodulators in humans in research studies on multiple disease states, such as rheumatoid arthritis and multiple sclerosis, though this effect is not as strong as immunosuppressing entirely.[23] This is because of the mevalonic acid being a part of the pathway for pro-inflammatory cytokine production as illustrated in figure 5.

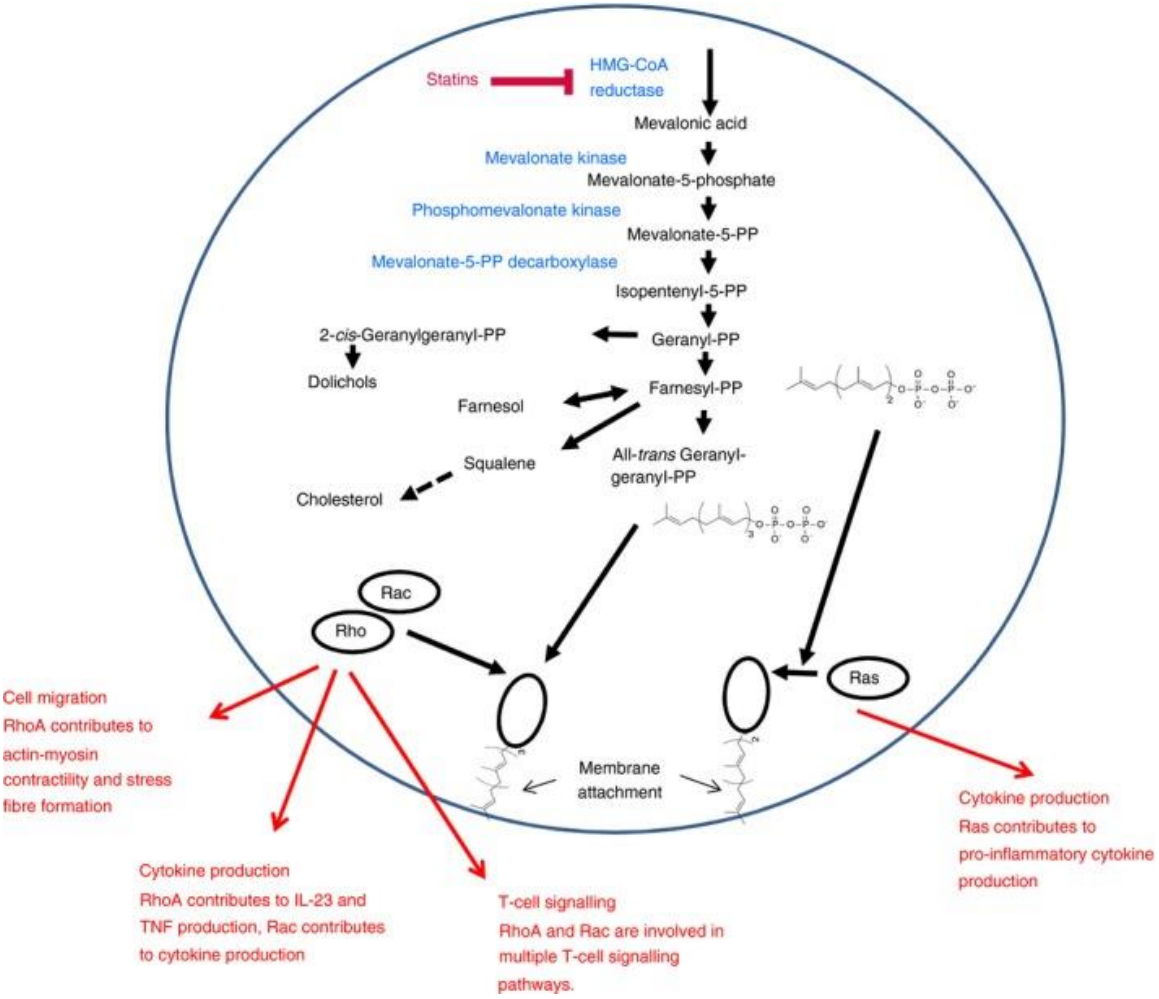


**Figure 5: Mevalonic Acid Pathway and Statin Mechanism**

**This image demonstrates how the inhibition of HMG-CoA reductase leads to a reduction in cytokines. Imported from Zeisser, 2018[23]**

These effects have been useful in analyzing whether or not statins are useful in reducing influenza morbidity and mortality in patients. Results of these studies have been mixed, possibly because of the varying length of administration of the drugs.[21]

A study by Frost et al., 2007 found that patients on a moderate dose statin had a reduction in mortality from influenza of about 40% when compared with patients not on a statin.[24] Another study by Enserink et al., 2005 observed that individuals who were

prescribed statins for cardiovascular reasons previously discussed, were less likely to have symptoms associated with influenza.[25] Although these studies were retrospective cohort studies, there is still promise shown in the results. Kruger et al., 2013, was a randomized control trial which found benefits of statins in decreasing sepsis mortality in patients that were in the intensive care unit.[26] This study was multicentered and all patients were prescribed atorvastatin. The study was 250 patients and measured cytokine levels, as well as mortality.[26] In conclusion. Patients who were previously on statin and continued statin had improved mortality outcomes but did not have significant differences in cytokine levels. The study did not specify cause of sepsis.[26]

CDC data has also been investigated and used to determine that statins did decrease death from laboratory confirmed influenza patients.[27] The evidence that the resulting decrease in influenza mortality is scientifically plausible, and is demonstrated by the fact that this decrease in mortality has also been demonstrated in sepsis and community-acquired pneumonia of various causes.[27]

In contrast, other studies have demonstrated that statins may not reduce mortality in influenza patients. This was seen in Kwong et al., 2009, which showed no significant reduction in mortality for elderly patients in 10 seasons.[28] This was also examined in a study of all influenza-like illnesses, including bronchitis, which does not include the specific infectious agent in analyzing the effects of statins on mortality.[29] The mixed results could be due to the pathogenicity of different pro-inflammatory states, or that the strength of statins in the studies have also varied. It is also difficult to assess compliance to statin therapy, and ability to adhere to oral medications when patients are critically ill.

With results from the data being mixed, and the potential benefit for influenza mortality to be reduced by a cheap, and readily available medication, it is important to analyze the studies which research the effects of statins, and design further studies which may help to explain the different findings and better elucidate the role and potential of statins in reducing mortality due to dysregulated production of cytokines. This could also be useful for possible reduction in mortality rates of other infectious agents which also lead to death via cytokine storms.

**Existing Research**

Janda et al., 2010 is a systematic review and meta-analysis which briefly discusses the need of additional therapies besides antimicrobials for infections that lead to sepsis because of the high mortality rate and treatment costs associated with sepsis.[7] Sepsis results from excessive proinflammatory cytokines, which may be able to be reduced by statins. The article discusses how previous meta analyses have found that there may be a benefit from statins on sepsis and pneumonia mortality, but there is a varying study design in the analyzed articles.[7] This study was meant to synthesize existing literature on the topic, and to examine the effect of statins on sepsis and other infections. The study was done following a checklist from Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), and analyzed RCTs, cohort studies, and case control studies, but not experimental, laboratory studies. Outcomes of various infections, and sepsis, were analyzed in both pediatric patients and adults based on, primarily mortality of any cause.[7] Articles were included following the search based on a full review by two reviewers and if there was not an agreement between the two, a third

reviewer was able to make the determination of whether or not the article should be included. The strength of cohort studies was analyzed based on inclusion criteria, how an exposure was defined, and how outcomes were defined and adjusted based on other variables. The data were compared quantitatively using Stata intercooled version 10.1 (StataCorp, College Station, Tx).[7] There were 20 articles which were analyzed, 12 of which were retrospective cohort studies, 6 were prospective cohort studies, and two were case controlled studies, a matched cohort study and an RCT.[7] These studies were ranked according to the rating system of the meta-analysis, and 18 were considered to be high quality. The pooling of the studies resulted in a total of 265,558 patients, but the majority (about 85%) of these patients were all a part of the same study.[7] In 15 of the studies, there was shown to be a mortality benefit from statins, with an OR of .06-.75, and the pooled analysis showed a mortality benefit as well, with an OR of .49 (95% CI .37-.61). Any removal of a single study did not result in a major difference in the OR.[7]

The study also further analyzed the data and divided the results based on how outcomes were defined, determining that there was a significant reduction in the pooled 30-day mortality in patients on a statin with sepsis (OR .61), a reduction in the pooled overall hospital mortality (OR .38), and an overall reduction in pooled sepsis mortality (OR .40).[7] There was also a reduction in the pooled pneumonia-related mortality (.63), which is important information for the possibility of the decreased mortality in influenza patients.[7] This analysis then suggests that the pleiotropic effects of statins do lead to a reduction of mortality from sepsis or infection based on the pooled OR. Not all studies found that there was a decrease in the mortality, although there was also found to be an

increase in influenza mortality, this could be attributed to the older population of this study when compared to other studies.[7] However, it is also potential that there is not a significant reduction in mortality for these individuals, or for patients of a certain age group and this should be further researched. The study proposes that the findings of studies indicating a statin-associated reduction in mortality in patients who develop diseases with dysregulated cytokine production support findings in animal models, and could be associated with the decrease in cytokine production. The study discusses that there may be a concern of increased risk in serious adverse effects of statins, such as rhabdomyolysis, in critically ill patients. As previously stated, this is a rare side effect, but it may be more likely in patients that are critically ill due to impaired metabolism resulting from organ dysfunction.[26] The paper concludes by discussing the need for more RCTs to better understand the effects of statins on sepsis and infection mortality, and states that though the information contained within the paper is beneficial, it is not significant enough to begin administering statins regularly to septic, or otherwise infected patients.[7]

Overall, there are many strengths to this study. The study was able to analyze many papers, using models to determine the significance of the findings of the various studies. The analysis was able to discuss the findings of the individual studies, as well as pool the data to reach conclusions. The pooled data is one of the most important aspects of the study because it increased the study size substantially, allowing for more statistically significant results. The criteria for inclusion in the systematic review is also a strength of the study, with the cohort inclusion criteria being specified and based on four

major aspects of the studies, and with guidelines in place for determining whether or not the studies should be included based on reviewers' consensus. Although establishing a guideline for the inclusion criteria is a strength, there is a chance of bias, especially when only a max of three reviewers were involved. One way that this could be improved is by simply increasing the number of reviewers for the studies that may be included in the meta-analysis. Another strength of the study was establishment of various outcomes when analyzing the pooled data. The study did not solely focus on the reduction in the mortality from sepsis, but also discussed the reduction in other outcome definitions, such as 30-day mortality, in hospital mortality; and the discussion of reductions in mortality from more specific causes, such as pneumonia, bacteremia or mixed infections. This was more easily analyzed because of the population of the study being so large, and the heterogeneity of the studies which were included in the meta-analysis. This is also a weakness because of the varied study designs, patients and outcome measures. The study is also made more generalizable by the fact that it did not focus on one population. This meta-analysis included studies which were from throughout the world, and this varying patient population, with varying environmental exposures, improves the strength of the study. This is also demonstrated by the fact that the study included pediatric patients and adults.

There are some key limitations to the study. First, the study is primarily a systematic review of cohort studies. This is discussed within the paper, and it is noted that these types of studies are much more likely to be affected by confounding factors, which is more of a worry in retrospective studies, and this meta-analysis included 12 such

studies. Another limitation of the study which is also discussed in the paper, is the variability of the exposure definition. Because statins are a class of drug, it is possible that there could be more of a reduction in mortality with a higher potency statin. Of the 20 studies included, only six stated the statin prescribed. The mortality benefit of statins could also be affected by the dose of the drug, which was only defined in two of the studies included. Overall, these weaknesses present a gap for an RCT to be designed, which could investigate various drugs within the class, and various doses.

The systematic review, as stated within the paper, is not evidence to begin treating septic or otherwise infected patients with statins, but the paper provides biologic plausibility, and supporting evidence for further investigating the effectiveness of statins in treating these patients.[7] Though this paper did not focus on influenza patients, the results could be used to guide research on this population of patients because of the cytokine storm observed in these patients being a major cause of death.

The goal of Brassard et al., 2016, was to discuss the possible effects of statins on the cytokine storm, particularly that associated with influenza infection.[30] The purpose was to investigate whether or not adverse outcomes from influenza infections are averted in individuals on a statin. The article discusses the importance of finding another possible treatment, because of the effectiveness of neuraminidase inhibitors varying. This study set out to determine how healthy user bias affects the results of other studies which examine the outcome of patients with influenza on a statin, as well as further analyzing timing of exposure to statins, and how this may affect outcomes for patients.[30]

The data for this study were collected from the UK Clinical Practice Research Datalink (CRPD), a large computer database which contains the record of about 11 million patients in the UK, and the Hospital Episodes Statistics, which are able to be linked to better understand both hospital and primary care of patients.[30] This is a cohort study which only included patients over the age of 30 who had been diagnosed with influenza between January 1997 and September 2010. This cohort was chosen in order to increase the likelihood that there would be individuals who had been prescribed a statin. The exposure in the cohort study was defined as "statin users" - individuals who were prescribed the medication within a year prior to the diagnosis of influenza, or recent statin users, who were prescribed a statin within 90 days of diagnosis.[30] The third group was the non-users. There were two outcome groups analyzed in the study, a primary and secondary outcome, based on when the patients were assessed. Primary outcome is assessing status after 30 days of an influenza diagnosis, and the secondary outcome is assessing status after 90 days.[30] The cases were individuals who were hospitalized or had died at each follow-up time point, from influenza or pneumonia. An individual was not considered a case if they were alive and had not been admitted to the hospital. The study used propensity score matching to reduce the effects of covariates on the study. Then a logistic regression was used to assess the effect of the medications. This was done comparing two groups of an identical number of patients, which had been matched using the propensity score, and flu season. The study also adjusted for other possible confounders, such as oseltamivir use, vaccinations for both influenza and pneumonia, gender and age.[30] Patients were also further stratified based on medications other than

statins or anti-flu or pneumonia medications, and other diagnoses. In order to achieve the goal of determining the healthy user bias effect, the study analyzed individuals who were observed at the start of their statin treatment, in what was called the new user design.[30]

The results of the study found that in the matched cohort, there was a statistically significant reduction in the 30-day influenza or pneumonia hospitalization or death (Cumulative incidence ratio 0.73), which was still true after the 90-day follow-up (CIR .73).[30] This observation was diminished if only examining hospitalization. The observation was stronger in groups of recent users as compared to the new users (within last 30 days). In the discussion, the paper states that the protective effects of statins seen in recent users was observed when assessing the reduction in influenza and pneumonia mortality and hospitalization. There was an observed greater effect in individuals who are "healthy" which the study deemed as individuals not on respiratory medications or without a history of heart disease.[30] The paper attributes the decrease in hospitalization and mortality to be healthy user bias, which states that individuals prescribed statins are more likely to get an influenza vaccination, and/or have a healthier lifestyle. The paper concludes that a reduction in hospitalization and death from influenza was observed in statin users, but that this was found in individuals who adhered to treatment and were more likely to be vaccinated or have a better lifestyle.[30]

This study has several strengths and weaknesses. First, this study was also large, allowing for better data collection. It is also beneficial that this study had a large database to examine multiple flu seasons, which could prevent any data being skewed by the possibility of one strain of the virus being more or less susceptible to the interfering

actions of statins or the immunomodulatory effects of the medication. The study examined the entire population of the UK in this database, which enhances the generalizability of the study. Another strength was the propensity matching, a technique which was used to help eliminate possible confounding factors. Some of the unique uses of the propensity matching that strengthen the study were vaccination and antiviral use. It is discussed that statin users were also more likely to take antivirals. The propensity matching compared two groups which each had about 50% vaccination rates.[30] The findings that the statins led to a reduction in morbidity and mortality rates with this information also leads to future possible studies. One possibility is examining synergistic effects of neuraminidase inhibitors and statins. This potentially synergistic effect could be skewing the data because of more statin prescribed patients also being more likely to take oseltamivir. Another possible study that this could lead to is a possible synergistic or opposing effect of statins and influenza vaccine efficacy. This has been researched and is also useful in better understanding how to control possible influenza outbreaks.[31] If the statin is interfering with effectiveness of the vaccine, then the strength of the reduction in morbidity and mortality in the statin user groups may be decreased. This is biologically plausible, because if statins do work as mild immunosuppressive drugs, the immune response mounted against the vaccine may be weak. Timing of vaccination and statin use is important, because some new statin users may have been vaccinated prior to starting statin therapy. If the statins are synergistic with vaccine effect, then the reduction in morbidity and mortality due only to statins may actually be exaggerated in this study.

One major limitation of the study was the inability to know whether or not the individuals adhered to their prescription, which the authors recognize could lead to an individual being assigned to the wrong groups of exposure.[30] Another limitation of this study, also discussed within the paper, is that most diagnoses of influenza were not actually laboratory confirmed.[30] This is a major weakness, and leads to the question of whether or not this is a reliable study to interpret how this class of drugs effects the outcome of influenza, versus another possible pneumonia. As previously stated, one of the possible mechanisms of action of the statin is interference with the virus itself,[21] and therefore the hypothesized reduction in morbidity and mortality depends on the diagnosis of influenza. Although the study did use propensity matching, this technique does not eliminate all of the possible confounding variables, evident by the fact that individuals that are unmatched are not included in the study. In this paper, the sample size was originally 71,308 which is a large population, but only 7,679 were statin users.[30] This led to a matched population comparing 5,181 users and non-users, about half of which were vaccinated.[30] This leaves a large number of patients out because those individuals were not matched. This amount of data could have contained patients whose outcomes were worse, and all of these individuals would have been non-users, which could falsely be skewing the data. The elimination of possible confounders through this technique is a strength, but the resulting loss of a lot of data is a major weakness of the study. Continuing with weaknesses related to the propensity matching, some of the variables which could be attributed to healthy user bias are not kept in the CPRD. This is important, because although BMI may be accounted for, there are other indicators of how

healthy an individual's lifestyle is, which are being missed. The paper discusses that the major omissions from the CPRD are physical activity and exercise. This means that it would be important for a study to adjust for lifestyle factors. This is especially important because of how they may affect pro-inflammatory markers, and how they affect resting cholesterol levels. Other weaknesses of the study are similar to the previous studies; the study is a retrospective cohort, and the specifics of the prescribed statin are not provided.

Overall the study also supports further investigation, but proposes some key factors, such as lifestyle and vaccination status which would have to be adjusted for to prevent confounding variables in a possible RCT of the effects of statins on morbidity and mortality.[30] This would have to be performed in groups of participants with known influenza infection to strengthen the study.

Vandermeer et al., 2011, is one of the major studies in this field which led to further research on the topic. The paper discusses the reduction in cytokines and inflammatory markers from statins, and how these cytokines, when elevated, are one of the main causes of mortality from avian influenza and the 1918 influenza pandemic.[27] The study aimed to gain clarity the inconsistent data, by better analyzing the exposure and outcomes of the populations being studied.

The study utilized CDC and the Emerging Infections data from 10 states, which tracked laboratory confirmed influenza.[27] The study also analyzed data from the patient charts pertaining to their demographics, and obtained data on patient vaccination records. Statin use was also clearly defined, whether the patient had been on a statin based on the past medical history, and whether statins continued to be administered based on the

medical record. Individuals were included in the study if older than 18, were admitted during the 2007-2008 flu season, and within 14 days of a confirmed influenza diagnosis. Individuals were not included if the infection was acquired during a hospital stay.[27] A 30-day post infection cutoff was used to allow for the possibility of opportunistic infections associated with influenza to also be analyzed. SAS software was utilized to analyze the data, and logistic regression was used to analyze the variables, primarily focusing on statin use as the dependent variable on mortality.[27]

The resulting data from the analysis yielded 3071 patients, of which 28 were excluded for consent reasons. The patients were mostly female (56%) and the median age was 70.4. A third of the patients had previously used statins, or used statins during hospital admission, and 57.1% of all patients included had been vaccinated. Only 11% of the statin users were on statins only during admission, and 13% were only on statins prior to admission, the remaining statin users were on statins prior to, and during admission.[27] The statin users had median age of 75.9 years, were mostly white men, with an underlying condition and more often vaccinated when compared to the cohort not on statin therapy.[27] The study found that statin use, when adjusted for age, race, underlying conditions, antiviral administration within 48 hours, and vaccination status, did decrease mortality (Adjusted Odds Ratio .59 95% CI .38-.92). This was only observed in the group who had been prescribed statins, and continued to take the drug while in the hospital.[27] The study did not analyze the patients who had been on statins until admission, or who had taken statins only during the admission because these groups were small. This was the first observational study which found such results. The study added to the findings of

previous cohort studies, adding specificity to the finding for influenza, rather than pneumonia due to other causes.

There are several strengths and limitations to the study. The first strength is again the size. This allows for the reduced likelihood of attributed confounding factors. Another strength regarding the population included in the study is the diversity of the participants with respect to geographic origin. This enhances the generalizability of the study. The patients included had diverse ethnic backgrounds, and underlying conditions. The population is about half men and half women, which also increases the generalizability of the study. Also, about half of the population included was vaccinated. Another strength of the study was how the groups were able to be subdivided and further analyzed. This was evident when adjusting data for race, age, underlying conditions, vaccination status and other treatments provided. One of the strongest aspects of the study, was that unlike other studies, it was known that the medication was being given. This was analyzed using the medical record for the patients' hospital stays. Unfortunately, this was not able to be analyzed when assessing whether or not the patients were compliant with the medication at home. Unlike Brassard et al., 2016,[30] this was only focusing on patients with a known diagnosis of influenza. This may miss some patients, but overall is a strength of the study. This is beneficial data because of the pathogenicity of the influenza virus and because of how the medication interacts with the virus. This allows for better understanding of how statins may affect viral replication in humans, rather than in vitro which was described in the overview. Lastly, the inclusion criteria for the study was very specific. This allowed for the findings to be very specific to the goal of the study. This was done by excluding

hospital-acquired infections which could potentially skew the data. The logistic regression model utilized is a good model for determining the effect of one variable on a given outcome.

Some limitations of this study are well described within the article. The article discusses that there may be some data missed because the study only included laboratory confirmed infections.[27] This is also a worry because some of the rapid tests used to determine infection may not be as sensitive as other available tests. Although this may result in some positive cases being missed, there was still a large population in the study. Another limitation cited within the study, is that much information obtained for the study was dependent on what was in the chart. This, therefore also depends on who is charting the data, although this was attempted to be mitigated by checking the local charting. An additional limitation stated by the study, recognized that it could not be determined whether or not the statin was still adhered to following patient discharge.[27] This is important because of many deaths occurring following discharge. This brings up another weakness of the study, the issue of adherence to the medication prior to admission. The study relies on the past medical history of patients to be accurate, which cannot be certain. Although this may not be as much of an issue for hospitalized patients, the study did not monitor status of patients during hospital stay, and therefore there could be missed doses because of being intubated, or other causes of missed doses. A major limitation is the inability to track other confounding variables. The statin users and non-users were not analyzed based on physical activity and diet. The study also describes that data on the race of participants was not enough to fully classify, and this could have led

to a misclassification which saw white individuals being more likely to die, even after adjusting for other factors. Although the study would have included individuals as long as the patient was older than 18, the median age is relatively old. Though age is controlled for when analyzing and comparing groups in the study, the higher age could affect the data. Because the study is an observational study, it could be subject to an observer bias. Another possible weakness is that, although the study had a diverse population, the study took place in one flu season, and the specific virus strain could be more or less susceptible to the effects of statins. Lastly, the weakness present in previous papers is still true, that the type of statin, and dose, was not described in this study.

This study overall strengthened the argument that statins decrease mortality from influenza, but the paper discusses the need for an RCT. The study focusing on influenza infection is a benefit, but the lack of specifics of the statin indicate the need for more research to be done.

Although Kruger et. Al, 2012, does not specifically discuss the effect of statins on influenza, this article is an RCT which examines the effect of atorvastatin on septic ICU patients.[26] This is useful in determining the biologic plausibility of reduction in inflammatory markers leading to reduction in mortality. The study discusses, like previous studies, how immunomodulation could benefit sepsis mortality, and this immunomodulation had been observed in animal models with statins.

The study was a double-blind, placebo controlled RCT which was conducted in 21 ICUs within Australia and New Zealand. Patients included were between the ages of 18 and 90, with a “strongly suspected” or confirmed infection, and evidence of sepsis,

based on SIRS criteria and organ dysfunction.[26] The resulting population was either treated with 20 mg of atorvastatin, or a placebo, for 14 days or until discharge. This would be stopped either by the physician, or withdrawn consent, based on severe side effects. Blood samples were regularly collected at days 1, 3, 5, 7 and 14, assessing inflammatory markers, with the primary outcome being IL-6 levels to assess the correlation to severity disease, and how statin is influencing these levels.[26] A total of 250 patients were included for the study, in order for the results to be considered clinically and statistically significant. The data were analyzed by various statistical methods, depending on the distribution of the data; if there was a normal distribution, the data were analyzed using the Student t test.[26] Data were further divided into subgroups; of the patients receiving statins, the group was separated into individuals who had previously taken the drug, and who were naive to the medication. Baseline characteristics were compared, and they found a statistically significant difference in age, existing COPD diagnosis, platelet count and heparin use, between the statin users and nonusers.[26]

Overall, the study did not find a difference between the statin user group and the statin nonuser group in regards to IL-6 serum levels. However, this was not true for patients who had been on a statin previously, and continued to take the statin during stay, which led to a statistically significant reduction in IL-6 when compared to the other groups.[26] This was still true after adjustment for other variables, such as age and weight. Secondary outcomes were not significantly different between groups, these outcomes included; length of stay and mortality.[26] The study does recognize that there was a trend of IL-6 levels and how those relate to ICU mortality. This means that more elevated IL-6

levels were related to worse outcomes. The authors concluded that prior statin users, who continued with statin therapy, had a reduction in both IL-6 levels and 28-day ICU mortality.[26] There were individuals in both the placebo and treatment arm who were removed from the study for adverse effects, which could not be attributed to either the statin or the placebo. This was present for 12.2% in the treatment group and 13.4% of the placebo group.[26] The study states the findings support continuation of statins during admission to improve sepsis mortality, but that *de novo* statin use does not improve patient outcomes.[26]

This study has several strengths and weaknesses. First, the study design is the gold-standard. Having a double blind randomized controlled trial limits bias and increases the reliability of the findings. Another strength is the generalizability because of the range of locations used. The generalizability is also improved by the range of ages of patients, and that all patients included in the study were critically ill, making this design useful for that specific cohort. This prevents the possibility of other patients who may not have been in the ICU from skewing the data. An important strength of this study, which was not discussed in any of the previously analyzed studies, is the specificity of the drug dosing. This study specified both the dose of the drug, and the length of administration of the drug. One possible issue with this, though, is that there are individuals who would have been discharged rather than completing their dose. Another possible issue with this is that individuals who had been on a statin prior, were assumed to have been compliant with the medication. This is a variable which is difficult to adjust for, just as diet and exercise are. The use of blood testing to monitor inflammatory markers was another

strength of the study. This allowed for there to be an objective marker of how the patients were doing, and eliminated possible biases. This was also verified by the fact that the study assessed the atorvastatin levels in the blood,[26] to ensure that this data were accurately represented. This was even further strengthened by the study also measuring the drops in cholesterol levels. The study adjusted for possible confounding variables, which helps the data be better represented, and makes up for the limitation of the differences in the baseline characteristics between the treatment and placebo arms.

One limitation of the study is the size. Although the study used a power to ensure that this study size would be statistically significant, the study could be improved if there were more patients included. This is also true of the subdivided statin user group, which only included 77 patients.[26] If this group was expanded, and then randomized to assess for outcomes related to whether or not it is beneficial to continue the medication during an ICU stay, the data would have been a better representation, and more generalizable. Another weakness of the study is the differences in the baseline characteristics besides the age and weight. Though these could have been adjusted for, the increased number of COPD patients in the statin group may have led to a misrepresentation of the data. These individuals may have been more likely to die due to complications related to the underlying condition, and this could make it appear as though the effects of statins are not as strong. A major weakness in the study was the varying of the length of the dosage. The patients in the treatment arm were given an average of four days dosage, and it is unknown how longer or shorter duration of treatment may affect the data. The study also focused on atorvastatin, and as previously stated, there is a possibility that certain statins

affect the clinical outcome differently, based on the potency. Atorvastatin, at the dose in the study, is a moderate potency statin,[32] and it is possible that stronger results would be yielded with a higher potency statin, or with a longer duration of treatment. The article does state that patients with lung injury may benefit from longer courses of statin treatment. The findings could be affected by alpha or beta errors in the study as well, and warrant further investigation. Pertaining to the specific hypothesis of this thesis, it would be beneficial to know the type of infections participants had who made up the populations of this study. This also relates to the weakness that not all infections were confirmed. When compared to Vandermeer et al., 2011,[27] where all infections were confirmed, this may lead to more cases included based on clinical suspicion, but this could also be subject to bias.

In conclusion, this study benefits from being a double-blinded, randomized controlled trial. The findings leave open the possibility of both further investigating different dosages and durations of statin treatment. This also proposes the possibility that the decreased mortality may be most associated with prior statin use. The decrease in IL-6, an important cytokine involved in the overactive immune response, is a marker that could be used for a future study which focuses primarily on influenza, which should be laboratory confirmed. The study provides further biologic plausibility to statins being used as a mild immunomodulator, supporting the studies in animal models. This could be used going forward in predicting patient outcomes and in possible treatments.

Frost et al., 2007, is study analyzed the reduction in pneumonia/influenza and COPD exacerbations leading to death.[24] The paper briefly discusses the proposed

immunomodulatory effects of statins and how these could be used to help decrease mortality from a severe strain of influenza. The paper acknowledges previous studies have found reduction in mortality from both COPD and community acquired pneumonia. The paper also discusses that H5N1 deaths have been primarily due to the rapid, overactive immune response.[24]

There was matched cohort study, which looked at individuals who had been on a statin for at least 90 days, versus those who were not exposed to statins. The statin group was subdivided into low (<4mg/day) and moderate dose (≥4mg/day).[24] The cohorts were matched based on sex, age and health maintenance organization enrollment periods. The mortality risk of the groups was based on ICD-9 classification, and was applied to both groups of the matched cohort.[24] Causes of death were based on pneumonia, influenza and COPD, and were analyzed using logistic regression to assess odds ratio. The results of this part of the study found that more statin users were likely to have comorbidities, but were also more likely to be vaccinated than non-statin users.[24] Moderate dose statin users were less likely to die of COPD (OR=.17), and after adjustment of co-occurrence of influenza and COPD, a reduction in influenza mortality was observed as well (OR=.63).[24]

This study also performed two case-control studies with the intention of confirming findings from the matched cohort study. The patients were taken from a patient database, and outcomes were defined using ICD-9 mentioning COPD or pneumonia/influenza for patient deaths, versus patients with these diagnoses and surviving inpatient or outpatient visits. This study also assessed low, moderate and no

statin use, and were adjusted based on the same criteria as the matched cohort study. The results of this study found that moderate statin users were less likely to die of influenza/pneumonia, but this was not the case for low dose statin.[24]

The results of this study found that COPD mortality was reduced from statin use significantly, and there was also a reduction in mortality due to pneumonia and influenza, which was seen in both aspects of the paper.[24] This supports previous studies. The paper suggests that the effect is dose dependent for both COPD and influenza/pneumonia.

This paper has several strengths and weaknesses. The first strength of the study is the utilization of multiple study types to corroborate the findings. The matched cohort study allowed for a minimization of the effects of possible confounding variables. The case control study was a large population (n=64,362) and this would likely diminish the effects of confounding factors as well.[24] Another strength of this study, is the various doses of the statins prescribed. This allowed for analysis into the effectiveness of mortality reduction based on the dose. A third strength of the study was that the study assessed statin compliance as well. This was not previously included in other studies, and is an essential component in assessing effectiveness of the intervention. If an individual was prescribed a statin but had not been compliant, this would skew the results. The paper discusses how this is an improvement on other studies that depend on serum markers because those studies may be assuming compliance, which could lead to a false conclusion of causation. Assessing compliance, and possibly coupling that information with serum markers, could improve a study. The study is generalizable as well because it involves about half men and half women. The generalizability of the study is improved

by the various ages of patients involved, and more importantly the various flu seasons. The study involved data from 1992 through 2003.[24] This would increase the various strains of the virus that are included in the study, and suggest more of a general effect of medication on the virus. One last important strength of the study was the matching for underlying comorbidities, this is useful for preventing skewing of the data.

A first limitation of the study is that the study relied on ICD-9 classification of disease. This is discussed in the paper as a potential source of a misdiagnosis because ICD-9 did not require laboratory confirmation of a pathogen.[24] This means that there could be some diagnoses wrongfully classified as influenza. Another weakness is that, although the doses of the statins were divided into low and moderate doses, the moderate dose included a large range, which could also be including high dose statins. The resulting decrease in mortality may be more associated with those statins that are high dose than moderate dose. A limitation discussed within the paper is that deaths that did not occur within the hospital were not included in the study. This could include data from both statin users and non-users, and would be beneficial to better understand the results. This is less likely to be a major limitation because of the large sample size. The study did not include "young" patients who could also be severely affected by influenza. This is possibly because statins are less likely to be prescribed to these individuals. The paper also discusses that the statin user group were more likely to have cardiovascular conditions,[24] which could be seen as a weakness. This would be difficult to adjust for, because, as discussed in the paper, this is the population which is most likely to be prescribed these medications. Other weaknesses in this study include weaknesses of other

studies, as well, i.e. a cohort study and the inclusion of all statins as a class, rather than separate drugs. This is especially important in this study, because different statins are meant to be low, high, or moderate potency, and could have different effects of mortality.

In conclusion, this study did a good job of further informing how statins affect mortality related to COPD, which could also affect mortality from influenza. This observed effect could contribute to the reduction in influenza mortality in COPD patients who are also on a statin. This study also better defines the dosage that is effective in decreasing the influenza mortality. This information is useful in devising a new study, which may not need to include examining the effect of low potency statins on influenza mortality. This also emphasizes the importance of an RCT including a group with COPD, to assess how this mortality associated with COPD is altered by a given statin for influenza patients. Although the reduction of mortality in the study was not as large as previous studies, this benefit suggests that further research into the topic could benefit patients. An RCT could be guided utilizing some of the principles used within this study, including dosage, including younger patients, and COPD arms of treatment. A new study would also benefit from using less subjective definitions of diagnoses. A new study would benefit from assessing compliance, as this study did. This further investigation will be useful in clarifying the data, and useful in finding other ways to reduce influenza mortality due to cytokine storm.

# METHODS

## Study Design

This study will be a double-blind randomized control study. Involving patients from the Boston hospitals and the 3 major NYC hospitals who have been diagnosed with influenza upon admission to the hospital. All patients will have been diagnosed using PCR. Previous studies have investigated the affects of statin use in decreasing influenza morbidity and mortality, but not many have been as specific in parameters nor have many been double-blind randomized controlled trials. The comparison between those patients previously on statins and continuing statins can be a predictor in adverse outcomes in patients with influenza infection which is severe enough to require the individual to be admitted to the hospital.

The goal of the study will be to better understand how to predict adverse outcomes in individuals with influenza as well as to better understand how intensity of statins affects these outcomes. Through assessing the inflammatory marker load, and mortality associated with infection, the effect of statins could be better determined. This could then be useful in predicting outcomes of other diseases with a similar mechanism of pathogenicity.

## Study Population and Sampling

The study will enroll men and women over the age of 30, who are admitted to the hospital with a diagnosis of influenza via PCR and who are on a medium to high intensity statin at the time of diagnosis. The study will enroll a total of 7188 patients. This is a large number and therefore there should be an aim to include many hospital systems over

multiple seasons. The goal will be to include greater Boston area hospitals and hospitals in NYC. This will be done in 3 seasons.

**Inclusion/Exclusion Criteria**

Men and women older than 30, who are admitted to the hospital with laboratory confirmed influenza diagnosis and on a moderate-high intensity statin at the time of admission will be eligible for the study, as long as there has been compliance with the medication. Patients included in the study will have their past medical history screened so that the individuals can be further stratified for data analysis. This is also important so that it can be known why the individuals are on the statin. Patients will also be screened for vaccination status against influenza as well, although this would not exclude individuals from the study population. If individuals are on other chronic corticosteroids, they will be excluded from the study population because of the proposed mechanism of action of statins possibly being interfered with or amplified by chronic steroid use. This also could be a risk factor for worse disease. Patients over the age of 30 will be included because of their likelihood to be on a statin. Patients with evidence of liver damage will be excluded from the study due to the possibility of statins worsening this damage. This will be determined by abnormal LFTs. If a patient is proposed to be placed on a drug which may interact with the statin during the admission, then these individuals will be excluded from the study. If there is any evidence of rhabdomyolysis, then these individuals will also be excluded from the study. Diabetics will be excluded from the study because there is a risk of worsening Hgb A1C on statins. To be included in the study, adherence to taking the statin in the past must be established. Lastly, should the

individual be unable to comply with the statin or placebo regimen, for any reason, such as dysphagia, or being intubated at any stage of hospital stay, they will be excluded from the study.

**Exposure Group**

Individuals who meet inclusion criteria with be randomized into one of two groups: continuing statin use, or discontinuing statin, but being given a daily placebo pill. The group continuing statin will be further analyzed based on the statin that is continuing during an analysis. Subgroup analysis should not need to be performed in this study because randomization with a large population should reduce any confounding variables.

**Study Variables and Measurement Tools**

The study variables are statin use in the patient. The primary outcome being studied is mortality at 30 days. Patient history will be indicative of previous statin use and PCR analysis will be used to determine infection status. Participants' mortality will be assessed at 15 and 30 days.

A secondary outcome which will be measured is pro-inflammatory marker IL-6, as performed in Kruger et al., 2012.[26] An IL-6 level will be added to the routine lab values being assessed in patients that belong to both groups. Lastly, length of stay will be measured and compared between the groups.

During each assessment of the patients, the population will be screened for possible side effects of the drugs which may require individuals to be removed from the study. Also, patients who are discharged before 15 or 30 days will be checked on at these points and scheduled for follow up visits. The 15- and 30-day mortality between the two

groups will be compared using CFR. It is also important that these individuals are routinely called to ensure that they are continuing to take their prescribed medications. Should an individual miss a dose, they will then be excluded from the study. Any death within 30 days will be counted as a part of the CFR.

IL-6 values will be measured because this is an important proinflammatory cytokine. The proposed mechanism of action of statins should lead to a reduction in this compound. These values will be charted for each group of patients. These will be continually assessed during routine follow up visits following discharge. Having these values assessed up until the end of a full 30 days will allow for a better comparison of groups when analyzing the data.

Hospital protocol for both groups of patients will be continued and assessed. Should there be new evidence of liver damage, rhabdomyolysis, or kidney damage, the statins will be discontinued. These patients will then be excluded from the study. To ensure that this is maintained at different sites, training will be provided for participating sites to instruct which labs must be conducted for all patients within the study.

**Recruitment**

Research assistants will discuss with patients being admitted to participating hospitals about the study being performed and ask if individuals would be willing to participate. Physicians will then be asked if they are willing to participate in the study, should their admitted patient also be willing. If the patient then meets the criteria to be involved in the study, then they will be included. The goal is to recruit equal numbers of men and women if possible at this site, as this could be a limitation in the data.

## Data Collection

Research assistants will input data for each patient at pertinent deadlines. These will include: routine collection of IL-6 values, discharge and length of stay, and outcomes at days 15 and 30. Research assistants will also record if patients are later excluded from the study and the reason for exclusion. This information will be stored within a spreadsheet for each patient.

## Data Analysis

The double-blind randomized control trial will separate patients admitted into the hospital with diagnosed influenza and on a statin into two groups: continuing medication and placebo. There will be more individuals in the treatment arm of the study. The primary outcome being measured will be mortality in the two groups. This will be assessed at 15 and 30 days. Secondary outcomes will be IL-6 values and length of stay in the hospital.

The goal is to recruit similar numbers of men and women to the study. In order to perform a study with an alpha error of .05 and a beta error of .8, a sample size of 7188 total patients will be needed. This is based on the sample-size.net/ calculator and through utilization of CFR from Vandermeer et al., 2011.[27] Because this is a large study, utilization of multiple flu seasons will be useful, as well as multiple sites. This assumption is based on Boston public health data which state that in the 2018-2019 influenza season, there were only a total of 588 hospital admissions.[33] As previously stated, the goal will be to enroll patients from NYC area hospitals as well, as this will increase the available population for the study.

The primary data will be analyzed using $\chi^2$ to compare the proportions of individuals who were on a statin that die to those who were not on a statin that die. The information obtained from the study will also be used to calculate the relative risk reduction between the two groups. The secondary outcome of IL-6 values will be compared between groups by using an independent t test because the variable is continuous. This will compare the average IL-6 values of each individual at the end of the hospital stay between groups. This will be looking to see if there is an association between statin use and pro-inflammatory cytokine levels. The last study that will be done is to assess the relationship between statin use, IL-6 values and mortality rate. This will be done by stratifying for IL-6 levels, and assessing for the relationship of statin use and death after this constant is adjusted for.

Subgroup analysis will be performed as needed, but should not be necessary because of the study design likely reducing the effect of confounding variables. Should a synergistic affect be observed, then further subgroup analysis will be performed.

**Timeline**

Year 1: Establishment of Study

- Submit study plan for approval and hire staff: Research assistants for data collection, and will need a PI to assist in running the study
- Establish relationship with participating hospitals and state DPH to assist with the study.
- Train hospital staff on which labs need to be collected and exclusion criteria. Also discuss with participating clinicians follow-up plans to obtain data.

Years 2, 3 and 4: Data collection.

- Collect data on patients to be enrolled and included in the study. Performed over three years to allow for multiple seasonal influenza seasons to be analyzed.

Year 5: Analysis:

- Statistician analyzes data that have been collected into excel spreadsheets. All participating sites will utilize the same statistical analysis software.
- Principle investigator will meet with participating clinicians and staff working on the project to discuss the data.

**Resources**

- Research assistants will need access to computers to input data.
- All participating hospitals will need a lab which can diagnose influenza with PCR testing.
- All participating hospitals will need to have an IL-6 blood test available to use.
- All statisticians and research assistants will need access to the same statistical software.

**Institutional Review Board**

This study, as with any study to be performed on human subjects, will be fully reviewed by the review board, especially because these individuals are being admitted to the hospital. Individuals should briefly be informed about the study design so that they understand that they will not know whether or not they are taking their statin. Participants should be counseled on known side effects of the medication and informed on how they will be excluded should any of these come up. Because there is not a known standard of

care, patients in the placebo group should be counseled about the possible risks of withholding this medication. Patients will also be specifically counseled on how patients with a similar medical history are treated within the hospital. There will be a detailed discussion on how the medication should be administered during the stay and what warrants discontinuing the medication and exclusion from the study. Patients will be informed of additional blood tests being performed, although this does not pose any increased risk because it is not an additional needle stick, rather an add on to labs which are already being performed. Patients will be instructed on what the study entails following discharge from the hospital. Lastly, the patients will be informed of what their stay looks like should they choose not to be a part of the study. Because there is not a standard of care when it comes to continuing this medication, the decision would then be up to the individual. With this information, patients should be able to make an informed decision as to whether or not they wish to participate in this study.

# CONCLUSION

## Discussion

This study will add new information regarding a possible treatment which could help reduce death in individuals with influenza. Although this is a double-blind randomized control study, it does have its limitations. First, the inclusion of multiple flu seasons introduces a possible effect of multiple variables affecting the data. For example, there is the variability in the seasonal strains for the flu. This is important because one strain may be more susceptible to the immunomodulating or antiviral effects of the statin than others. Another limitation related to the multiple year nature of the study is the variation in the vaccine. Different vaccines may be more or less efficacious which could skew the interpretation of the role of the vaccine on the effects of the statin treatment. However, the size of the study and the multiple seasons will reduce confounding factors of this. Due to the fact that the study needed a relatively large population, the inclusion of multiple sites could make the study difficult and expensive, but this should not be a limitation as it will help lead to stronger data.

Though there are limitations with utilizing multiple sites, and performing the study over multiple seasons, this makes the study more generalizable. This has been a limitation of some of the existing studies. Another aspect of the proposed study that makes it more generalizable is the aim to have better recruitment. Some existing studies, such as Kwong et al., 2009, have mostly elderly white men.[28] This has been due to the population being studied, such as the VA system.  Through including multiple sites in

different locations, this should expand the demographics of the study, and the specific aim to include more women should also assist in this goal. The study is large enough to be generalizable as well.

In comparison to previous studies, the design of this study should give more of a definitive answer to the effect of statins on mortality rates from influenza. This is because the double-blind randomized controlled study should be able to eliminate or at least minimize confounding variables. This study also decreases biases.

Some anticipated obstacles to the study include; getting many sites to agree to be involved, having patients agree to be involved, and delays in the study because of how specific the population is. Firstly, it could be difficult to get all sites that are intended to be involved to agree to be a part of the study. This is to be expected with any study not affiliated directly with a given site. Secondly, it may be difficult to get patients to agree to this study. This is because patients will likely assume that they should remain on a medication that they are meant to be taking every day normally. This could be alleviated by an in-depth discussion mentioned in the IRB section. Thirdly, there is a chance that the study has to be extended in time. This is because of how the flu seasons can vary, and it is possible that one season may not have as many flu hospitalizations as others, but the inclusion of many sites should help decrease the likelihood that this occurs. Another reason that this may occur is because this is a very specific patient population which is meant to be included in the study. Should this occur, there would likely be additional costs associated with the study.

In conclusion, should this study be carried out, it would be the most specific study in this field, as well as being the gold standard of study designs to get answers in a way as to how to reduce mortality from influenza. This will also lead to further research into how statins could be added for patients who were not previously on the medication, as well as further research in other disease states.

**Summary**

As previously stated, influenza remains a massive public health problem throughout the world. This is indicative by burden of disease that the virus causes. According to CDC estimates, the 2019-2020 influenza season led to about 38 million infections and 22,000 deaths in the U.S. alone.[1] Although antivirals exist for treatment of influenza infections, there are still other possible treatments which could reduce deaths from influenza.[18,19] Originally proposed by Vandermeer et al., 2011, statins may be effective in reducing the burden of disease from influenza.[27] Because many individuals who die of influenza have underlying comorbidities, should evidence from an RCT suggest maintaining this medication during hospitalization, the CFR of influenza could be reduced in this specific population.

Studies have been performed in the past on this topic, but there have not been RCT comparing the CFR of individuals who continued to take the statin to those who had the medication discontinued during hospital stays. This could then be further analyzed to assess the mechanism by which statin acts in reducing death, as proposed by studying IL-6. Through utilizing an RCT in a large study, confounding variables could be accounted for. With more studies on immunomodulatory medications such as statins in disease

states that cause an overactive immune response, there could be a reduction in influenza deaths, as well as further research in diseases with similar mechanisms of pathogenesis. This could have a large impact on reducing the hospital burden of influenza, which can have pandemic potential.

**Public Health Significance**

Given the large burden of disease, improving treatment of influenza would have great public health significance. First, reducing the burden of any infectious disease improves public health outcomes. A reduction in mortality and morbidity from influenza would ease burden on the healthcare system and allow resources to be utilized for other issues, e.g. the coronavirus pandemic. Another positive implication, should the results of the proposed study be conclusive, would be how accessible the treatment is. Some newer designer drugs will be expensive and not quickly available to the public, whereas statins are cheap and very accessible, allowing the treatment to be given to individuals throughout the world dealing with flu epidemics. It is also beneficial that many people already take the medication.

The results from the study could also be useful in suggesting further studies which will also have public health impacts by evaluating the efficacy of the same medication in treating other viral infections, or testing other medications with similar effects/mechanisms of action for beneficial effects in patients with influenza. Having multiple ways to decrease mortality and morbidity from an infectious disease allows there to be more normal life. This then has further downstream implications such as societal and social impacts. Finding cheap, effective ways to treat influenza which can cause a

pandemic, or other similar acting viruses, can be key to lowering the burden on hospitals and allow for there to be less of a socioeconomic impact of the virus. This is evident with the current coronavirus situation, and with pandemic influenza a concern, it is important to approach the virus in a way that minimizes the many adverse ways it can impact society.

## LIST OF JOURNAL ABBREVIATIONS

| | |
|---|---|
| ACC | American College of Cardiology |
| Acta Virol. | Acta Virologica |
| AHA | American Heart Association |
| Biomed Res Int. | BioMed Research International |
| Epidemiol Infect. | Epidemiology and Infection |
| Front Immunol. | Frontiers in Immunology |
| JAMA | The Journal of the American Medical Association |
| J Infect Dis. | The Journal of Infectious Diseases |
| Nat Rev Cardiol. | Natural Reviews Cardiology |
| P T. | Pharmacy and Therapeutics |
| Respir Crit Care Med. | American Journal of Respiratory and Critical Care Medicine |

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## CURRICULUM VITAE