Persistent Pathogens and Incident Stroke in Mexican Americans

by

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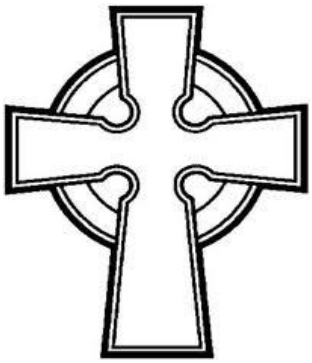

Dedication

To Kj and Kortney.

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Abstract

Stroke is the fourth leading cause of death and a leading cause of disability in adults in the US. Nearly 800,000 people suffer a stroke annually, and more than 143,000 deaths occur as a result. Hispanic Americans are the fastest growing minority population in the US, as well as the most numerous. The majority of Hispanic Americans are of Mexican descent. Mexican Americans (MAs) have an increased risk of stroke especially at younger ages. Traditional risk factors are not likely to fully account for the increased stroke burden in MAs. Chronic infectious pathogens have been proposed as risk factors for stroke, although the evidence remains inconclusive.

Using data from the Sacramento Area Latino Study on Aging, we investigated the associations between Immunoglobulin G (IgG) antibody levels to five persistent pathogens including: Cytomegalovirus (CMV), Herpes Simplex Virus Type 1 (HSV1), *Toxoplasma gondii (T. gondii)*, Varicella Zoster Virus (VZV) and *Helicobacter pylori (H. Pylori)*, and incident stroke. Next, we examined the effect of pathogen burden, or concurrent exposure to several pathogens, on stroke risk. Third, we sought to elucidate the mechanism of the *H. Pylori*- incident stroke association by examining the mediating effects of interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor (TNF), homocysteine, folate, Vitamin B12, systolic and diastolic blood pressure, and glucose.

We found a significant association between high antibody levels to *H. Pylori* and incident stroke, in fully adjusted models. There were no significant associations between stroke risk and either antibody levels to CMV, VZV, *T. gondii*, or HSV1 or seropositivity

to each pathogen. We report a significant association between sum of the number of pathogens eliciting a high antibody level, and incident stroke; however attenuation and loss of statistical significance occurred with multivariable adjustment. No associations between summed seropositivity to 0-5 pathogens or average antibody level to 0-5 pathogens and incident stroke were found. Lastly, we found no evidence of mediation of the *H. Pylori*-incident stroke association by 9 candidate mediators. This research has implications for future population-based research in MAs, as well as studies intervening on persistent pathogen exposure, for reductions in stroke risk.

Chapter 1

Introduction

1.1 Introduction

In the United States, a stroke occurs approximately every 40 seconds.[1] Stroke is the 4th leading cause of death, and a major contributor to adult disability.[1] Nearly 800,000 people suffer a new or recurrent stroke annually, and more than 143,000 deaths occur as a result.[1] Eighty-seven percent of all strokes are ischemic, 10% occur as a result of intracerebral hemorrhage, and 3% result from subarachnoid hemorrhage.[1]

Hispanic Americans are the fastest growing minority population in the United States, as well as the most numerous.[2] The majority of Hispanic Americans in the United States are of Mexican descent.[2] Mexican Americans (MAs) have an increased risk of stroke compared to Non-Hispanic Whites (NHWs), especially at younger ages when disability has the greatest impact.[3] Reasons for increased stroke risk in MAs are not fully understood but traditional risk factors for stroke, such as hypertension, heart rhythm disorders, tobacco usage, hyperlipidemia, low leisure time physical activity, and diabetes, are not likely to completely account for the disparity, since ethnic differences in these factors either do not exist or are not substantial enough to account for the large difference in stroke risk.[1]

In the US, seroprevalence to persistent pathogens, such as *Helicobacter pylori (H. Pylori*), Cytomegalovirus (CMV), Herpes Simplex Virus 1 (HSV1), *Toxoplasma*

gondii (T. gondii) and Varicella Zoster (VZV), is common. These pathogens are acquired in early life and many persist throughout the lifespan of the host, often in latent form.[4] Upon reactivation, persistent pathogens may increase systemic inflammation, which negatively impacts stroke risk.[5, 6] It is plausible that persistent pathogen exposure, traditional risk factors and genetics work together to promote atherosclerosis, which may lead to incident ischemic stroke.[6] Although infections caused by exposure to individual persistent pathogens have been proposed as risk factors for stroke, the evidence from prospective studies remains inconclusive with only one[7] of the three existing studies[8, 9] reporting a significant association. Further, pathogen burden, or exposure to several persistent pathogens concurrently, may be a stronger risk factor for stroke than exposure to individual pathogens, through an inflammatory pathway.[10, 11] Unfortunately, the existing studies that investigated the link between pathogen burden and stroke risk have also had conflicting results.[8, 9]

Among MAs, disparate rates of infectious disease have been reported.[12, 13] In particular, MAs have higher overall burden of infection caused by *H. Pylori,* CMV, *Hepatitis B*, HSV-1, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, suggesting that persistent pathogen exposure, as a stroke risk factor, may be particularly relevant in this population.[12-15] Similarly, MAs have also been reported to have higher sero-prevalence to several pathogens concurrently compared to NHWs.[16, 17]

The existing prospective studies that investigate the links between persistent pathogens and stroke risk have used seropositivity to persistent pathogens in defining pathogen exposures versus antibody response, which is thought to be a more

consistent predictor of inflammatory outcomes.[18] Furthermore, the study population of one of the existing studies included NHW, non-Hispanic blacks and Cuban and Puerto Rican Hispanics,[8] and the remaining two cohorts were comprised entirely of NHWs.[7, 9] Importantly, none of the previous studies focused on the MA population, which is an understudied group with a disproportionate burden of stroke.[3] The previous studies also did not examine the pathways by which persistent pathogens may increase stroke risk.

Given the potential links between persistent pathogen exposure and incident stroke in MAs and the gaps in the existing literature, the goals of this dissertation research were to (1) to investigate the effects of exposure to five persistent pathogens, including CMV, HSV-1, VZV, *H. Pylori* and *T. gondii,* on incident stroke (2) to examine the effect of pathogen burden, operationalized as (I.) summed seropositivity to 0-5 pathogens (II.) average antibody level to 0-5 pathogens and (III.) sum of the number of pathogens eliciting high antibody levels (0-5), on incident stroke, and (3) to explore the mechanisms involved in any associations found between persistent pathogens and incident stroke, using an elderly cohort of MAs, aged 60+ years old, from the Sacramento Area Latino Study on Aging (SALSA).

1.2 Specific Aims and Hypotheses

Specific Aim 1: To determine the association between Immunoglobulin G (IgG) antibody levels to CMV, *H. Pylori,* HSV1, VZV and *T. gondii* and incident stroke in MAs from SALSA, after accounting for traditional risk factors.

Hypothesis 1: Higher IgG antibody levels to each individual pathogen will be associated with greater stroke risk, even after adjusting for traditional risk factors for

stroke, such as age, gender, hypertension, diabetes, smoking, hyperlipidemia, atrial fibrillation, coronary heart disease/PAD and socioeconomic status.

Specific Aim 2: To examine the association between pathogen burden, as measured by (1) summed seropositivity to the five pathogens (2) average IgG antibody levels across all five pathogens and (3) sum of the number of pathogens eliciting a high antibody response, and incident stroke in MAs after accounting for traditional risk factors.

Hypothesis 2: Greater pathogen burden will be associated with greater stroke risk, even after adjusting for traditional risk factors as described above.

Specific Aim 3: To determine whether inflammatory markers such as Interleukin-6 (IL-6), C-reactive protein (CRP), and Tumor necrosis factor (TNF), partially mediate the association between increased IgG antibody levels to five persistent pathogens and stroke risk in MAs.

Hypothesis 3: The association between increased IgG antibody levels to five persistent pathogens and incident stroke will be partially mediated by TNF, CRP, and IL-6.

1.3 Background

Stroke is an important public health issue. The next 40 years are pivotal for the health of our nation as the "baby boom" generation becomes senior citizens and the morbidity and costs from chronic diseases skyrocket to unprecedented levels. Due to the aging population, the number of strokes will increase dramatically; exceeding 2 million per year by 2050.[19] Stroke is the fourth leading cause of death in the US, and is the leading cause of long-term disability in adults.[20] Between 15-30% of stroke survivors

are permanently disabled and many require long-term nursing care.[21] High rates of disability contribute to enormous costs associated with stroke; over the next 50 years the cost of stroke will exceed $2 trillion in the US.[22] The Healthy People 2020 objectives for heart disease and stroke include increasing overall cardiovascular health, which emphasizes the need for more stroke research.[23]

Eighty-seven percent of all strokes are ischemic in nature, 10% occur as a result of intracerebral hemorrhage, and 3% are caused by subarachnoid hemorrhage.[1] Ischemia results from low vascular blood supply to the brain.[24] Ischemic stroke pathophysiology is complex, and strokes of this type may manifest from a thrombus (clot), embolus (traveling clot), systemic hypoperfusion, or a venus system thrombus.[24] During ischemic stroke, the brain parenchyma, or inner core, dies instantly as blood flow to this area decreases to 10-25%, whereas the penumbra, or the brain tissue surrounding the core, may only be partially affected by the ischemia, and could be spared from death as a result of collateral blood vessel formation and/or timely pharmacologic intervention.[24]

Atherosclerosis or hardening of arteries is the major precipitating event leading to ischemic stroke.[25] Arterial changes leading to atherosclerosis begin with an injury to the arterial wall, which can be caused by high cholesterol and triglyceride levels, smoking cigarettes, increased serum glucose, and/or hypertension.[25] The next step involves the accumulation of cholesterol, platelets, calcium and other cellular debris at the site of arterial damage and formation of plaque, which thickens the endothelium, and eventually impedes blood flow.[25] Atherosclerotic plaque is also deleterious when it becomes fragile and ruptures causing blood clots, which can either block a blood vessel

(thrombus) or break off and travel to another location in the body (emboli).[25] Traveling emboli can block blood vessels that supply blood to the: (1) heart, resulting in a heart attack, (2) brain, causing a stroke, or (3) arms and legs, causing difficulty walking and ultimately gangrene.[25] The process of atherogenesis begins in childhood, usually affects medium to large-sized arteries, and slowly progresses throughout life.[25]

The largest and fastest growing minority population in the United States is the Hispanic American population.[26] The majority of Hispanic Americans, nationally, are MAs.[26] The incidence of stroke among MAs is higher than that of their NHW counterparts, especially at younger ages.[3] The MA population is currently youthful and will, in the coming years, suffer an even greater burden of stroke as the population ages. The disproportionate stroke burden in Hispanic Americans poses a significant financial obligation to society, with projected direct and indirect costs from 2005-2050 exceeding $300 billion for this group.[22]

While MAs have different stroke risk factor profiles than NHWs, traditional risk factors are not likely to fully account for the stroke disparities seen in this group. For instance, higher prevalence rates of diabetes have been reported in MAs,[27] while the prevalence of hypertension and hyperlipidemia is similar to that seen in NHWs.[28-30] Next, MAs have been reported to have lower prevalence rates of smoking and atrial fibrillation compared to NHWs.[31, 32] On the other hand, higher rates of kidney disease[33] and decreased amounts of leisure time physical activity[34] have been documented in MAs compared to their NHW counterparts. There are also important differences in hypertension awareness, treatment and control among MAs and NHWs, with MAs less likely to be aware of disease status, to be prescribed adequate treatment, and to have

the disease controlled.[29] Similarly, evidence exists that MAs are less likely to have their diabetes properly controlled by pharmacologic treatments, as compared to NHWs.[35] Given that MAs have higher rates of some stroke risk factors compared to NHWs, but lower rates of others, and the fact that we currently do not have a conclusive explanation for the disparate stroke burden in this group, novel intervention targets should be identified, in order to avert the impending death and disability that stroke will cause in this population in the coming years.

Infections caused by HSV-1, CMV, VZV, *T. gondii* and *H. Pylori* are quite common in US populations. The *Herpesviridae* family of viruses consist of 8 human pathogens including HSV-1 and CMV.[4] HSV-related diseases are the most widespread in the family, affecting 60-95% of adults worldwide.[36, 37] These infections are currently incurable and persist through the life span of the host, most commonly in latent form.[4] HSV-1 accounts for most of the non-genital HSV-induced infections in humans.[38] Next, CMV affects 50-80% of US adults by their 40th birthday.[39] Close contact with body fluids of an infected person facilitates transmission of this pathogen.[39] Most people infected with CMV have no symptoms and are unaware of the infection.[39] VZV is caused by Human (alpha) herpes virus 3.[39] In temperate climates, at least 90% of the population acquires the infection caused by VZV by age 15, and at least 95% by young adulthood.[40] Life-long immunity is usually conferred with the original VZV infection, with secondary attacks common in immunodeficient individuals.[40] This viral infection may remain latent, with recurrence years later as herpes zoster in about 15% of older adults.[40] *T. gondii* is an intracellular coccidian protozoan characterized by asymptomatic infections.[40] However, *T. gondii* cysts containing viable organisms may remain in the

tissues, with reactivation of cysts during immune system compromise.[40] The duration and degree of immunity are unknown, but it is assumed to be long-lasting or permanent, with antibodies persisting for years, even throughout life.[40] *H. Pylori* is a small, curved microaerophilic gram negative rod.[41] It is one of the most common bacterial infections in the world, with a 70% prevalence in developing countries and 30-40% prevalence in industrialized regions.[41] Exact modes of transmission of this pathogen are unknown, however, fecal-oral or oral-oral routes are most plausible.[41] Infection generally occurs in childhood, and could persist throughout life if untreated.[41]

Certain persistent pathogens are more prevalent in MAs.[12, 13] MAs have a higher prevalence of exposure to several individual infections than their NHW counterparts.[17] For example, using data from NHANES III, Everhart et al reported an age-adjusted sero-prevalence of *H. Pylori* of 62% in MAs compared with 26% among NHWs.[13] Also from NHANES III, Staras et al reported a CMV sero-prevalence of 82% in MAs compared with 51% in NHWs.[12] MAs have also been shown to have a higher prevalence of exposure to multiple persistent pathogens (termed pathogen burden) compared with NHWs.[16, 17] In analyzing data from a representative sample of US adults, Zajacova et al reported higher burden of infection caused by six persistent pathogens (*H. Pylori*, CMV, *Hepatitis B*, HSV-1, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*) in MAs compared to NHWs.[17] Disparities seen in MAs with regard to infectious disease burden do not appear to be accounted for by differences in education or income.[17]

Several mechanisms exist that may link persistent pathogens to incident stroke. Persistent pathogens are thought to increase inflammation, thereby producing migration

of inflammatory cells to vessel walls which damages the endothelium, and increases risk of atherosclerosis and clot formation.[42, 43] As early as the 1970s, Mareks disease, caused by an avian herpes simplex virus, was implicated in the formation of atherosclerosis in animal models, which is similar to the atherosclerotic changes seen in humans.[44] CMV has recently been linked with initial carotid artery changes leading to atherosclerosis, as indicated by increased intima-media thickness (IMT) and later carotid stenosis.[45] CMV may also increase the speed at which plaques mature and/or increase plaque instability and rupture as a result of immune cell activation.[6, 46] Inconclusive evidence suggests that elevated plasma homocysteine concentration (a known risk factor for stroke) is associated with decreased folic acid induced by *H. Pylori* infection.[47-53] Persistent pathogens may also directly infect vascular cells.[54] For instance, it has been preliminarily shown that *H. Pylori* contributes to atherosclerotic changes in arteries, in retrospective analyses;[55] however, prospective studies have yet to confirm this association.[56] Furthermore, *C. pneumoniae,* has been isolated from atherosclerotic plaques, but rarely from normal cells of the arterial wall.[57-60] Persistent pathogens, traditional risk factors, and genetic predisposition may work together in stimulating inflammatory pathways to increase risk of atherosclerosis and therefore risk of stroke.[6]

Persistent pathogens have also been associated with established stroke risk factors, although prospective studies are few in number, and are rarely focused on MAs. In a recent report using the data from the Sacramento Area Latino Study on Aging, Jeon et al. reported a significant increase in diabetes risk among individuals sero-positive to *H. Pylori* (hazard ratio (HR): 2.69; 95% CI: 1.10-6.60).[61] In a population-based study

conducted in Taiwan, HSV infection was associated with an increased risk of atrial fibrillation (HR: 1.39; 95% CI: 1.20-1.60).[62] Further, an association between high antibody levels to CMV and elevated systolic and diastolic blood pressure among men (p-value: 0.053 and p-value: 0.002, respectively) was found in analyses using data from the Cardiovascular Risk in Young Finns Study.[63]

Although it is plausible that persistent pathogens are risk factors for stroke, the existing literature is limited. Studies have been conducted to examine *C. pneumoniae,* CMV, HSV-1, and *H. Pylori* as risk factors for stroke.[10, 11, 64-80] However, most studies have been small, and results from existing studies have been inconsistent. There have been few prospective studies on persistent pathogens and stroke risk, with most data coming from case-control studies.[64-71, 73, 75-80] For instance, investigators using data from the Framingham Heart Study reported no association between seropositivity to *C. pneumoniae, H. Pylori and* CMV infections and incident stroke.[9] In this study, a combined cardiovascular endpoint, including incident MI, fatal CHD and fatal and nonfatal atherothrombotic stroke was used, and 199 total outcome events occurred, however, the number of stroke cases was not reported. Next, in a prospective five year follow-up study of patients with early stages of atherosclerosis, seropositivity to cytotoxic associated gene-A (CAG-A) strains of *H. Pylori (*which is a strain of the bacteria that induces systemic inflammation[7]) was associated with transient ischemic attack (TIA) (p = 0.018) and ischemic stroke (p = 0.046), and seropositivity to CMV was associated with TIA (p = 0.007).[81] However, Elkind et al reported no significant association between seropositivity to 5 infectious pathogens (*C. pneumoniae*, *H. Pylori*, CMV, HSV-1 and HSV-2) and stroke risk, in a multi-ethnic prospective cohort (n = 1625)

with an 8 year (median) follow-up.[82] Most recently, in a five year prospective cohort study, no significant association was found between incident stroke and seropositivity to CAG-A negative or positive strains of *H. Pylori.*[7]

There is evidence that overall pathogen burden versus exposure to particular pathogens may be more important for the promotion of atherosclerosis and therefore, possibly ischemic stroke. Espinola-Klein and colleagues reported an association between pathogen burden, defined as the summed seropositivity to *C. pneumoniae, H. Pylori, Haemophilis influenza, Mycoplasma pneumonaie,* CMV*, EBV* and HSV-1 and 2, and progression of atherosclerosis, as measured by an increase in IMT or the progression of carotid stenosis.[83] This same group also reported an association between pathogen burden and advanced atherosclerosis defined as atherosclerosis in greater than two vascular territories.[84] In a prospective study, persistent pathogens amplified the risk of atherosclerosis (RR = 4.1 for any versus no infection) and the association persisted after adjustment for vascular risk factors.[85]

Although overall pathogen burden may be associated with stroke, this has not been well studied. In one small case-control study no association was detected between overall pathogen burden and stroke risk.[86] Haider et al using data from the Framingham Heart Study reported no association between pathogen burden and risk of a composite cardiovascular endpoint which included stroke.[9] Most recently, researchers from the Northern Manhattan Study reported a positive association between an infectious disease burden index and risk of all strokes after multivariable adjustment (HR=1.39; CI: 1.02-1.90).[8]

The existing studies examining exposure to pathogens and stroke have focused on seropositivity, which may be limited in terms of measuring underlying inflammation. Nazmi et al examined circulating levels of IL-6, CRP and fibrinogen in relation to five pathogens: CMV, HSV-1, Hepatitis A, *H. Pylori* and *C. pneumoniae*.[18] High antibody response to multiple pathogens, including the total number of pathogens with antibody response in the highest quartile, showed graded and significant associations with IL-6 (p-value: <0.001), CRP (p-value: 0.04) and fibrinogen (p-value: 0.001), whereas sero-positive pathogen burden did not.[18] For each pathogen exhibiting a high antibody response, there was a 2-9% increase in inflammatory marker level.[18] The conclusion from this work was that antibody response, which provides data on the extent of immune response to pathogens, may be more closely linked to underlying inflammation and the progression of atherosclerosis.[18]

Persistent pathogen exposure may increase stroke risk, though the exact biologic mechanism has yet to be elucidated. We hypothesize, however, that inflammatory markers, such as IL-6 and TNF, as well as the acute phase-reactant, CRP, may lie on the causal pathway. Exposure to *H. Pylori* causes activation of the innate immune response, or the body's first line of defense against invaders, when lipopolysaccharides on its outer membrane bind to toll like-receptors of endothelial, monocyte and macrophage cells,[87] precipitating damage to the endothelium and the initiation and/or progression of atherosclerosis.[87-89] Further, *H. Pylori* antigens are presented by dendritic cells, which activate both B and T cells, thereby inducing a proinflammatory state.[90] CagA strains of *H. Pylori* are associated with increased levels of systemic inflammation,[91, 92] and have been linked to atherosclerotic changes in arteries, in

retrospective analyses.[56] Further, several periodontal pathogens have been linked to vascular inflammation, with data suggesting that periodontal disease causes increased systemic cytokines as well as CRP, which would promote inflammation and be detrimental to vascular health.[93-95] Inconsistent data exists, however, linking CMV infection to elevated IL-2, IL-4, TNF-α, and interferon-gamma,[96, 97] however, human herpes viruses, in general, have been shown to induce chemokine production.[54] Stroke incidence has also been linked to systemic concentrations of inflammatory markers such as IL-6,[98] as well as baseline CRP levels,[99, 100] however, some studies report only a weak association.[101-104] These data support the hypothesis that inflammatory markers may partially mediate the association between persistent pathogens and stroke risk.

The existing literature on the role of persistent pathogens as well as pathogen burden on incident stroke is limited for several reasons. First, none of the existing studies considered the effect of antibody response to persistent pathogens on stroke risk, rather, they defined pathogen exposure using serostatus, which may not be the best predictor of inflammatory outcomes.[18] Furthermore, Elkind et al, when investigating the effect of pathogen burden on incident stroke risk, used at a weighted index of infection burden, based on parameter estimates from the Cox proportional hazard models containing positive serological test results in relation to stroke versus antibody response to the persistent pathogens to define pathogen burden.[8] To our knowledge, no study to date has elucidated the mechanisms by which exposure to persistent pathogens increases stroke risk. Elkind et al, in a recent prospective cohort study, found no change in the effect estimate for the association between infectious burden and stroke risk, when adjusting for high sensitivity CRP (HR (unadjusted), 1.39; 95% CI,

1.04-1.90; HR (adjusted for demographics and risk factors) 1.39; 95% CI, 1.02-1.90; HR (adjusted for demographics, risk factors and hs-CRP) 1.39; 95% CI, 1.02-1.90).[82] The authors did not, however, focus specifically on the MA population, consider other inflammatory markers, formally assess mediation in their analysis, or use antibody response to operationalize pathogen burden, as we previously discussed.[82]

This dissertation research builds on the existing literature by examining antibody response to several persistent pathogens as risk factors for incident stroke in the MA population, a population known to experience greater stroke and infectious disease burden. Further, we investigated the effect of pathogen burden, as defined as: (1) summed seropositivity to 0-5 pathogens, (2) average IgG antibody level across five pathogens and (3) count sum of the number of pathogens eliciting a high antibody response, on stroke risk. Lastly, this dissertation adds clarification to the literature by investigating the mechanism(s) involved in the associations between persistent pathogens and incident stroke, which has received little attention in the literature.

1.4 Public Health Significance

Stroke is the leading cause of disability and the 4th leading cause of death in the US.[1, 20] MAs have an increased risk of stroke, compared to their NHW counterparts, and this disparity is currently unexplained. Persistent pathogens, some of which are more prevalent in MAs, have been proposed as risk factors for stroke, although prospective studies in diverse populations are lacking. This dissertation research is novel in that the association between persistent pathogens and stroke is investigated using data from a cohort of elderly MAs, a population particularly vulnerable to stroke. In the first specific aim of this dissertation, the associations between IgG antibody levels to five persistent

pathogens and incident stroke were investigated. In aim 2, associations between overall pathogen burden and incident stroke were explored, using novel measures of pathogen burden. In specific aim 3, this dissertation sought to elucidate the possible mechanisms underlying the association between persistent pathogens and incident stroke. Importantly, this dissertation overcomes a large limitation of previous work by focusing on antibody response, which may be a more consistent predictor of inflammatory outcomes but has not yet been studied with respect to stroke.[18] This dissertation research could have important implications for the prevention of stroke in MAs and for reducing the stroke health disparity in the MA population in several ways. First, if exposure to high antibody levels to individual persistent pathogens is shown to increase stroke risk in MAs and is confirmed in other populations, new pharmacological intervention trials aimed at pathogen eradication may be effective at decreasing the burden of stroke in this group. Similarly, if concurrent exposure to several persistent pathogens is shown to increase stroke risk and is replicated in other groups, interventions aimed at decreasing exposure to these pathogenic organisms, especially during childhood, may be effective at decreasing the stroke burden in MAs. Furthermore, by clarifying the mechanisms involved in any associations between persistent pathogens and incident stroke, the dissertation may inform future prospective studies seeking to confirm intermediate variables on the causal chain from persistent pathogens to incident stroke, as well as clinical trials seeking to intervene on the potential mediators. Given the disparities in stroke rates in the MA population, the identification of novel and modifiable risk factors in this subgroup is imperative.

Chapter 2

Persistent Pathogens and Incident Stroke in Mexican Americans

2.1 Introduction

Hispanic Americans make up the largest minority group in the United States, with Mexican Americans (MAs) comprising the largest subgroup.[2] In the United States, MAs are among the fastest growing populations.[2] Stroke incidence in MAs is higher than in Non-Hispanic whites (NHWs), especially at younger ages.[3] Traditional risk factors for stroke are unlikely to fully account for the ethnic difference in stroke incidence.

MAs have a higher prevalence of several persistent infections that have been linked to chronic diseases, including Cytomegalovirus (CMV),[12] *Helicobacter pylori* (*H. Pylori*),[13] *Toxoplasma gondii* (*T. gondii*) [15] and Herpes simplex virus 2 (HSV2).[14] Reactivation of these infections may result in higher levels of systemic inflammation.[5] Inflammation increases stroke risk through several interconnected mechanisms.[6] Persistent pathogens, traditional risk factors, and genetic predisposition may work together in stimulating inflammatory pathways that promote atherosclerosis, which in turn may increase stroke risk and contribute to ethnic differences in stroke.[6]

Unfortunately, there are few prospective studies specifically linking persistent pathogens to incident stroke. Investigators using data from the Framingham Heart Study (n=1,187) reported no association between seropositivity to *C. pneumoniae (C. pneumoniae*), *H. Pylori* and CMV infections and incident stroke.[9] Elkind et al. reported

no significant association between seropositivity to five infectious pathogens (*C. pneumoniae, H. Pylori*, CMV, HSV-1 and HSV-2) and stroke risk, in a multi-ethnic prospective cohort (n = 1625) with an 8 year (median) follow-up.[8] Most recently, in a five year prospective cohort study, no significant association was found between seropostivity to cytotoxin associated A-gene (CAG-A) negative or positive strains of *H. Pylori* and stroke risk.[7]

Importantly, existing studies on the link between persistent pathogens and stroke risk were not focused on the MA population.[3, 12-15] Further, these studies were limited by their use of seropositivity instead of antibody level to infection, which may predict inflammatory outcomes more consistently,[18] and by considering only a small subset of infectious diseases. To build on this earlier research, our objective was to examine the associations between both seropositivity and antibody level to five infectious agents, including previously studied (CMV, HSV1, and *H. Pylori*) as well as novel pathogens (VZV and *T. gondii*), and stroke risk in an elderly cohort of MAs from the Sacramento Area Latino Study on Aging (SALSA). The novel pathogens were included in our analysis because of the potential for reactivation of latent infections, which may produce a chronic inflammatory response.[5]

2.2 Methods

Study population

Our analysis sub-sample (n=1621) included participants from SALSA, a longitudinal study of community-dwelling Latinos residing in the Sacramento Valley of California. At baseline (1998-1999), 1,789 Latino participants who were 60-101 years of age were enrolled. Details of the recruitment and enumeration process, as well as

survey design, have been published previously.[105, 106] Over the course of the 10 year study period, total attrition from deaths (n=452), refusals (n=169) and loss to follow up (n=147) in the cohort averaged 5.4% per year. Attrition due to deaths averaged 3% per year, refusals 1.2% and loss to follow-up 1%. We excluded 168 participants who had a self-reported history of stroke at baseline.

Study Procedures

Study participants underwent a baseline in-home interview and medical exam, where a trained bilingual interviewer collected a fasting serum sample, data on socio-demographics, medical history, medication usage, behavioral risk factors, and clinical, cognitive and functional status (Figure 1). Seven in-home interviews were conducted at 12-15 month intervals, to update the majority of the information collected during the baseline interview. Fasting serum samples were collected at baseline and, during the 3-6th annual in-home interviews. Study subjects also participated in semi-annual telephone interviews (n=6), where information on medical history, medication usage, and demographics was collected. This study was approved by the Institutional Review Boards (IRB) at the University of Michigan, and the University of California, San Francisco and Davis, and all study subjects gave consent.

Exposure Ascertainment

Serum samples were tested at the Stanley Neurovirology Laboratory of the Johns Hopkins University School of Medicine for Immunoglobulin G (IgG) antibody levels to *H. Pylori*, CMV, HSV1, VZV, and *T. gondii*, measured by optical density ratio units, using a commercially available solid-phase enzyme-linked immunosorbent assay (ELISA), with instructions from the manufacturers used to determine categorical

serostatus. Individuals were categorized as sero-negative to CMV, HSV1, *H. Pylori*, and VZV if their absorbance values were ≤ 0.9; equivocal if > 0.9 and < 1.1; and sero-positive if ≥ 1.1.[107] The cut-point for seropositivity to *T. gondii* was 10 international units/ml.[107] For the analysis, pathogen variables were defined using serostatus (positive/negative, with equivocal values categorized as negative) and antibody level, based on IgG antibody levels.

Outcome Ascertainment

Incident strokes were identified from study participants using the following methods. At the baseline visit, participants were asked “Has a doctor ever told you that you had a stroke?". At each subsequent follow-up visit and semi-annual telephone conversation, participants were asked whether a doctor ever told them that they had a stroke or cerebrovascular accident since the last interview. Fatal strokes were identified from death certificates using ICD-10 code 164. Death certificates were obtained on 90.2% (n=414) of those reported to be deceased by their families and/or by vital statistics, and only those stroke deaths confirmed by death certificate were included as outcome events.

Covariates

Variables included in the analysis as potential confounders included hypertension, diabetes, hyperlipidemia, smoking, atrial fibrillation, coronary heart disease, education, age and gender. Two blood pressure measurements were taken using an automatic digital blood pressure monitor (OMRON MODEL: HEM-747 IC). Individuals were categorized as hypertensive (yes/no) if they self-reported a physician diagnosis of hypertension, reported use of anti-hypertensives, or had a sitting systolic

blood pressure of at least 140 mm Hg and/or a diastolic blood pressure of at least 90 mm Hg. Diagnosis of diabetic status (yes/no) was determined by the presence of a serum fasting glucose >125 mg/dl, self-report of physician diagnosis of diabetes, or use of a diabetic medication. Morning fasting serum samples were used to test for total cholesterol using Reagent for Cholesterol (number 3313018; Roche Diagnostics, Indianapolis, IN), low density lipoprotein (LDL) cholesterol, using the LDL Direct Liquid Select (number 7120; Equal Diagnostics) and high density lipoprotein (HDL) cholesterol using the HDL Direct Reagent (number 3034569; Roche Diagnostics, Indianapolis, IN). Hyperlipidemia (yes/no) was defined as: LDL > 100mg/dl and HDL < 40mg/dl or total cholesterol level > 200mg/dl. Smoking status was categorized as ever/never for the analysis. Several variables were used to determine heart disease and/or peripheral artery disease (PAD) status. History of atrial fibrillation (yes/no) was determined by self-reported physician diagnosis within the previous 5 years. Diagnosis of myocardial infarction, angina pectoris, intermittent claudication, congestive heart failure, and heart/coronary catheterization was determined by self-report of any prior physician diagnosis of these conditions. A coronary heart disease/PAD indicator variable was defined as having a history of at least 1 of these conditions versus no history. Years of education was categorized as 0-3, 4-11 and ≥12.

Statistical Approach

Baseline descriptive statistics for socio-demographics as well as prevalence of stroke risk factors were calculated and compared across incident stroke status using χ^2 tests for categorical variables and Wilcoxon rank-sum test for continuous variables. Correlation among the five persistent pathogens was assessed with chi-squared tests of

independence (serostatus) and spearman correlations (antibody levels). Discrete-time logistic regression analyses were performed to investigate the association between seropositivity and antibody level to the pathogens of interest and incident stroke risk using data from 13 discrete time points. We used baseline covariate data and updated with longitudinally collected covariate information when available and relevant. Since not all of the variables of interest were ascertained at semi-annual visits, we used the previous annual visit data when available, to fill in semi-annual information on hypertension, diabetes, hyperlipidemia, pathogen serostatus and antibody level. The number of individuals with missing data for all 5 pathogens at baseline, and at follow-up visits 3-6 was: 523, 1214, 1193, 1499 and 1057, respectively. To justify carrying forward pathogen data from follow-up visits with serum samples to subsequent visits without samples, we assessed agreement between serostatus at baseline and each subsequent follow-up visit, and between each follow-up visit and succeeding visits (Kappa, Gamma, Somers D, Lambda (symmetric and asymmetric), and McNemar's Test); the same comparisons were made for IgG antibody level to each pathogen (intraclass correlation and coefficient of variation). There was good agreement between previous and subsequent serostatus and antibody level to each pathogen under study (see Tables 2.1-2.10).

For the discrete time logistic models, antibody levels for each pathogen were modeled continuously and were re-scaled by dividing by their corresponding interquartile range, to allow us to interpret our regression coefficients as the stroke risk for individuals in the 75^{th} versus the 25^{th} percentile of antibody levels. Two levels of covariate adjustment were used: 1) age, gender, education, and 2) age, gender,

education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia. Interactions between the five pathogens were tested, with the inclusion of product terms in our fully adjusted models. P-values <0.05 were considered significant. All statistical procedures were performed using SAS version 9.2(SAS Institute, Inc., Cary, North Carolina).

2.3 Results

Characteristics of the baseline study population by incident stroke status (n=1621) are shown in Table 2.11. During follow-up 164 incident strokes occurred. The mean age of the study participants was 70.4 (Standard deviation (SD)=7.04), with 41% male. Individuals who experienced an incident stroke were more likely to be older and to have diabetes and hypertension than individuals who did not have an incident stroke during the follow-up. Roughly 47% of the population was born in the United States with most of the participants completing less than a high school education and earning less than 2,000 dollars per month. At baseline, 60.3% of participants were sero-positive to CMV, 65.6% to *H. Pylori*, and 62.5% to HSV1. However, only 20.7% of the participant's sero-positive to VZV and 24.1% were sero-positive to *T. gondii*. Finally, 4.1% of the baseline sample was sero-positive to all five pathogens concurrently.

Seropositivity to each pathogen and antibody levels to *H. Pylori* were associated with baseline history of stroke (data not shown). Table 2.12 presents odds ratios (OR) and 95% confidence intervals (CI) for risk of stroke associated with seropositivity to each of the persistent pathogens. For all pathogens, seropositivity was not significantly associated with increased risk of stroke, either before or after adjustment for covariates. We further analyzed the effect of IgG antibody levels to the 5 pathogens on stroke risk

(Table 2.13). There were no associations between antibody levels to CMV, HSV1, *T. gondii* or VZV and stroke risk before or after adjustment for covariates. However, individuals with high *H. Pylori* antibody level (IgG in the 75th versus 25th percentile) had significantly increased risk of stroke (OR: 1.53; 95% CI: 1.11, 2.12). We present results of the fully adjusted models in Table 2.14. Lastly, we performed a post-hoc sensitivity analysis, limiting the analysis of the effect of antibody level on stroke risk, to individuals who, at baseline, were sero-positive to *H. Pylori* (n=959), comparing 75th versus 25th quartiles and we found similar trends to those reported in Table 2.13 (OR: 1.51; 95% CI: 1.06, 2.15). The association between *H. Pylori* and incident stroke was not statistically significantly modified by antibody levels to CMV, HSV-1, VZV, or *T. gondii* (data not shown). We found no correlations between antibody levels to any of the persistent pathogens, except for between CMV and VZV (p-value: 0.0003). Lastly, given the potential for nativity to impact persistent pathogen exposure, we re-ran all of our fully adjusted models after adding country of birth variables (Mexico, other Latin countries and US, with the later serving as the referent category), and found no change in our risk estimates (data not shown).

2.4 Discussion

In our analysis of an elderly cohort of Latinos residing in Sacramento County, California, we found a significant association between IgG antibody levels to *H. Pylori* and incident stroke in fully adjusted models. In contrast, we found no association between seropositivity to five pathogens and stroke risk, or between IgG antibody levels to CMV, HSV1, *T. gondii*, or VZV and risk of stroke. In cross-sectional analyses, we found significant associations between seropositivity and antibody levels to all five

persistent pathogens, and baseline history of stroke. This finding is likely the result of reverse causation, given the lack of an association in our prospective analyses.

Our findings of null associations between seropositivity to five persistent pathogens and incident stroke are in general agreement with existing prospective studies. Haider et. al using data from the Framingham Heart Study reported no association between seropositivity to *C. pneumoniae*, *H. Pylori* and CMV infections and incident stroke.[9] Elkind et al. also reported no significant association between seropositivity to 5 infectious pathogens (*C. pneumoniae*, *H. Pylori*, CMV, HSV-1 and HSV-2) and stroke risk, in a multi-ethnic prospective cohort (n = 1625) with an 8 year (median) follow-up.[8] Most recently, Schottker, et al., reported no significant association between seropostivity to Cag-A negative or positive strains of *H. Pylori* and stroke risk, in their five-year prospective cohort study (n=9953).[7] In contrast to these studies based on serostatus, our findings pertaining to antibody levels to *H. Pylori* and stroke risk may be the result of our ability to more accurately assess the effect of exposure to this pathogen, given the evidence that antibody level may be a more consistent predictor of inflammatory outcomes.[18]

It is unclear why *H. Pylori* alone was associated with incident stroke. However, there are biologically plausible pathways by which *H. Pylori* may influence incident stroke. Lipopolysaccharides on the outer membrane of *H. Pylori* have been reported to bind to Toll-like receptors on endothelial, monocyte and macrophage cells, which results in endothelial damage and the initiation of atherosclerosis.[87] A retrospective analysis suggests a direct link between *H. Pylori* and atherosclerotic changes in arteries, however, prospective studies have yet to confirm this association.[56] It has also been

reported that *H. Pylori* strains with CagA are more likely to produce a systematic immune response.[91, 92] However, we did not have data on *H. Pylori* strain-type in our cohort, which precluded us from addressing this hypothesis.

There are also several indirect pathways that may link *H. Pylori* to incident stroke. For instance, *H. Pylori* was recently reported to increase diabetes risk in the SALSA cohort, and diabetes is a known stroke risk factor.[61] However, when we adjusted for diabetes status in our analysis, the ORs were not significantly attenuated, suggesting that diabetes does not substantially mediate the *H. Pylori*-stroke association. Another possible pathway linking *H. Pylori* to incident stroke is through folic acid and Vitamin B12, both of which are decreased with exposure to this pathogen, which results in increased serum plasma homocysteine, a known risk factor for cardiovascular disease and stroke.[47, 49-52, 108]

Our study has several strengths. Although earlier studies looked at the effect of persistent pathogens on stroke risk, they all used serostatus in defining exposures. Therefore, our work is the first study to use prospectively collected, population-based data to investigate the link between IgG antibody levels to persistent pathogens and stroke risk in MAs. We used discrete-time regression, which allowed us to analyze time-varying covariates, which may have permitted us to more accurately assess the time-varying effect of pathogen exposure on stroke risk, and to account for changes in risk factors for stroke over time. Next, antibody level to persistent pathogens is thought to be a more specific predictor of inflammation,[18] and our study is the first to prospectively examine its effect on incident stroke in MAs. Finally, our research focused specifically on MAs, a group disproportionately affected by stroke.[3]

The following limitations of the current study should be considered. First, our primary endpoint, incident stroke, was largely based on self-report, and could be under or over-estimated, which, may bias our findings. However, sensitivity rates for self-reported stroke ranging from 80-98% have been reported in the elderly.[109, 110] Next, we could not distinguish between stroke types, which may bias our associations towards the null, as infection is more likely to be associated with ischemic stroke through atherosclerotic mechanisms. Further, additional pathogens, such as *C. pneumoniae* and periodontal pathogens, which have been previously studied for their links to stroke, were not available.[68, 111-114] When we carried forward the risk factor and pathogen data from either baseline or the previous follow-up visit to semiannual visits, we assume that this information is constant, which may not be true for all individuals. Next, the possibility exists, that our multivariable models over-adjusted the associations between pathogens and stroke risk, because some of the variables such as diabetes may lie on the causal pathway of the pathogen-stroke association.[61] However, we did not observe a large attenuation of our ORs in fully adjusted models suggesting this may not be the case. Further, it is possible that individuals with infections caused by *H. Pylori* are more likely to seek medical attention, and as a result, are more likely to have mild strokes diagnosed. Lastly, the generalizability of our study results is limited to elderly MAs. Nevertheless, the MA population suffers disproportionate effects from stroke, which makes identification of novel risk factors in this group particularly important.

2.5 Summary

We report a significant association between IgG antibody levels to *H. Pylori* and risk of stroke in MAs, but no associations between exposure to several other persistent

infections and incident stroke. Further study of *H. Pylori's* effect on stroke risk is warranted, as is the potential for treatment of this pathogen to decrease stroke risk.

Table 2.1. Correlations of CMV serostatus by visit, SALSA, California, 1998-2008.

	BL vs. FV3	BL vs. FV4	BL vs. FV5	BL vs. FV6	FV3 vs. FV4	FV5 vs. Fv6
Gamma	0.9157	0.8754	0.7832	0.8406	0.9147	0.8846
Kappa	0.6662	0.6002	0.5397	0.5615	0.6054	0.6798
Somers D	0.6739	0.6129	0.5316	0.5614	0.7426	0.6872
Lambda*	0.4464	0.4000	0.4211	0.4051	0.4737	0.4571
Lambda†	0.4359	0.4043	0.4026	0.3757	0.4444	0.4507
McNemar's p-value	0.4168	0.2378	0.7851	0.0644	0.5319	0.9643

*Asymmetric; †Symmetric; BL, baseline; FV3-6, follow-up visit 3-6;

CMV: cytomegalovirus; SALSA: Sacramento Area Latino Study on Aging

Table 2.2. Variability of CMV Immunoglobulin G antibody levels by visit, SALSA, California, 1998-2008.

	Intra-class correlation coefficient	Coefficient of Variation
Baseline	0.5135	48.5461
Follow-up visit 3	0.5228	46.3835
Follow-up visit 4	0.5380	49.0630
Follow-up visit 5	0.5146	47.2572
Follow-up visit 6	0.5500	47.8570

SALSA: Sacramento Area Latino Study on Aging; CMV: cytomegalovirus

Table 2.3. Correlations of HSV1 serostatus by visit, SALSA, California, 1998-2008.

	BL vs. FV3	BL vs. FV4	BL vs. FV5	BL vs. FV6	FV3 vs. FV4	FV5 vs. FV6
Gamma	0.9323	0.9159	0.9450	0.9452	0.9496	0.8870
Kappa	0.7008	0.6136	0.6002	0.6992	0.7967	0.6354
Somers D	0.7236	0.6692	0.7209	0.7372	0.8432	0.7036
Lambda*	0.5102	0.3704	0.5185	0.4923	0.6842	0.5172
Lambda†	0.5049	0.3860	0.4310	0.4929	0.6842	0.5345
McNemar's p-value	0.5044	0.3387	0.1059	0.0785	0.9536	0.4980

*Asymmetric; †Symmetric; BL, baseline; FV3-6, follow-up visit 3-6; Gamma: range (-1, 1); Kappa interpretation: (0.0< Kappa >0.4= marginal agreement, 0.4< kappa >0.75= good agreement, Kappa >0.75: excellent agreement; Somers D: range (-1,1); Lambda (asymmetric and symmetric): range (0,1);

Table 2.4.Variability of HSV1 Immunoglobulin G antibody levels by visit, SALSA, California, 1998-2008.

	Intra-class correlation coefficient	Coefficient of Variation
Baseline	0.6065	51.0447
Follow-up visit 3	0.6324	51.7672
Follow-up visit 4	0.5970	51.0291
Follow-up visit 5	0.6630	56.9176
Follow-up visit 6	0.6490	51.4561

HSV1: Herpes simplex virus type 1; SALSA: Sacramento Area Latino Study on Aging

Table 2.5. Correlations of *H. Pylori* serostatus by visit, SALSA, California, 1998-2008.

	BL vs. FV3	BL vs. FV4	BL vs. FV5	BL vs. FV6	FV3 vs. FV4	FV5 vs. FV6
Gamma	0.8920	0.8829	0.7980	0.9190	0.9230	0.8722
Kappa	0.5873	0.4444	0.4081	0.4820	0.7159	0.5783
Somers D	0.6379	0.6305	0.5565	0.7324	0.7581	0.6369
Lambda*	0.3462	0.1923	0.2308	0.2500	0.5714	0.3939
Lambda†	0.3163	0.1538	0.1515	0.1527	0.5641	0.3731
McNemar's p-value	0.2867	0.1038	0.0906	0.0002	0.5319	0.7932

*Asymmetric; †Symmetric; BL, baseline; FV3-6, follow-up visit 3-6; Gamma: range (-1, 1); Kappa interpretation: (0.0< Kappa >0.4= marginal agreement, 0.4< kappa >0.75= good agreement, Kappa >0.75: excellent agreement; Somers D: range (-1,1); Lambda (asymmetric and symmetric): range (0,1); SALSA: Sacramento Area Latino Study on Aging

Table 2.6. Variability of *H. Pylori* Immunoglobulin G antibody level by visit, SALSA, California, 1998-2008.

	Intra-class correlation coefficient	Coefficient of Variation
Baseline	0.7830	50.7843
Follow-up visit 3	0.7857	57.0787
Follow-up visit 4	0.8016	59.2287
Follow-up visit 5	0.7958	61.8262
Follow-up visit 6	0.7958	61.0098

H. Pylori: Helicobacter pylori; SALSA: Sacramento Area Latino Study on Aging

Table 2.7. Correlations of VZV serostatus by visit, SALSA, California, 1998-2008.

	BL vs. FV3	BL vs. FV4	BL vs. FV5	BL vs. FV6	FV3 vs. FV4	FV5 vs. FV6
Gamma	0.4446	0.3900	0.4011	0.4315	0.4147	0.5309
Kappa	0.5492	0.4741	0.4292	0.4678	0.5460	0.5856
Somers D	0.3537	0.2824	0.2960	0.3117	0.3154	0.4192
Lambda*	0.5333	0.3812	0.3697	0.4452	0.5541	0.5556
Lambda†	0.4956	0.3571	0.3451	0.3779	0.5484	0.5200
McNemar's p-value	0.0008	0.0010	0.1701	<0.0001	0.2896	0.1069

*Asymmetric; †Symmetric; BL: baseline; FV3-6:followup visit 3-6; Gamma: range (-1, 1); Kappa interpretation: (0.0< Kappa >0.4= marginal agreement, 0.4< kappa >0.75= good agreement, Kappa >0.75: excellent agreement; Somers D: range (-1,1); Lambda (asymmetric and symmetric): range (0,1); SALSA: Sacramento Area Latino Study on Aging

Table 2.8. Variability of VZV Immunoglobulin G antibody levels by SALSA, California, 1998-2008.

	Intra-class correlation coefficient	Coefficient of Variation
Baseline	0.1903	52.6606
Follow-up visit 3	0.2278	52.1039
Follow-up visit 4	0.1990	51.5351
Follow-up visit 5	0.2199	54.0146
Follow-up visit 6	0.2331	52.4651

VZV: varicella zoster virus; SALSA: Sacramento Area Latino Study on Aging

Table *2.9*. *T. gondii* serostatus correlations by visit, SALSA, California, 1998-2008.

	BL vs. FV3	BL vs. FV4	BL vs. FV5	BL vs. FV6	FV3 vs. FV4	FV5 vs. FV6
Gamma	0.8387	0.8725	0.6834	0.8001	0.8613	0.6993
Kappa	0.7711	0.7913	0.6568	0.7118	0.7475	0.7810
Somers D	0.7143	0.7495	0.5029	0.6423	0.7077	0.6342
Lambda*	0.6988	0.7624	0.5429	0.6505	0.7536	0.7143
Lambda†	0.7069	0.7636	0.5789	0.6490	0.7500	0.7007
McNemar's p-value	0.0587	0.3973	0.1321	0.4461	0.0937	0.1386

*Asymmetric; †Symmetric; BL: baseline; FV3-6: follow-up visit 3-6; Gamma: range (-1, 1); Kappa interpretation: (0.0< Kappa >0.4= marginal agreement, 0.4< kappa >0.75= good agreement, Kappa >0.75: excellent agreement; Somers D: range (-1,1); Lambda (asymmetric and symmetric): range (0,1); SALSA: Sacramento Area Latino Study on Aging

Table 2.10.Variability of *T. gondii* Immunoglobulin G antibody levels by visit SALSA, California, 1998-2008.

	Intra-class correlation coefficient	Coefficient of Variation
Baseline	0.5957	99.0583
Follow-up visit 3	0.6174	103.1960
Follow-up visit 4	0.6016	100.8542
Follow-up visit 5	0.5783	111.7730
Follow-up visit 6	0.6011	100.5119

T. gondii: *Toxoplasma gondii*; SALSA: Sacramento Area Latino Study on Aging

Table 2.11. Baseline Participant Characteristics by Incident Stroke Status, SALSA, California, 1998-2008.

	No Stroke (n=1457)	Incident Stroke (n=164)	P
Characteristic	N (%)	N (%)	
Age (SD)*	70.2 (6.9)	72.6 (7.9)	0.0004
Men	602 (41.3)	71 (43.3)	0.6265
Nativity			0.8955
Mexico	677 (46.5)	71(43.3)	
United States	686 (47.1)	82 (50.0)	
Other	85 (5.8)	10 (6.1)	
Education (years)			0.2878
0-3	464 (31.8)	59 (36.0)	
4-11	552 (37.9)	59 (36.0)	
≥12	432 (29.6)	45 (27.4)	
Smoking Status			0.8795
Ever	769 (52.8)	92 (56.1)	
Never	677 (46.5)	71 (43.3)	
Income			0.7760
<1,000/month	635 (43.6)	70 (42.7)	
1,000-1999/month	441 (30.3)	54 (32.9)	
≥2,000/month	349 (24.0)	37 (22.6)	
Hypertension	928 (63.7)	126 (76.8)	0.0008
Diabetes	433 (29.7)	66 (40.2)	0.0215
Hyperlipidemia	846 (58.1)	92 (56.1)	0.2907
Atrial Fibrillation	74 (5.1)	15 (9.2)	0.0901
Coronary Heart Disease/PAD	260 (17.8)	42 (25.6)	0.0532
Seropositivity			
Cytomegalovirus	882(60.5)	97 (59.2)	0.1889
Helicobacter pylori	959 (65.8)	105 (64.0)	0.4574
Varicella zoster	299 (20.5)	36 (22.0)	0.6192
Herpes simplex virus1	920(63.1)	94 (57.3)	0.3564
Toxoplasma gondii	353 (24.2)	38 (23.2)	0.7506
To all 5 pathogens	56 (3.8)	11 (6.7)	0.0807

*Mean(Standard Deviation (SD)); N: number; SALSA: Sacramento Area Latino Study on Aging

Table 2.12. Seropositivity to Time-Dependent Persistent Pathogens and Incident Stroke, SALSA, California, 1998-2008.

	CMV		HSV1		*T. GONDII*		VZV		*H. PYLORI*	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Model1[*]	1.31	(0.76, 2.25)	0.86	(0.52, 1.42)	1.08	(0.75, 1.56)	1.12	(0.77, 1.61)	1.93	(0.85, 4.40)
Model2[†]	1.12	(0.62, 2.04)	0.82	(0.48, 1.40)	1.13	(0.76, 1.68)	0.91	(0.62, 1.36)	1.56	(0.68, 3.57)
Model3[‡]	1.10	(0.60, 1.98)	0.78	(0.45, 1.34)	1.06	(0.71, 1.58)	0.88	(0.59, 1.31)	1.63	(0.71, 3.76)

CMV, Cytomegalovirus; HSV1, Herpes simplex virus 1; *T. gondii*, *Toxoplasma gondii*; VZV, Varicella Zoster; *H. Pylori*, *Helicobacter pylori*; CI, confidence interval; OR, odds ratio; [*]Model 1: unadjusted; [†]Model 2: age, gender, education; [‡]Model 3: age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia; SALSA: Sacramento Area Latino Study on Aging

Table 2.13. IgG Antibody Levels to Time-Dependent Persistent Pathogens, Comparing 75th versus 25th Quartiles, and Incident Stroke, SALSA, California, 1998-2008.

	CMV		HSV1		*T. gondii*		VZV		*H. Pylori*	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Model1*	0.97	(0.76, 1.23)	0.93	(0.73, 1.18)	1.05	(0.88, 1.26)	1.04	(0.86, 1.26)	1.27	(0.94, 1.72)
Model2†	0.85	(0.65, 1.12)	0.92	(0.71, 1.19)	1.10	(0.91, 1.33)	0.95	(0.76, 1.19)	1.38	(1.00, 1.91)
Model3‡	0.87	(0.66, 1.15)	0.91	(0.70, 1.19)	1.07	(0.88, 1.30)	0.93	(0.75, 1.16)	1.53	(1.11, 2.12)

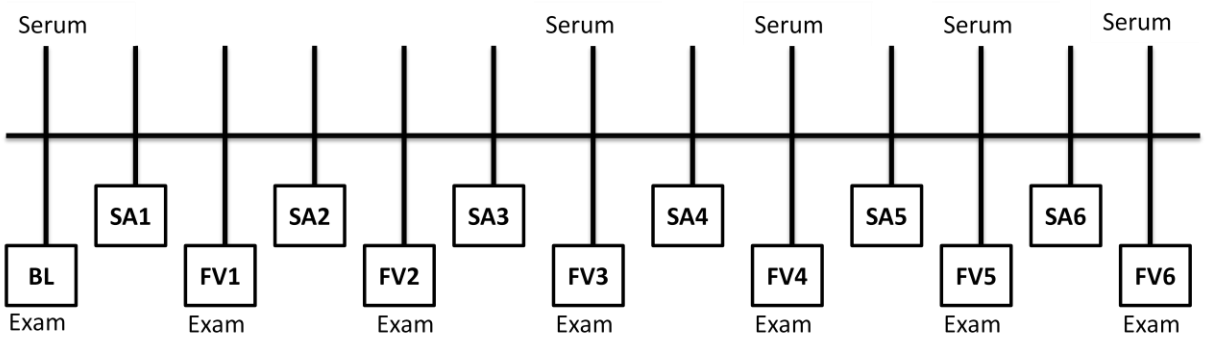
CMV, Cytomegalovirus; HSV1, Herpes simplex virus 1; *T. gondii*, *Toxoplasma gondii*; VZV, Varicella zoster virus; *H. Pylori*, *Helicobacter pylori*; CI, confidence interval; OR, odds ratio; *Model 1: unadjusted; †Model 2: age, gender, education; ‡Model 3:age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia; SALSA: Sacramento Area Latino Study on Aging

Table 2.14. Odds Ratios and Associated 95% Confidence Intervals for Associations Between Covariates and Incident Stroke by Infection Status, SALSA, California, 1998-2008.

	CMV	*H. Pylori*	VZV	*T. gondii*	HSV1
Characteristic	OR (95% CI)	OR (95% CI)*	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age	1.04 (1.01, 1.07)	1.03 (1.01,1.06)	1.03 (1.01, 1.06)	1.03 (1.01, 1.06)	1.03 (1.00, 1.06)
Women	0.85 (0.55, 1.32)	0.84 (0.55, 1.29)	0.81 (0.53, 1.25)	0.81 (0.53, 1.24)	0.81 (0.53, 1.24)
Education (years)					
≥12	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
0-3	1.31 (0.82, 2.10)	1.31 (0.82, 2.11)	1.29 (0.80, 2.07)	1.30 (0.81, 2.08)	1.33 (0.83, 2.12)
4-11	1.28 (0.77, 2.13)	1.21 (0.73, 2.01)	1.22 (0.73, 2.04)	1.22 (0.73, 2.04)	1.27 (0.76, 2.12)
Smoking Status					
Never	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Ever	1.19 (0.78, 1.83)	1.19 (0.78, 1.83)	1.19 (0.77, 1.83)	1.18 (0.77, 1.81)	1.18 (0.77, 1.81)
Hypertension	1.70 (0.77, 3.76)	1.74 (0.79, 3.84)	1.72 (0.78, 3.79)	1.72 (0.78, 3.79)	1.70(0.77, 3.75)
Diabetes	1.40 (0.97, 2.06)	1.38 (0.93, 2.05)	1.41 (0.95, 2.10)	1.40(0.94, 2.06)	1.40(0.94, 2.07)
Hyperlipidemia	0.90 (0.60, 1.34)	0.91 (0.61, 1.36)	0.89 (0.60, 1.33)	0.90 (0.60, 1.35)	0.90(0.60, 1.34)
Atrial Fibrillation	1.53 (0.94, 2.50)	1.59 (0.97, 2.60)	1.53 (0.94, 2.50)	1.52 (0.94, 2.49)	1.56 (0.95, 2.54)
Coronary Heart Disease	3.28 (2.19, 4.93)	3.42 (2.28, 5.13)	3.28 (2.18, 4.92)	3.27 (2.18, 4.91)	3.27 (2.18, 4.91)

CMV, Cytomegalovirus; HSV1, Herpes simplex virus 1; *T. gondii*, *Toxoplasma gondii*; VZV, Varicella Zoster; *H. Pylori*, *Helicobacter pylori*; CI, confidence interval; OR, odds ratio; SALSA: Sacramento Area Latino Study on Aging

Figure 2.1. Data collection details for the Sacramento Area Latino Study on Aging, 1998-2008.



BL: baseline; FV1-6: follow-up visit 1-6; SA1-6: semi-annual telephone interviews

Chapter 3

Pathogen Burden and Incident Stroke in Mexican Americans

3.1 Introduction

Roughly 795,000 people have a stroke each year.[115] Racial/ethnic minority groups, including Mexican Americans (MAs) suffer disproportionately from stroke.[3] MAs also have higher seroprevalence rates of infectious diseases caused by Cytomegalovirus (CMV),[12] *Helicobacter pylori* (*H. Pylori*),[13] *Toxoplasma gondii* (*T. gondii*) [15] and Herpes Simplex Virus Type 2 (HSV-2) than non-Hispanic whites (NHWs).[14] Many of these pathogens are never completely cleared from the host, and as a result, are prone to reactivation, which may cause increased levels of inflammation.[4] If concurrent exposure to these pathogens is related to elevated stroke risk, the increased prevalence in MAs may partially explain the higher stroke risk in this population.

The majority of prospective cohort studies investigating the effect of exposure to individual persistent pathogens on stroke risk have reported null findings.[9, 82] However, in a recent analysis of data from the Sacramento Area Latino Study on Aging (SALSA), we found a significant association between high immunoglobulin G (IgG) antibody levels to *H. Pylori* and incident stroke (Odds Ratio (OR): 1.53, 95% CI: 1.11, 2.12, comparing IgG antibodies in the 75th versus 25th percentiles, unpublished data). To our knowledge, this is the first report of an association between high antibody levels to *H. Pylori* and incident stroke in MAs, using prospectively collected data.

Aside from exposure to individual pathogens, total pathogen burden, or exposure to multiple pathogens concurrently, may have an impact on stroke risk. Pathogen burden may cause increased inflammation, which leads to the promotion and/or worsening of atherosclerosis.[10, 11] However, the existing studies that examined the effect of pathogen burden on incident stroke have had conflicting results. For instance, in one small case-control study, no association was reported for overall pathogen burden and stroke risk.[86] Similarly, researchers using data from the Framingham Heart Study found no association between pathogen burden and risk of a composite cardiovascular endpoint which included stroke.[9] Alternatively, Elkind et. al, using data from the Northern Manhattan Study, reported a positive association between an infectious disease burden index and risk of all strokes after multivariable adjustment (Hazard Ratio (HR):1.39; 95% CI: 1.02, 1.90).[8] None of these studies focused specifically on the MA population, a group that is infrequently studied, and experiences increased stroke risk.[3]

The literature examining pathogen burden and incident stroke using prospectively collected data has defined pathogen exposure with serostatus, which is a dichotomous (positive/negative) variable.[8, 9] However, in recent work, continuous antibody levels to persistent pathogens were better predictors of inflammatory outcomes compared to serostatus.[18] High antibody response to some persistent pathogens may be more reflective of sub-clinical pathogen reactivation.[116, 117] Whether a persistent infection has reactivated or remains latent likely contributes differently toward the development of inflammation, atherosclerosis and therefore incident stroke.

Given the gaps in the literature, our study objective was to examine the association between pathogen burden and incident stroke risk, with pathogen burden operationalized in several novel ways utilizing antibody levels, in an elderly cohort of MAs from SALSA.

3.2 Methods

Study Population

We studied participants from SALSA, which is a longitudinal study of community-dwelling Latinos residing in the Sacramento Valley of California. At baseline (1998-1999), 1,789 Latino participants who were 60-101 years of age were enrolled. Details of the survey design, recruitment and enumeration process have been published elsewhere.[105, 106] Over the course of the 10 year study period, total attrition in the cohort averaged 5.4% per year, which included: deaths (n=452), refusals (n=169) and lost to follow-up (n=147). Deaths averaged 3% per year, refusals 1.2% and loss to follow-up 1%. One hundred sixty-eight participants who had a self-reported history of stroke at baseline were excluded from the analysis.

Study Procedures

Study participants took part in an in-home interview and medical exam at baseline, where a trained bilingual interviewer collected a fasting serum sample, data on socio-demographics, medical history, medication usage, behavioral risk factors, and clinical, cognitive and functional status. Seven in-home interviews were conducted at 12-15 month intervals to update much of the information collected during the baseline interview. In addition to the baseline samples, fasting serum samples were ascertained during the 3rd-6th annual follow-up visits. Study subjects also underwent semi-annual

telephone interviews (n=6), where data on medical history, medication usage, and demographics was collected. This study was approved by the Institutional Review Boards (IRB) at the University of Michigan, and the University of California, San Francisco and Davis, and all study subjects gave consent.

Pathogen Burden

Serum samples were tested at the Stanley Neurovirology Laboratory of the Johns Hopkins University School of Medicine for Immunoglobulin G (IgG) antibody levels to *H. Pylori*, CMV, HSV-1, Varicella Zoster (VZV), and *T. gondii* using a commercially available enzyme-linked immunosorbent assay (ELISA), with serostatus determined using instructions from the manufacturers. Individuals were categorized as sero-negative to CMV, HSV1, *H. Pylori*, and VZV if their absorbance values were ≤ 0.9; equivocal if > 0.9 and < 1.1; and sero-positive if ≥ 1.1.[107] The cut-point for seropositivity to *T. gondii* was 10 international units/ml.[107] For the analysis, pathogen burden was operationalized using the following approaches: (1) summed seropositivity to the 5 pathogens (continuous variable ranging from 0 to 5) for comparison with previous studies, (2) sum of the number of pathogens with high antibody level (continuous variable ranging from 0 to 5), defined as IgG antibody response in the top quartile of the distribution for the pathogen, and (3) average IgG antibody level across all 5 pathogens, including those individuals sero-positive to at least one pathogen, with a weight created that accounted for high antibody response to *H. Pylori* given the previous finding in this cohort of a significant association between this pathogen and stroke risk. Since the pathogens are measured on different scales, z-scores were calculated for each pathogen, at each time point, before taking the average.

Stroke Ascertainment

Incident stroke was identified from study subjects using the following methods. At the baseline visit, each participant was asked “Has a doctor ever told you that you had a stroke?". At each subsequent follow-up visit and during semi-annual telephone conversations, participants were asked “Since we last interviewed you on [DATE], has a doctor ever told you had a stroke or cerebrovascular accident?” Fatal strokes were identified from death certificates using ICD-10 code I64. Death certificates were obtained on 90.2% (n=414) of those reported deceased by family and/or vital statistics, and only those stroke deaths verified by death certificate were included as events.

Covariates

Variables included in the analysis as potential confounders included hypertension, diabetes, hyperlipidemia, smoking, atrial fibrillation, coronary heart disease and/or peripheral artery disease (PAD), education, age and gender. Two blood pressure measurements were taken using an automatic digital blood pressure monitor (OMRON MODEL: HEM-747 IC). Study subjects were categorized as hypertensive (yes/no) if they reported a physician diagnosis of hypertension, reported use of anti-hypertensives, or had a sitting systolic blood pressure of at least 140 mm Hg and/or a diastolic blood pressure of at least 90 mm Hg. Diagnosis of diabetic status (yes/no) was determined by the presence of a serum fasting glucose >125 mg/dl, self-report of physician diagnosis of diabetes, or use of a diabetic medication. Fasting serum samples were used to test for total cholesterol, low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. Hyperlipidemia (yes/no) was defined as LDL > 100mg/dl and HDL < 40mg/dl or total cholesterol level > 200mg/dl and/or use of statins.

Smoking status was categorized as ever/never for analysis, since only 11.10% of the study population were current smokers (n=180). History of atrial fibrillation (yes/no) was determined by self-reported, physician diagnosis within the previous 5 years. Diagnosis of myocardial infarction, angina pectoris, intermittent claudication, congestive heart failure, and heart/coronary catheterization was determined by self-report of physician diagnosis for these conditions. A coronary heart disease variable was defined as having a history of at least 1 of these conditions versus no history. Years of education was categorized as 0-3, 4-11 and ≥12.

Statistical Approach

Baseline descriptive statistics for socio-demographics and risk factors for stroke were calculated and compared across incident stroke status using χ^2 tests for categorical variables and Wilcoxon rank-sum test for continuous variables. Discrete-time logistic regression was used to investigate the association between pathogen burden and incident stroke risk using data from 13 discrete time points. Baseline data was used for the analysis, with updated longitudinally collected covariate information, if available. Since not all of the variables of interest were ascertained at semi-annual visits, we used the previous visit data to fill in semi-annual information on hypertension, diabetes, hyperlipidemia, pathogen serostatus, and antibody level.

The number of individuals with missing information for all 5 pathogens of interest at baseline and follow-up visits 3-6 were: 523, 1214, 1193, 1499 and 1057, respectively. To justify carrying forward pathogen data from follow-up visits to the semi-annual visits, we conducted tests of agreement comparing serostatus at baseline and each subsequent follow-up visit, and for each follow-up visit with succeeding visits (Kappa,

McNemar's Test); the same comparisons were made for IgG antibody level to each pathogen (intraclass correlation and coefficient of variation). There was good agreement between previous and subsequent serostatus and antibody response to each pathogen under study (see Tables 2.1-2.10).

The average IgG antibody level variable was re-scaled by dividing it by the corresponding interquartile range, to allow the comparison of stroke risk for the 75th versus the 25th percentile of average antibody levels. Likelihood ratio tests were performed to determine the appropriate functional form of pathogen burden variables. Comparisons were made between models with continuous and dummy variables for both summed seropositivity to 0-5 pathogens and sum of the number of pathogens with high antibody level (range 0-5). Based on these analyses, variables for summed seropositivity and sum of the number of pathogens with high antibody level were modeled continuously. Models were adjusted for: (1) age, gender, education and (2) age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia. All statistical procedures were performed in SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

3.3 Results

Table 3.1 presents the baseline characteristics of the study participants, by incident stroke status (n=1621). Individuals with an incident stroke during follow-up were older at baseline than those without incident stroke (72.6 versus 70.2 years, p-value: 0.0004). Nearly 60% of the study population was female. Study participants who experienced an incident stroke during follow-up, were more likely to report hypertension and diabetes at baseline (p-value: 0.0008 and 0.0215, respectively). Seropositivity to

each individual pathogen was highest for *H. Pylori* (66%), HSV1 (63%) and CMV (60%), with the prevalence of both VZV and *T. gondii* falling below 25%. There was no difference in individual pathogen exposure by incident stroke status. We also observed higher average IgG antibody levels at baseline among those with an incident stroke during follow-up (p-value: 0.0095).

Table 3.2 shows the baseline distribution of pathogen burden defined as summed seropositivity to 1-5 pathogens, by incident stroke status. The percentage of individuals who were concurrently sero-positive to 1-5 pathogens was similar among those who did and did not have an incident stroke during follow-up (p-value: 0.4942). Table 3.3 shows the sum of the number of pathogens eliciting a high antibody level by incident stroke status. Here, no difference was observed by incident stroke status (n=0.1957), and 16.7% of the study participants had zero pathogens eliciting a high antibody level. The majority of the study population had high antibody levels to only one pathogen (30.8%), followed by those individuals with two (15.9%), three (7.2%) and four or five (1.2%) pathogens concurrently eliciting a high antibody level.

The risk of stroke associated with summed seropositivity to 5 pathogens and with the number of pathogens eliciting high antibody levels is shown in Table 3.4. We observed a positive association between summed seropositivity to the 5 pathogens, however, the association was not significant before or after adjustment for covariates. We found a positive association between sum of the number of pathogens eliciting high antibody response and stroke risk (OR: 1.18, 95% CI: 1.04, 1.35), however, attenuation and loss of statistical significance occurred after covariate adjustment.

In table 3.5, we present the association between incident stroke and pathogen burden defined as average antibody levels in individuals sero-positive to at least one pathogen (with all 5 pathogens carrying the same weight and with *H. Pylori* weighted higher than the other 4 pathogens). There were positive associations between average antibody level and stroke risk among those sero-positive to at least one pathogen, in both weighted and unweighted analyses, but these results were not statistically significant. Lastly, given the potential for nativity to impact persistent pathogen exposure, and thus, pathogen burden, we re-ran all of our fully adjusted models after adding country of birth variables (Mexico, other Latin countries and US, with the later serving as the referent category), and our risk estimates were unchanged (data not shown).

3.4 Discussion

From our analysis of an elderly cohort of Latinos participating in the SALSA study, we found a positive association between pathogen burden defined as the sum of the number of pathogens eliciting antibody levels in the fourth quartile (Odds Ratio (OR): 1.18, 95% CI: 1.04, 1.35), however, the association was attenuated and statistical significance was lost after multivariable adjustment. Null associations were present between incident stroke and pathogen burden operationalized as summed seropositivity to 5 pathogens as well as average antibody level, in fully adjusted models.

After adjusting for socio-demographics (age, gender, and education), the association between the number of pathogens producing a high antibody level and incident stroke was attenuated and no longer significant (OR: 1.11; 95% CI: 0.96, 1.30). Similarly, in fully adjusted models, the association between this measure of pathogen

burden and incident stroke was further reduced (OR: 1.09; 95% CI: 0.94, 1.27). Given the attenuation in stroke risk from unadjusted to the fully-adjusted models, it is possible that one or more of our adjustment variables lies on the causal pathway linking this measure of pathogen burden to incident stroke. For example, inflammation may partially explain the association of pathogen burden on incident stroke. Inflammatory cytokines are associated with cerebral blood vessel constriction,[118-120] which links inflammation to hypertension, a predominant modifiable risk factor for stroke.[121-125] Furthermore, pathogen burden, including sero-prevalence to *C. pneumoniae, mycoplasma pneumoniae, H. Pylori* and Coxsackie virus, have been associated with hypertension.[126] Given these data, the idea that exposure to more than one pathogen increases systemic inflammation and hypertension risk, thereby increasing incident stroke, is conceivable. The development of diabetes mellitus, an established stroke risk factor, is also predicted by inflammatory markers, such as white blood counts, CRP and interleukin-6 (IL-6).[127-129] The inflammatory pathway that is induced by pathogen burden could increase diabetes risk, especially given the recent report of an association between *H. Pylori* and incident diabetes in the SALSA cohort,[61] and as a result, increase stroke risk, especially in the MA population, given the disproportionate burden of diabetes in this subgroup.[115] The interplay between pathogen burden, inflammation and incident stroke is complex, and requires further study to clarify the potential mechanisms involved.

Our report of no association between pathogen burden and incident stroke after multivariable adjustment is in agreement with the majority of existing studies. For instance, in one case-control study of 59 ischemic stroke cases and 59 controls, Kis et

al. reported no significant differences in pathogen burden between stroke cases and controls (p-value: 0.657).[86] Similarly, investigators using data from the Framingham Heart study (n=1,187), reported no statistically significant associations between concurrent seropositivity to CMV, *H. Pylori,* or *C. pneumoniae* and a pooled endpoint of incident cardiovascular disease that included atherothrombotic stroke, myocardial infarction, and coronary heart disease deaths (HR: 0.77, 95% CI: 0.44, 1.35).[9] Alternatively, in an 8 year, multi-ethnic prospective cohort study (n=1625), Elkind et al. reported a significant positive association between a weighted index of pathogen burden (including *H. Pylori, C. pneumoniae*, CMV, Herpes simplex virus 1 and 2) and incident stroke (HR: 1.39, 95% CI: 1.02-1.90).[82] The infectious disease burden index used in this analysis was based on summed parameter estimates from models containing individual sero-positive pathogen exposures. Comparisons across these studies with different study populations, exposure and outcome definitions, as well as variables included in multivariable adjustment, is difficult. It is possible that the studies reporting null associations, including ours, were biased towards the null due to adjustment for factors on the causal pathway as described above. It is also possible that in order to detect significant associations between pathogen burden and incident stroke, larger sample sizes are required, especially given the relatively small effect sizes reported in this and previous studies

Our study has several strengths that distinguish it from the existing literature. To our knowledge, we are the first to analyze IgG antibody levels to five persistent pathogens for associations with incident stroke, using longitudinally collected data. Given our study design, we were able to establish temporality between pathogen

exposures and stroke. Discrete-time logistic regression was used for our analysis, which allowed us to account for the potential time-dependency of stroke risk factors and other potential confounders in our analysis. Next, we operationalized pathogen burden several different ways, including summed seropositivity, sum of the number of pathogens eliciting high antibody levels, and average IgG immune response to persistent pathogens, (including weighted and un-weighted analyses). As a result, we were able to comprehensively examine the effect of pathogen burden on stroke risk. Our study is the first to focus specifically on MAs in examining the link between exposure to more than one pathogen at a time and incident stroke, which is important given the disproportionate stroke burden in this group.[3]

There are limitations to the current work that should be considered. First, our primary endpoint was self-reported physician diagnosis of incident stroke, and may be under or over-estimated, potentially biasing our findings. However, self-reported stroke rates in the elderly have reported sensitivity rates of 80-98%.[109, 110] Next, we could not distinguish between stroke types, and our associations may be biased towards the null, since infection is more likely to be associated with ischemic stroke through an atherosclerotic pathway. Further, additional pathogens, such as *C. pneumoniae* and periodontal pathogens, which have been previously studied for their links to stroke, were unavailable for analysis in our study.[68, 111-114] Next, when we operationalized pathogen burden, we assumed biologic interaction between the persistent pathogens was absent, which may not be the true for all potential combinations of pathogen exposures. As a result, our results do not improve upon our understanding which specific combination(s) of pathogens exerts the most detrimental effect on stroke risk.

Next, when we carried forward the risk factor and pathogen data from either baseline or the previous follow-up visit to semiannual visits, we assume that this information is constant, which may not apply to all individuals. It is also possible, that our multivariable models adjusted for mediators, because some of the variables such as hypertension and diabetes may lie on the causal pathway. We also did not adjust for multiple comparisons in our analyses. Lastly, the generalizability of our study results is limited to elderly MAs with similar risk factor and exposure histories. Nevertheless, it is important to identify novel risk factors in the MA population given their disproportionate stroke burden.

3.5 Summary

In our 10 year prospective cohort, we report no significant associations, in fully-adjusted models, between pathogen burden operationalized as (1) summed seropositivity and (2) average immune response to infection and (3) sum of the number of pathogens eliciting a high antibody level, and incident stroke in an elderly MAs residing in Sacramento County, California.

Table 3.1. Baseline Participant Characteristics by Incident Stroke Status, SALSA, California, 1998-2008.

	No Stroke (n=1457)	Incident Stroke (n=164)	P
Characteristic	N (%)	N (%)	
Age (SD)*	70.2 (6.9)	72.6 (7.9)	0.0004
Men	602 (41.3)	71 (43.3)	0.6265
Nativity			0.8955
Mexico	677 (46.5)	71(43.3)	
United States	686 (47.1)	82 (50.0)	
Other	85 (5.8)	10 (6.1)	
Education (years)			0.2878
0-3	464 (31.8)	59 (36.0)	
4-11	552 (37.9)	59 (36.0)	
≥12	432 (29.6)	45 (27.4)	
Smoking Status			0.8795
Ever	769 (52.8)	92 (56.1)	
Never	677 (46.5)	71 (43.3)	
Income			0.7760
<1,000/month	635 (43.6)	70 (42.7)	
1,000-1999/month	441 (30.3)	54 (32.9)	
≥2,000/month	349 (24.0)	37 (22.6)	
Hypertension	928 (63.7)	126 (76.8)	0.0008
Diabetes	433 (29.7)	66 (40.2)	0.0215
Hyperlipidemia	904 (62.0)	98 (59.8)	0.7032
Atrial Fibrillation	74 (5.1)	15 (9.2)	0.0901
Coronary heart disease/PAD	260 (17.8)	42 (25.6)	0.0532
Pathogen Status			
Seropositivity			
Cytomegalovirus	882(60.5)	97 (59.2)	0.1889
Helicobacter pylori	959 (65.8)	105 (64.0)	0.4574
Varicella zoster	299 (20.5)	36 (22.0)	0.6192
Herpes simplex virus1	920(63.1)	94 (57.3)	0.3564
Toxoplasma gondii	353 (24.2)	38 (23.2)	0.0807
Average IgG Antibody Level*	0.793 (0.3)	0.796 (0.4)	0.0095

*Mean(Standard Deviation (SD)); N: number; P: p-value; SALSA: Sacramento Area Latino Study on Aging

Table 3.2. Baseline Pathogen Burden Defined As Summed Seropositivity By Incident Stroke Status, SALSA, California, 1998-2008.

	No Stroke (n=1457) N (%)	**Incident Stroke (n=164) N (%)**	**P**
Summed Seropositivity			0.4942
Missing	405 (27.8)	52 (31.7)	
1 pathogen	27 (1.9)	2 (1.2)	
2 pathogens	144 (9.9)	16 (9.8)	
3 pathogens	440 (30.2)	47 (28.7)	
4 pathogens	368 (25.3)	34 (20.7)	
5 pathogens	56 (3.8)	11 (6.7)	

N: number; P: p-value; SALSA: Sacramento Area Latino Study on Aging

Table 3.3. Baseline Pathogen Burden Defined as Number of Pathogens Eliciting High Antibody Levels by Incident Stroke Status, SALSA, California, 1998-2008.

	No Stroke (n=1457) N (%)	Incident Stroke (n=164) N (%)	P
High IgG Antibody Level			0.1957
Missing	405 (27.8)	52 (31.7)	
0 pathogens	243 (16.7)	28 (17.1)	
1 pathogen	458 (31.4)	41 (25)	
2 pathogens	224 (15.4)	34 (20.7)	
3 pathogens	108 (7.4)	8 (4.9)	
4 or 5 pathogens	19 (1.3)	1 (0.6)	

P: p-value; n: number; IgG: immunoglobulin G; SALSA: Sacramento Area Latino Study on Aging

Table 3.4. Pathogen Burden Defined as Summed Seropositivity and Sum of the Number of Pathogens Eliciting High Antibody Levels, and Stroke Risk, SALSA (n=1621), California, 1998-2008.

	Model 1[a]		Model 2[b]		Model 3[c]	
	OR	95% CI	OR	95% CI	OR	95% CI
Summed Seropositivity (0-5)	1.10	(0.91, 1.33)	1.05	(0.86, 1.29)	1.01	(0.82, 1.23)
Number of Pathogens Eliciting High Antibody Response (0-5)	1.18	(1.04, 1.35)	1.11	(0.96, 1.30)	1.09	(0.94, 1.27)

OR, Odds Ratio; CI, Confidence Interval; [a]Model 1: unadjusted; [b]Model 2: age, gender, education; [c]Model 3: age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia; SALSA: Sacramento Area Latino Study on Aging

Table 3.5. Pathogen Burden Defined as Average Antibody Level to Persistent Pathogens (weighted and unweighted) and Stroke Risk, SALSA, (n=1621), California, 1998-2008.

	Model 1[a]		Model 2[b]		Model 3[c]	
	OR	95% CI	OR	95% CI	OR	95% CI
Average Antibody Level	1.19	(0.90, 1.57)	1.07	(0.78, 1.46)	1.02	(0.75, 1.40)
Weighted Average Antibody Level	1.26	(0.90, 1.76)	1.12	(0.77, 1.62)	1.07	(0.74, 1.54)

OR, Odds Ratio; CI, Confidence Interval; [a]Model 1: unadjusted; [b]Model 2: age, gender, education; [c]Model 3: age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia; SALSA: Sacramento Area Latino Study on Aging

Chapter 4

H. Pylori and Incident Stroke: Potential Mediating Mechanisms

4.1 Introduction

We previously found a significant association between high immunoglobulin G (IgG) antibody levels to *Helicobacter pylori (H. Pylori)*, and increased risk of stroke, in an elderly cohort of Mexican Americans (MAs) from the Sacramento Area Latino Study on Aging (SALSA) (OR: 1.53, 95% CI: 1.11, 2.12, comparing 75th versus 25th percentiles). To the best of our knowledge, this was the first report of an association using prospectively collected data, between high antibody levels to *H. Pylori* and incident stroke in this population. In contrast to this finding, the two existing prospective studies examining the effect of *H. Pylori* serostatus on incident stroke have reported null findings.[7-9]

The exact mechanism(s) by which high antibody levels to *H. Pylori* may increase stroke risk is unknown. However, it is plausible that the association is, at least, partially mediated by diabetes, elevated systolic and/or diastolic blood pressure, inflammatory markers and/or homocysteinemia. In a recent analysis using the SALSA cohort, Jeon et al reported a significant increase in diabetes risk, among individuals sero-positive to *H. Pylori* (hazard ratio (HR): 2.69; 95% CI: 1.10-6.60).[61] These results were in agreement with two cross-sectional studies that reported a significant association between *H. Pylori* and diabetes prevalence,[130, 131] however, other studies have failed to report such an

association.[132-134] Given the newly reported link between diabetes and *H. Pylori* in a prospective study, and the fact that diabetes is an established stroke risk factor,[115] an investigation into the potential mediating effect of diabetes on the *H. Pylori*-incident stroke association is warranted.

The literature on the association between *H. Pylori* exposure and hypertension is scarce. In one small case-control study conducted in Rome (n=142), blood pressure was significantly decreased among hypertensive patients after *H. Pylori* eradication.[135] In a population-based, cross-sectional analysis using data from the Bristol Helicobacter Project (n=10,537), significantly increased mean systolic blood pressure was reported for those infected with *H. Pylori*.[136] Hypertension is also the most potent stroke risk factor,[1] which makes its mediating effect of *H. Pylori* on incident stroke, plausible.

Another possible mechanism linking *H. Pylori* to incident stroke is through inflammation. Gram negative bacteria, such as *H. Pylori*, have lipopolysaccharides on their outer membrane that bind to toll-like receptors of endothelial, monocyte and macrophage cells.[87] This binding precipitates damage to the endothelium and the initiation and/or progression of atherosclerosis.[87-89] Furthermore, cytotoxin associated gene-A strains (CagA) of *H. Pylori* are associated with increased levels of systemic inflammation,[91, 92] and have been linked to atherosclerotic changes in arteries, in retrospective analyses.[56] Stroke incidence has also been linked to systemic concentrations of inflammatory markers such as interleukin-6 (IL-6),[98] and baseline C-reactive protein levels (CRP).[99, 100] These data support the hypothesis that inflammatory markers may partially mediate the association between *H. Pylori* and stroke risk.

Finally, decreased folate and Vitamin B12 levels, and the resulting elevation of serum homocysteine, has been associated with infections caused by *H. Pylori*.[49] Furthermore, in a German cohort, decreased levels of Vitamin B12 and folate were associated with increased risk of ischemic stroke (relative risk (RR), 2.24; 95% CI, 1.10-4.54).[137] Elevated total homocysteine levels have also been associated with ischemic stroke in several studies.[138-140] As a result, it is plausible that folate, Vitamin B12 and/or homocysteine mediate the association between *H. Pylori* and incident stroke.

If the association between *H. Pylori* and incident stroke is corroborated in future studies, and the association is found to be partially mediated by diabetes, elevated blood pressure, inflammatory outcomes and/or folate, Vitamin B12, and homocysteine, pharmacological interventions for both *H. Pylori* as well as the potential mediators may be possible, which could decrease stroke burden. Our objective was to assess the mediating effects of diabetes, blood pressure, inflammatory markers (IL-6, CRP and tumor necrosis factor (TNF)), folate, Vitamin B12, and homocysteine, on the *H. Pylori*-stroke association, using a population-based cohort of elderly MAs from the SALSA study (Figure 4.1).

4.2 Methods

Study Population

The original SALSA cohort included 1,789 community dwelling Latinos, 60-101 years old who resided in California's Sacramento Valley from 1998-2008. The recruitment, participant enumeration and study design details have been previously published.[105, 106] From the original cohort, we excluded 168 individuals who had a history of stroke at baseline. Thus, our analysis sub-sample included 1,621 individuals,

which represented 90.4% of the baseline cohort. Total attrition in the original cohort averaged 5.4% per year, and included attrition from death (n=425), refusals (n=169) and loss-to-follow-up (n=147).

Study Procedures

At the start of the 10 year follow-up period, an in-home baseline interview was performed, where a trained bilingual interviewer obtained a fasting serum sample, and collected data on socio-demographics, medical history, medication usage, behavioral risk factors, as well as data on clinical, cognitive and functional status. Seven annual in-person interviews were performed at 12-15 month intervals, in order to update data collected at the baseline interview, if applicable. Fasting serum samples were collected at baseline, and during the 3rd-6th follow-up visits. At six month intervals, semiannual visits were conducted (n=6), for the purpose of updating data collected during the annual visits. This study was approved by the Institutional Review Boards (IRB) at the University of Michigan, and the University of California, San Francisco and Davis, and all study subjects gave consent.

***H. Pylori* Ascertainment**

During the baseline interview, as well as during follow-up visits 3-6, serum samples were collected and frozen, and were tested at the Stanley Neurovirology Laboratory of the Johns Hopkins University School of Medicine for Immunoglobulin G (IgG) antibody levels to *H. Pylori*, using optical density ratio units, from a commercially available solid-phase enzyme-linked immunosorbent assay (ELISA).

Potential Mediators

Frozen (-70˚ C), fasting serum or whole blood samples were used to determine inflammatory marker, glucose, folate, homocysteine, and Vitamin B12 levels, with measurement performed at the University of Michigan. High sensitivity CRP levels were assessed from serum samples collected during the baseline interview and follow-up visits 3-6, using a Genzyme Diagnostics kit (formerly Equal Diagnostics), which is wide range CRP assay that measures 0-160 mg/L. TNF and IL-6 levels were both only available from serum collected during the baseline interview and follow-up visit 4 and 5. TNF and IL-6 levels were quantified using a chemiluminescent immunoassay (R&D Systems, Q60000B). Glucose levels were assessed using serum from the following study points: baseline, annual visit 1, and follow-up visit 3-6, and were determined with the Cobas Mira Chemistry Analyzer assay (Roche Diagnostics, Indianapolis, IN, USA). Total plasma Vitamin B12 and folate concentrations were determined by radioassay (Quantaphase II; BioRad Diagnostics, Hercules, CA). Total plasma homocysteine was quantified by high performance liquid chromatography (HPLC) with post-column fluorescence detection. Only baseline serum samples were tested for Vitamin B12, folate and homocysteine levels. During medical exams conducted every 12-15 months, blood pressure measurements were performed twice, using an automatic digital blood pressure monitor (OMRON MODEL: HEM-747 IC).

Stroke Ascertainment

At baseline, each follow-up visit, and during semi-annual telephone conversations, incident stroke status was ascertained from study subjects, who were asked if a doctor ever told them that they had a stroke since their last interview. Death certificates were searched for ICD-10 code 164 to verify fatal strokes. Of the study subjects reported deceased by family and/or vital statistics, death certificate verification

occurred in 90.2% (n=414) of participants. Fatal strokes verified with a death certificate were counted as incident strokes.

Covariates

Only sociodemographic variables (age, gender and education) were included in multivariable analyses as potential confounders, given the potential for traditional stroke risk factors to affect the pathways of interest. Age was self-reported at each annual and semi-annual visit. Gender and years of education were ascertained at the baseline interview only.

Statistical Approach

Statistical analyses were performed with SAS version 9.2 (SAS Institute, Inc., Cary, NC). The analysis dataset included 13 discrete-time points, including a baseline interview, six annual visits and six semi-annual telephone interviews. We used baseline covariate data and updated with longitudinally collected covariate information when available and relevant. Since not all of the variables of interest were ascertained at semi-annual visits, we used the previous annual visit data when available, to fill in semi-annual information on systolic and diastolic blood pressure, glucose, CRP, IL-6, TNF and *H. Pylori* antibody level. Since Vitamin B12, folate and homocysteine, were only collected at baseline, we carried these data forward to fill in the remaining 12 time points. The number of individuals with missing data for *H. Pylori* antibody levels at baseline, and at follow-up visits 3-6 was: 523, 1214, 1193, 1499 and 1057, respectively. To justify carrying forward pathogen data from follow-up visits with serum samples to subsequent visits without samples, we assessed agreement between IgG antibody to *H. Pylori* levels using intraclass correlation and coefficient of variation. There was good

agreement between previous and subsequent IgG antibody level to *H. Pylori* (see Tables 2.1- 2.10).

Baseline descriptive statistics for participant characteristics were compared across incident stroke status using χ^2 tests for categorical variables and Wilcoxon rank-sum test for continuous variables. Multivariable linear and logistic models including age, gender, and education were used to assess potential mediation for each candidate mediator (glucose, systolic and diastolic blood pressure, folate, homocysteine, Vitamin B12, CRP, IL-6 and TNF), separately, with each mediator modeled as a continuous variable. Potential mediators (each modeled continuously) of the *H. Pylori*-stroke association were investigated, using criteria based on the Baron and Kenny method.[141, 142] Given our previous finding of a significant association between high antibody levels to *H. Pylori* and incident stroke, the Baron and Kenny criteria were adapted to assess the following relationships: (1.) Does *H. Pylori* significantly affect each potential mediator? (2.) Does each potential mediator have a significant effect on incident stroke? and (3.) Does the effect of *H. Pylori* on incident stroke attenuate upon addition of each potential mediator to the model? We performed a bootstrap re-sampling technique, using the Preacher and Hayes method, in which, the difference between the total and direct effects of *H. Pylori* on incident stroke was calculated, in an effort to approximate the indirect effect through each potential mediator as well as associated 95% confidence intervals.[143-145] One-thousand re-samples of our study cohort were taken, with replacement, to generate sampling distributions. It is possible that chronic diseases such as diabetes and hyperlipidemia may be aggressively treated following diagnoses, thereby decreasing their potential impact on inflammatory processes. Similarly,

treatments for hypertension would affect systolic and diastolic blood pressure, and hinder our ability to assess potential mediation of the *H. Pylori*- incident stroke association, by blood pressure. As a result, we performed mediation analyses restricted to individuals without a history of statin usage (for inflammatory markers), diabetes medication usage (for glucose as a potential mediator), and medications used to treat hypertension (for systolic and diastolic blood pressure as a potential mediator).

4.3 Results

Table 4.1 shows descriptive statistics for the study population (n=1621) by incident stroke status. The study population had a mean age of 70.4 years (standard deviation (SD) =7.04), and over half were women. Approximately half of the cohort was born in Mexico, and most had not attained a high school education, and earned less than 2,000 dollars per month. Individuals who experienced an incident stroke during follow-up were older, more likely to have diabetes and hypertension, as well as elevated IL-6, homocysteine, and systolic blood pressure levels.

In Table 4.2 we present the regression models for the effect of *H. Pylori* on six potential mediators (step 1 of Baron and Kenny relational criteria for mediation).[143, 145] We found a significant association between *H. Pylori* and CRP (p-value: 0.0004), homocysteine (p-value: 0.0356), and folate (p-value: 0.0006), in socio-demographic adjusted models. However, there were no significant associations between *H. Pylori* and: IL-6, TNF, or Vitamin B12. Table 4.3 shows the associations between six potential mediators and incident stroke (step 2 of Baron and Kenny criteria for mediation). We found no significant effects of inflammatory markers, folate, Vitamin B12 or homocysteine on incident stroke. In our full sample, none of the candidate mediators

met the 1st and 2nd criteria for mediation, and as a result, the final step in our mediation analysis was not performed.

In table 4.4, we present results of our analysis of the mediating effect of glucose on the *H. Pylori*-incident stroke association, among individuals with no self-reported history, during follow-up, of diabetes medication usage. In these analyses, the 1st and 2nd criteria for mediation were not met. Table 4.5 depicts the associations between *H. Pylori* and inflammatory markers (CRP, TNF, and IL-6), among individuals with no history of statin usage during follow-up (step 1 of Baron and Kenny criteria for mediation). We found a significant association between *H. Pylori* and CRP (p-value: 0.0415), however, no significant associations were present for *H. Pylori* and IL-6 or TNF. In Table 4.6, we present no significant associations between IL-6 and TNF and incident stroke among those reporting no history of statin use, during follow-up (step 2 of Baron and Kenny's criteria for mediation). However, the association between CRP and incident stroke approached statistical significance (p-value: 0.1755). Given the null results for the 1st and 2nd relational criteria assessing mediation of the *H. Pylori*-incident stroke association by IL-6 and TNF, among study participants with no history of statin usage, the 3rd step of Baron and Kenny's criteria for mediation was not warranted for these mediators. On the other hand, given the significant association between *H. Pylori* and CPR, and the association between CRP and incident stroke that approached statistical significance, we performed the 3rd step of the mediation analysis for CRP. Table 4.7 depicts the indirect effect of *H. Pylori* on incident stroke through CRP. We found a null association between *H. Pylori* and incident stroke through CRP, as evidenced by the 95% bootstrap confidence intervals that included zero. In table 4.8, we

present the effect of *H. Pylori* on blood pressure, among those without a history of medications used to treat hypertension during follow-up. We found a significant effect of *H. Pylori* on diastolic blood pressure (p-value: 0.0243), and a null association between *H. Pylori* and systolic blood pressure (p-value: 0.9285). Table 4.9 shows the effect of systolic and diastolic blood pressure on incident stroke, among individuals without a history of hypertensive medication usage during follow-up. Both systolic and diastolic blood pressure significantly predicted incident stroke, in this analysis sub-sample (p-values: 0.0086, 0.0420, respectively). Table 4.10 depicts the 3rd step in Baron and Kenny's criteria for mediation for diastolic blood pressure, given the concurrent significant associations for the 1st and 2nd steps for this potential mediator. We found no evidence of mediation of the *H. Pylori*-incident stroke association by diastolic blood pressure (bootstrap 95% CI: -0.0527, -0.0014).

4.4 Discussion

This study investigated whether the association between *H. Pylori* and incident stroke is partially mediated by glucose, systolic and diastolic blood pressure, folate, Vitamin B12, homocysteine, TNF, CRP and IL-6 in a population-based cohort of elderly MAs aged 60+ years old, residing in Sacramento County, California. In analyses of our full cohort, we found no evidence that CRP, IL-6, TNF, homocysteine, folate, or Vitamin B12 lie on the causal pathway from *H. Pylori* to incident stroke. In analyses limited to individuals with no pharmacologic treatment for diabetes during follow-up, no evidence of mediation by glucose was found. Similarly, in analyses restricted to study subjects with no history of statin usage, no evidence of mediation of the association between *H. Pylori* and incident stroke, by CRP, IL-6 or TNF was present. In analyses restricted to

individuals without a history of hypertensive medication usage during follow-up, no evidence of mediation by systolic or diastolic blood pressure was found.

Figure 1 shows the hypothesized indirect effect of *H. Pylori* on incident stroke through 9 candidate mediators. In terms of the potential mediation of the *H. Pylori*-incident stroke association, by inflammatory markers (CRP, IL-6 and TNF), null associations were found for the effect of *H. Pylori* on IL-6 and CRP, while a significant effect was found for TNF. For the associations between each inflammatory marker and incident stroke null associations were found. We found significant associations for the effect of *H. Pylori* on homocysteine and folate (p-values: 0.0356, and 0.0006, respectively), and a null association for Vitamin B12 (p-value: 0.2124). Furthermore, null associations were found for the effect of homocysteine, Vitamin B12 and folate on incident stroke (p-value: 0.6295, 0.6295 and 0.1600, respectively). In assessing the mediating effect of glucose among those without a history of diabetes medication usage during follow-up, no association was found for *H. Pylori*- and glucose (p-value: 0.3968) or for glucose and incident stroke (p-value: 0.3481). In terms of the mediating effect of blood pressure on the *H. Pylori*- incident stroke association, among those without a history of hypertensive medication usage, a non-significant association was found for *H. Pylori* and systolic blood pressure (p-value: 0.9285), and a significant association between *H. Pylori* and diastolic blood pressure (p-value: 0.0243). Furthermore, in assessing the effect of blood pressure on incident stroke, we found significant effects for both systolic and diastolic blood pressure (p-value: 0.0086 and 0.0420, respectively). Since we found concurrent associations between *H. Pylori* and diastolic blood pressure, as well as diastolic blood pressure and incident stroke, we performed the 3rd step of

Baron and Kenny's criteria for mediation, and found null results, as evidenced by a bootstrap 95% confidence interval that contained the null value (-0.0527, -0.0014). Given the lack of concurrent significant associations for the other eight candidate mediators for associations between (1) *H. Pylori* and each potential mediator (step 1 of Baron and Kenny) and (2) each potential mediator and incident stroke (step 2 of Baron and Kenny), in the full or restricted study populations, we conclude that criteria for mediation by IL-6, CRP, TNF, glucose, homocysteine, folate and Vitamin B12, was not met in our study. In an analysis that was intended for hypothesis generation, we performed step 3 of Baron and Kenny's criteria for mediation by CRP in individuals reporting no history of statin usage, given the significant association between *H. Pylori* and CRP (p-value: 0.0415), as well as the association between CRP and incident stroke that approached statistical significance, (p-value: 0.1755, respectively). We found no evidence of a significant indirect effect of *H. Pylori* on incident stroke through CRP, among individuals not taking statins during study follow-up, however this association merits further investigation.

It is possible that *H. Pylori* may affect incident stroke risk, through pathways different from the ones of interest in our study. As an example, ghrelin, which is a 28-amino acid peptide secreted in the stomach, and is involved in the regulation of body weight, has been shown to decrease as a result of *H. Pylori* infection.[146, 147] Furthermore, ghrelin is known to decrease apoptotic and inflammatory processes, both of which are part of the pathogenesis of cerebral ischemia.[148-150] As a result, it is conceivable that decreased ghrelin levels may mediate the association between *H. Pylori* and incident stroke.

Although our mediation analysis produced null results, it is still possible that one or more of the variables is on the causal pathway linking *H. Pylori* to stroke, but methodological issues hindered our ability to identify mediators of the *H. Pylori*-incident stroke association. For instance, our use of years of education as a proxy for socioeconomic status may not completely capture the true socioeconomic status of our study participants, which is likely a strong confounder of the *H. Pylori* stroke association. Next, we did not have information on *H. Pylori* strain type, which has been shown to be highly predictive of systemic inflammation, and therefore may have a stronger association with stroke as well as the candidate mediators.[91, 92] Since our primary outcome was primarily based on self-reported physician diagnosis of stroke, we suspect that the potential under-report of stroke diagnoses as well as our inability to differentiate between stroke sub-types, may have biased our associations between: (1) *H. Pylori* and incident stroke and (2) each potential mediator and stroke, toward the null. We also had varying amounts of data for several of our potential mediators, and as a result, carried forward data for folate, homocysteine and Vitamin B12 from the baseline interview to subsequent follow-up visits, which poses several potential problems: (1) we assumed consistency of these measures over time, which may not be true for every individual, and (2) reverse temporality cannot be ruled out, since we had longitudinal stroke and *H. Pylori* data, but information for several of our candidate mediators only at baseline. Finally, it is plausible that our analyses were underpowered to detect mediation of the *H. Pylori*-incident stroke association.

Our study has several strengths that distinguish it from the existing literature. First, we used data from a population-based cohort of MAs to examine potential

mediation of the association between high antibody levels to *H. Pylori* and incident stroke, by CRP, IL-6, TNF, glucose, systolic and diastolic blood pressure, homocysteine, folate and Vitamin B12. Our study is focused on MAs, which is important, given the disparate rates of stroke and the lack of a conclusive explanation for the excess stroke burden in this group.[3] Next, several advantages exist for bootstrapping techniques in mediation analyses. First, the assumptions about the shape of the sampling distribution, which are required for parametric statistics, are not required for bootstrap re-sampling methods.[145] Second, the asymmetric confidence intervals generated with bootstrapping are linked more closely to the sampling distribution of normal random variables.[145] Further, according to Fritz et al., bootstrapping results in lower type I error rates and higher statistical power than parametric procedures.[151]

4.5 Summary

For the association between antibody levels to *H. Pylori* and incident stroke, we found no evidence of mediation by IL-6, CRP, TNF, Vitamin B12, homocysteine, folate, blood pressure or glucose, using data from the SALSA study. More research aimed at elucidating the mechanism(s) by which high antibody levels to *H. Pylori* increases stroke risk, using large population-based cohorts, is warranted.

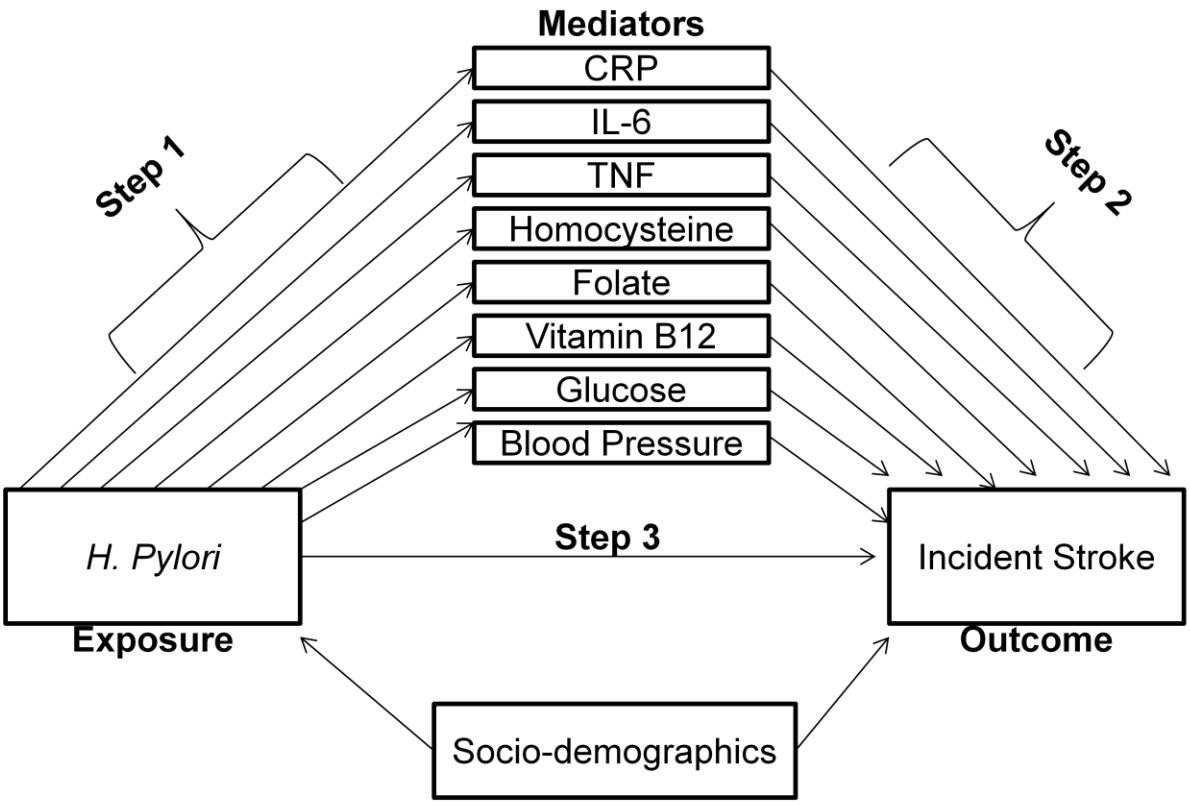


Figure 4.1. Potential mediators of the *H. pylori*- incident stroke association.

*IL-6: interleukin-6; CRP: c-reactive protein; TNF: tumor necrosis factor; *H. pylori*: *Helicobacter pylori;* Blood pressure: systolic and diastolic blood pressure; socio-demographics: age, gender, and education; Steps 1-3: Baron and Kenny's relational criteria for mediation

Table 4.1. Baseline Participant Characteristics By Incident Stroke Status, SALSA, 1998-2008.

	No Stroke (n=1457)	Incident Stroke (n=164)	P-value
Characteristic	N (%)	N (%)	
Age (SD)*	70.2 (6.9)	72.6 (7.9)	0.0004
Men	602 (41.3)	71 (43.3)	0.6265
Nativity			0.8955
Mexico	677 (46.5)	71(43.3)	
United States	686 (47.1)	82 (50.0)	
Other	85 (5.8)	10 (6.1)	
Education (years)			0.2878
0-3	464 (31.8)	59 (36.0)	
4-11	552 (37.9)	59 (36.0)	
≥12	432 (29.6)	45 (27.4)	
Smoking Status			0.8795
Ever	769 (52.8)	92 (56.1)	
Never	677 (46.5)	71 (43.3)	
Income			0.7760
<1,000/month	635 (43.6)	70 (42.7)	
1,000-1999/month	441 (30.3)	54 (32.9)	
≥2,000/month	349 (24.0)	37 (22.6)	
Hypertension	928 (63.7)	126 (76.8)	0.0008
Diabetes	433 (29.7)	66 (40.2)	0.0215
Hyperlipidemia	846 (58.1)	92 (56.1)	0.2907
Atrial Fibrillation	74 (5.1)	15 (9.2)	0.0901
Coronary Heart Disease/PAD	260 (17.8)	42 (25.6)	0.0532
Potential Mediators*			
IL-6	5.02 (7.2)	5.70 (4.8)	0.0002
CRP	5.94 (13.4)	5.24 (5.7)	0.8272
TNF	4.16 (2.5)	4.15 (2.0)	0.6043
Glucose	113.24 (44.7)	121.72 (56.9)	0.2068
Homocysteine	10.72 (6.9)	11.22 (4.0)	0.0007
Folate	21.82 (79.4)	18.74 (62.8)	0.2405
Vitamin B12	475.59 (252.7)	455.50 (236.8)	0.2640
Systolic blood pressure*	137.75 (19.2)	143.36 (20.8)	0.0028
Diastolic blood pressure*	75.78 (10.6)	76.77 (10.0)	0.3784

*Mean(Standard Deviation (SD)); N: number; IL-6: interleukin-6; CRP: c-reactive protein; TNF: tumor necrosis factor.

Table 4.2. Effect Of *H. Pylori* Antibody Level On Six Potential Mediators, Using The Preacher And Hayes Method (Step 1 Of Baron And Kenny's Criteria For Mediation); SALSA, 1998-2008.

Mediator	Coefficient	SE	P-value
IL-6	-0.1709	0.1541	0.2674
CRP	-0.2935	0.1915	0.1254
TNF	0.2194	0.0615	0.0004
Homocysteine	-0.2607	0.1240	0.0356
Folate	3.5681	1.0386	0.0006
Vitamin B12	7.7887	6.2449	0.2124

SE: Standard error; IL-6: interleukin-6; CRP: c-reactive protein; TNF: tumor necrosis factor; *H. Pylori*: *Helicobacter pylori*

Table 4.3. Effect Of Six Potential Mediators On Incident Stroke, Using The Preacher And Hayes Method (Step 2 Of Baron And Kenny Criteria For Mediation); SALSA, 1998-2008.

Mediator	Coefficient	SE	P-value
IL-6	0.0344	0.0200	0.0853
CRP	-0.0258	0.0301	0.3910
TNF	0.0393	0.0389	0.3126
Homocysteine	0.0337	0.0189	0.6295
Folate	0.0021	0.0015	0.1600
Vitamin B12	0.0003	0.0007	0.6295

SE: standard error; IL-6: interleukin-6; CRP: c-reactive protein; TNF: tumor necrosis factor; *H. Pylori*: *Helicobacter pylori*

Table 4.4. Effect Of Glucose On The Association Between *H. Pylori* And Incident Stroke Among Individuals With No Self-Report Of Diabetes Medication Usage; Step 1 and 2 of Baron and Kenny's Criteria For Mediation, Preacher And Hayes Method; SALSA, 1998-2008.

Criteria	Coefficient	SE	P-value
Step 1:			
H. Pylori→glucose	0.3113	0.3674	0.3968
Step 2:			
Glucose→stroke	0.0059	0.0063	0.3481

SE: standard error; *H. Pylori*: *Helicobacter pylori*; SALSA: Sacramento Area Latino Study on Aging

Table 4.5. Effect Of *H. Pylori* On Inflammatory Markers (CRP, IL-6, And TNF), Among Individuals With No Self-Reported Statin Usage During Follow-Up; Step 1 Of Baron And Kenny's Criteria For Mediation, Preacher And Hayes Method; SALSA, 1998-2008.

Mediator	Coefficient	SE	P-value
CRP	-0.3524	0.1728	0.0415
IL-6	-0.2743	0.1724	0.1118
TNF	0.2807	0.2003	0.1611

SE: standard error; *H. Pylori*: *Helicobacter pylori*; SALSA: Sacramento Area Latino Study on Aging; CRP: c-reactive protein; IL-6: interleukin-6; TNF: tumor necrosis factor

Table 4.6. Effect Of Inflammatory Markers (CRP, IL-6, And TNF) On Incident Stroke, Among Individuals With No Self-Reported Statin Usage During Follow-Up; Step 2 Of Baron And Kenny's Criteria For Mediation, Preacher And Hayes Method; SALSA, 1998-2008.

Mediator	Coefficient	SE	P-value
CRP	0.0181	0.0133	0.1755
IL-6	0.0047	0.0131	0.7214
TNF	-0.0012	0.0213	0.9546

SE: standard error; *H. Pylori*: *Helicobacter pylori*; SALSA: Sacramento Area Latino Study on Aging; CRP: c-reactive protein; IL-6: interleukin-6; TNF: tumor necrosis factor

Table 4.7 Effect Of *H. Pylori* On Incident Stroke Through CRP, Among Individuals With No History Of Statin Usage During Follow-Up; Step 3 Of Baron And Kenny's Criteria For Mediation, Using The Preacher And Hayes Method; SALSA, 1998-2008.

	Coefficient	Boot SE	Boot LLCI	Boot ULCI
CRP	-0.0064	0.0087	-0.0295	0.0062

CRP: c-reactive protein; BootSE: bootstrap standard error; BootLLCI: bootstrap 95% lower limit confidence interval; BootULCI: bootstrap 95% upper limit confidence interval

Table 4.8. Effect Of *H. Pylori* On Blood Pressure (Systolic and Diastolic), Among Individuals With No Self-Reported History of Hypertensive Medication Usage During Follow-Up; Step 1 Of Baron And Kenny's Criteria For Mediation, Preacher And Hayes Method; SALSA, 1998-2008.

Mediator	Coefficient	SE	P-value
Systolic	0.0406	0.4526	0.9285
Diastolic	0.5466	0.2426	0.0243

SE: standard error; *H. Pylori*: *Helicobacter pylori*; SALSA: Sacramento Area Latino Study on Aging; CRP: c-reactive protein; IL-6: interleukin-6; TNF: tumor necrosis factor

Table 4.9. Effect Of Blood Pressure (Systolic and Diastolic) On Incident Stroke, Among Individuals With No Self-Reported History of Hypertension Medication Usage During Follow-Up; Step 2 Of Baron And Kenny's Criteria For Mediation, Preacher And Hayes Method; SALSA, 1998-2008.

Mediator	Coefficient	SE	P-value
Systolic	0.0201	0.0077	0.0086
Diastolic	-0.0332	0.0163	0.0420

SE: standard error; *H. Pylori*: *Helicobacter pylori*; SALSA: Sacramento Area Latino Study on Aging; CRP: c-reactive protein; IL-6: interleukin-6; TNF: tumor necrosis factor

Table 4.10. Effect Of *H. Pylori* On Incident Stroke Through Systolic and Diastolic Blood Pressure, Among Individuals With No History Of Hypertensive Medication Usage During Follow-Up; Step 3 Of Baron And Kenny's Criteria For Mediation, Using The Preacher And Hayes Method; SALSA, 1998-2008.

	Coefficient	Boot SE	Boot LLCI	Boot ULCI
Diastolic Blood Pressure	-0.0182	0.0122	-0.0527	-0.0014

BootSE: bootstrap standard error; BootLLCI: bootstrap 95% lower limit confidence interval; BootULCI: bootstrap 95% upper limit confidence interval; SALSA: Sacramento Area Latino Study on Aging

Chapter 5

Conclusion

5.1 Conclusion

This dissertation investigated the links between five persistent pathogens, including CMV, VZV, *H. Pylori* and *T. gondii*, and incident stroke among elderly MAs from the SALSA study. In our analysis of whether exposure to each individual persistent pathogen increased risk of stroke, we found a significant association between high antibody levels to *H. Pylori* and incident stroke. However, there were no significant associations between (1) high antibody levels to VZV, CMV, HSV1 or *T. gondii* or (2) seropositivity to each individual pathogen and incident stroke. Furthermore, since it is possible that total pathogen burden, or concurrent exposure to several persistent pathogens, is more important to incident stroke development than exposure to individual persistent pathogens, we addressed this hypothesis in Chapter 3. We operationalized pathogen burden in the following manner: (1) summed seropositivity (2) sum of the number of pathogens eliciting a high antibody level, and (3) average antibody level to 5 pathogens. We reported a significant association between the number of pathogens eliciting a high antibody level and incident stroke that attenuated and was no longer significant after multivariable adjustment for demographics and stroke risk factors. Further, we found no statistically significant associations between summed seropositivity or average IgG antibody level and stroke risk. Finally, we sought to

elucidate the mechanisms underlying the association between high antibody levels to *H. Pylori* and incident stroke that we reported in Chapter 2, by assessing the mediating effect of nine candidate mediators, including TNF, IL-6, CRP, glucose, systolic and diastolic blood pressure, homocysteine, Vitamin B12 and folate. In this investigation, we found no evidence of mediation by inflammatory markers, folate, Vitamin B12, homocysteine, glucose, or blood pressure (systolic and diastolic), as evidenced by a lack of concurrent significant associations for steps 1 and 2 of Baron and Kenny's relational criteria for mediation. Next, given the potential for aggressive treatment following diagnosis of hyperlipidemia and diabetes, which could decrease the impact that these diseases have on inflammatory processes, we conducted the following secondary analyses to assess: (1) the mediating effect of inflammatory markers (TNF, IL-6 and CRP) among those with no history during follow-up of statin usage, and (2) the mediating effect of glucose among individuals with no diabetes medication history during the course of the study. Here, we found no statistically significant evidence of mediation by inflammatory markers or glucose; however, the mediating effect of CRP, based on Baron and Kenny's 1st and 2nd criteria for mediation approached statistical significance. As a result, we conducted a test of the indirect effect of *H. Pylori* on incident stroke through CRP, among those not taking statins during follow-up. While the 95% bootstrap confidence interval contained the null value for this analysis, these results are suggestive of an association, and deserve further investigation.

In this dissertation research, antibody levels to five persistent pathogens were used to predict incident stroke, which is important given that the existing studies on the topic have relied on serostatus to define exposures.[7-9] Antibody levels are thought to

predict inflammatory outcomes more consistently than seropositivity.[18] This dissertation adds to the literature the first report of a statistically significant association between antibody levels to *H. Pylori* and stroke risk. We also comprehensively examined the effect of pathogen burden, again, using antibody levels, which has not been previously done. Lastly, we assessed the effect of seven potential mediators on the association between antibody levels to *H. Pylori* and incident stroke. While there was no statistically significant evidence of mediation by IL-6, TNF, CRP, glucose, folate, Vitamin B12, blood pressure or homocysteine, this was an important contribution as the pathways by which pathogens may increase stroke risk have not been well investigated.

This work suggests that future replication studies, in other populations are warranted, in order to confirm our significant association between *H. Pylori* and incident stroke. Moreover, our finding of a significant association between the sum of the number of pathogens eliciting a high antibody response in unadjusted models, requires further study given that the attenuation and loss of statistical significance after multivariable adjustment may suggest that one or more of the adjustment variables was on the causal pathway. Although we found no evidence of mediation by the candidate mediators, we hypothesize that future studies, if able to overcome some the methodological limitations of our study including missing data, limited sample size, residual confounding, and self-reported stroke, may uncover significant mediation of the *H. Pylori*-incident stroke association, by inflammatory markers, glucose, blood pressure, homocysteine, folate and/or Vitamin B12 or other factors.

5.2 Aim 1

The first aim of this dissertation examined whether exposure to CMV, HSV-1, VZV, *T. gondii*, and *H. Pylori*, defined using serostatus and antibody levels, increased stroke risk, using data from the SALSA study. Individuals with antibody levels in the 75th percentile of antibody levels to *H. Pylori* had higher stroke risk compared to those in the 25th percentile of antibody level to (OR: 1.53, 95% CI: 1.11, 2.12) in models adjusted for age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia. We found no significant associations (in unadjusted and adjusted models) between antibody levels to CMV, HSV-1, VZV, or *T. gondii* and incident stroke, or between seropositivity to each persistent pathogen and stroke risk.

Aim 1 fills several gaps in the current knowledge about the effect of persistent pathogens on stroke risk. First, we are the first to establish an association between antibody levels to *H. Pylori* and incident stroke among MAs using prospectively collected data. Previous longitudinal studies on the link between persistent pathogens and stroke have limited their exposure definition to seropositivity,[7-9] which may be less sensitive as a marker for inflammatory outcomes as antibody levels.[18] While the results of this analysis are hypothesis generating, the newly uncovered association between *H. Pylori* and stroke risk does suggest the potential for a new stroke intervention target in MAs. We also add to the literature, a confirmation of null associations between seropositivity to persistent pathogens and incident stroke, with a focus on the MA population. Haider et al., using data from the Framingham Heart study (n=1,187), reported no significant associations between seropositivity to *C. pneumoniae*, *H. Pylori*

or CMV and stroke risk.[9] Further, using data from the multi-ethnic Northern Manhattan Stroke Study (n=1,625), Elkind et al, reported positive but non-significant associations between seropositivity to 5 persistent pathogens and incident stroke.[8] Similarly, in a prospective cohort of German men and women (n=9953), no significant association was found between seropositivity to *H. Pylori* and stroke risk.[7] In summary, future studies that seek to confirm our finding of a significant effect of high antibody levels to *H. Pylori* on stroke risk are needed and may inform interventions aimed at infection eradication for reduction in stroke risk.

5.3 Aim 2

The second aim of this dissertation examined whether concurrent exposure to several pathogens, or pathogen burden, significantly increases stroke incidence, in elderly community dwelling MAs from the SALSA study. It has been purported that total pathogen burden, compared to exposure to individual pathogens, more effectively increases systemic inflammation, which leads to atherosclerosis development or exacerbation.[10, 11] We defined pathogen burden as (1) summed seropositivity, (2) sum of the number of pathogens eliciting a high antibody level, and (3) average antibody level across 5 pathogens. We found that with each additional pathogen to which an individual had high antibody levels to, stroke risk increased 18%, in unadjusted models (OR: 1.18, 95% CI: 1.04, 1.35); however, with multivariable adjustment for age, gender, education, diabetes, atrial fibrillation, smoking, coronary heart disease/PAD, hypertension, and hyperlipidemia, there was attenuation of the association and statistical significance was lost. Pathogen burden defined as summed seropositivity to 0-5 pathogens and average antibody level to 5 pathogens (in analyses with all

pathogens weighted equally, and with *H. Pylori* carrying a higher weight than the other 4 pathogens) were not shown to predict incident stroke in this cohort. Our report of no significant associations between our pathogen burden variables are in accord with one of the two existing cohort studies addressing this research question. In particular, investigators using data from the Framingham cohort found no significant associations between concurrent seropositivity to CMV, *H. Pylori* and *C. pneumoniae* and a pooled endpoint of incident cardiovascular disease that included atherothrombotic stroke, myocardial infarction and coronary heart disease deaths (HR: 0.77, 95% CI: 0.44, 1.35). [9] On the other hand, Elkind et al, found a significant positive association between a weighted index of pathogen burden based on serostatus that included *H. Pylori*, *C. pneumoniae*, CMV, and HSV1 and 2, and incident stroke (HR: 1.39, 95% CI: 1.02, 1.90).[82]

Aim 2 fills the gap in the literature resulting from the lack of studies assessing the effect of pathogen burden on stroke risk using antibody levels and by studying MAs. We add a significant association between number of pathogens eliciting a high antibody response and incident stroke in unadjusted models. Given the attenuation and loss of statistical significance with multivariable adjustment, one or more of the adjustment variables may lie on the causal pathway from pathogen burden to incident stroke. Our results suggest that future studies examining the effect of pathogen burden defined as the sum of the number of pathogens eliciting a high antibody level on stroke risk are warranted as are studies seeking to clarify the potential mechanism(s) involved.

5.4 Aim 3

The third aim of this dissertation was conceived based on the significant association we uncovered between high antibody level to *H. Pylori* and incident stroke, as discussed in Chapter 2. Since the pathway by which *H. Pylori* increases stroke risk remains unknown, we sought to assess the mediating effect of CRP, IL-6, TNF, glucose, systolic and diastolic blood pressure, homocysteine, folate and Vitamin B12. Baron and Kenny's relational criteria were used to assess the following relationships: (1) *H. Pylori*'s effect on each potential mediator and (2) each potential mediator's effect on incident stroke and (3) the effect of *H. Pylori* on stroke risk controlling for each mediator. If concurrent associations were found for criteria 1 and 2, the third criteria were assessed. The candidate mediators were selected based on the literature suggesting that exposure to *H. Pylori* is associated with inflammation,[87-89 91, 92] decreased folate and Vitamin B12 and the associated increase in serum homocysteinemia,[49, 137] as well as incident diabetes[61] and hypertension[135, 136] (Step 1 of Baron and Kenny's criteria). As evidence for step 2 of Baron and Kenny's criteria for mediation, stroke risk has been associated with inflammatory outcomes,[99, 100,98] diabetes,[115] hypertension,[1] and homocysteinemia.[138-140] We found no evidence of mediation of the *H. Pylori*-incident stroke association by inflammatory markers, glucose, blood pressure, folate, Vitamin B12, or homocysteine in the SALSA study population. When we limited our analysis to individuals without a history of statin or diabetes medication usage, our results were similar to those in the full cohort. However, as a purely hypothesis generating analysis, given the significant association between *H. Pylori* and CRP (p-value:0.0415), as well as the association between CRP and incident stroke that approached statistical significance (p-value: 0.1755) among individuals not taking statins for the treatment of

dyslipidemia, we performed the last step of Baron and Kenny's criteria for mediation, and while the 95% bootstrap confidence interval contained the null value (-0.0295, 0.0062), this association merits further investigation given our suggestive evidence of mediation by CRP in this analysis subset.

Analyses from Aim 3 add to the literature the first investigation into the mechanism(s) underlying the association between high antibody levels to *H. Pylori* and incident stroke. Although we found no statistically significant evidence of an indirect effect of *H. Pylori* on incident stroke through inflammatory markers, glucose, blood pressure, Vitamin B12, folate or homocysteine, we believe these mechanisms are still plausible, and should be investigated further. Importantly, we used bootstrap resampling methods, as proposed by Preacher and Hayes,[143-145] which affords our analysis the following advantages: no distributional assumptions about the sampling distribution, [145] asymmetric confidence intervals that are more reflective of the sampling distribution of normal random variables, [145] and have lower type I error rates and higher statistical power than parametric procedures.[151]

5.5 Strengths and Limitations

This dissertation research has several strengths. We are the first to examine the effects of exposure to individual persistent pathogens as well as the comprehensive effect of total pathogen burden on incident stroke using IgG antibody levels. We used discrete time logistic regression, which allowed us to incorporate time-varying covariates into our analyses, which may have allowed us to more accurately account for changes in pathogen exposures over time and confounding by stroke risk factors than analyses that do not have time varying data.[152] Next, antibody levels may be more

specific predictors of inflammatory outcomes than serostatus of pathogen exposure.[18] We were also able to establish temporality between pathogen exposures and incident stroke given the longitudinal nature of our data and the exclusion of those with prevalent stroke at baseline. We performed a mediation analysis using Preacher and Hayes' bootstrap resampling techniques, which have less strict assumptions about the shape of the sampling distribution than parametric statistics.[145] Another advantage of bootstrapping is that asymmetric confidence intervals are generated that are more closely linked to the sampling distribution of normal random variables.[145] Bootstrapping has been reported to have lower type I error rates and higher statistical power than parametric procedures.[151] Finally, our study is the first to focus solely on investigating the link between IgG antibody levels and incident stroke in MAs, which is important given the higher stroke burden, higher seroprevalence of infectious diseases, and lack of an explanation for the disparities seen in stroke in this population.

Several limitations to the current research should be considered. First, and likely most important, our endpoint incident stroke was based on self-report and could be over or underestimated, which may bias our findings toward the null since it is most likely that error in stroke recall by study participants is non-differential, meaning that they probably occur to a similar extent among those exposed and unexposed to persistent pathogens. Unfortunately, there are no published stroke validation studies using the SALSA cohort, which would have allowed us to assess the sensitivity and specificity of self-reported stroke in this study population. Further, the evidence on the validity of self-reported stroke is inconclusive. Existing studies were mostly conducted among NHWs, and reported positive predictive values for self-reported stroke ranging from 50-80%.[153-162] In

a validation study conducted in a cohort of elderly NHW, Hispanic and African Americans, low sensitivity and specificity were reported for self-reported stroke compared to brain magnetic resonance imaging, and the authors concluded that neuroimaging techniques are a superior to self-report in determining stroke history.[163]

Next, the mean age of our study population is about 70 years old, which prohibits any investigation into why MAs have higher stroke risk at younger ages, compared to NHWs. Future research in younger populations of MA and NHWs would be needed to address this research question. We also excluded individuals with a history of stroke at baseline, from our analysis, which potentially removes those at highest risk from our study cohort. Another limitation of our work is that we could not distinguish between stroke subtypes, which could bias our findings toward the null since persistent pathogen exposure is more likely to be associated with ischemic stroke through atherosclerotic mechanisms. Next, we used education level as a proxy for socioeconomic status, which may not completely capture true socioeconomic status of the study participants. An individual is said to have low socioeconomic status when he/she has a combination of the following attributes: low income, low educational achievement, occupational status and/or area based deprivation.[164-166] Hagger-Johnson et al recently reported links between life-course variables including parental socioeconomic status and childhood intelligence, and inflammation, as well as low socioeconomic status in adult life, both of which, negatively affect cardiovascular disease risk.[167, 168] As a result of our limited control for socioeconomic status in our analysis, our results may be residually confounded by measures of socioeconomic status such as wealth, as well as life-course trajectories of socioeconomic status.

We also did not have IgG antibody levels for several pathogens that have been previously linked to stroke, such as *C. pneumoniae*, and periodontal pathogens, nor did we have information on *H. Pylori* strain type. In structuring our analysis dataset, we carried forward the risk factor and pathogen data from either baseline or the previous follow-up visit to semi-annual visits given the large amount of missing data, and assumed that this information is constant, which may not be true for all individuals. In future research, multiple imputation methods could be used in order to deal with the large missing data problem in the SALSA study and to see if results remain consistent. It is also possible that our multivariable models adjusted for mediators, because some of the control variables may lie on the causal pathway. For example, in our analysis for aims 1 and 2, we included variables such as hypertension, and diabetes in our multivariable models, which may mediate the effect of persistent pathogen and pathogen burden exposure on incident stroke. Further, in aim 2, we operationalized pathogen burden in several ways, however, we did not consider the possibility that specific combinations of pathogen exposures differentially affect stroke risk. This information would facilitate the planning of more tailored stroke interventions aimed at eradicating persistent pathogens that exert the highest risk. We also may have had limited power to detect significant associations given our sample size and the amount of missing data in our cohort. Lastly, since we did not adjust for multiple comparisons in our analysis, an important caveat is that our results must be considered hypothesis-generating.

5.6 Next Steps

Population-based research allows more reliable definitions of race/ethnicity as well as the outcome of interest, which yields more accurate estimates of disease burden.[3] As a result, large population-based longitudinal cohort studies, with serum for ascertaining IgG antibody levels to persistent pathogens known to increase inflammatory levels, such as CMV, HSV1, *H. Pylori*, *T. gondii*, and VZV, as well as neurologist-validated incident stroke cases, would be ideal for replicating the results of this dissertation. Since we are the first to report a significant association between high antibody levels to *H. Pylori* and incident stroke, and to investigate the mechanism underlying this association, confirmatory population-based research in MAs and other populations is warranted. Our results, in combination with future studies that provide additional evidence for an independent effect of persistent pathogens, specifically *H. pylori*, on stroke risk, could inform new interventions aimed at eradication of infectious disease, especially among individuals with mild pre-existing atherosclerosis, in an effort to reduce and/or eliminate stroke disparities.

Three existing cohort studies could be used to replicate the results of this dissertation research. For instance, from 1993-1996 the Hispanic Established Population for the Epidemiologic Study of the Elderly (H-EPESE), probability sampled community-dwelling MAs from California, Colorado, New Mexico, Arizona and Texas.[169] Eligibility requirements included being at least 65 years old at baseline, self-designated as MA, resident of one of the selected states, and non-institutionalized. Study subjects (n=3,050) underwent in-home interviews where data on socio-demographics, functional status, medical history, health behaviors, medication usage, and measures of

acculturation were collected. The objectives of the study were (1) to estimate the prevalence of health conditions, mental health problems, as well as functional abnormalities among elderly MAs, and (2) to make morbidity and mortality comparisons across racial/ethnic groups. This sample would improve upon our study by increasing the sample size of MAs. However, the limitation of our study regarding our use of self-reported incident strokes would not be overcome with this data set. If serum samples are available for this study, IgG antibody levels to persistent pathogens could be quantified, which would allow an investigation into the links between persistent pathogens and incident stroke. Alternatively, future studies with characteristics similar to that of the Hispanic EPESE could add serum sample collection, stroke validation, and IgG antibody level measurement for persistent pathogens to their protocol, and investigate our research question.

Next, data from the Northern Manhattan Study could easily be used to explore the links between antibody levels to individual persistent pathogens as well as pathogen burden and incident stroke.[8] One-thousand six hundred twenty five African Americans, Hispanics (including Dominican, Puerto Rican and Cuban) and NHWs, who had no history of stroke at baseline and were at least 39 years old, were included in the study cohort. Here, incident stroke cases occurring during the study were validated by two neurologists. Importantly, antibody levels for multiple persistent pathogens, including C. pneumoniae, *H. Pylori*, CMV, and HSV1 and 2 are available in this study. Even though the Hispanic population comprising this study cohort is different from ours, replication studies in other racial/ethnic groups are warranted. In terms of investigating the effect of antibody levels to more than one pathogen concurrently, this dataset could be used to

explore different combinations of persistent pathogen exposures that take into account potential biologic interaction between pathogens, which was not included in our pathogen burden definition. Another important question that an analysis of this dataset would be able to investigate is whether persistent pathogen exposure explains part of the disparity in stroke risk among younger MAs compared to their NHW counterparts,[3] which we were not able to examine, given that the population did not include NHWs.

Lastly, the National Health and Nutrition Examination Survey (NHANES) began in the 1960s, as a program to document the health and nutritional condition of US residents.[170] Study participants undergo interviews assessing socio-demographics, dietary and health status, as well as physical examinations where medical, dental, physiological measurements and laboratory tests are performed. Each year, the survey assesses a nationally representative group of roughly 5,000 individuals. Results from this survey could be used to further investigate our research questions, if the following changes and/or additions to the study protocol are made: validation of all stroke cases by a neurologist, prospectively following individuals without history of stroke, at baseline, and testing serum samples for antibody levels to the persistent pathogens of interest. This survey could overcome our limited power to detect significant effects between persistent pathogens and incident stroke, given its large sample size.

After population-based studies are conducted to replicate and/or extend the results of our research, the next step to consider would be interventions aimed at persistent pathogen eradication, in an effort to reduce stroke risk. Several antibiotic trials have already been conducted to assess the effect of treating *C. pneumoniae* on cardiovascular disease, and have routinely found no long-term benefit. However, in the

Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders study of 7,747 individuals with a history of myocardial infarction, the risk of death and myocardial infarction after 6 weeks of treatment with Azithromycin, was decreased by 33%, but the protection was lost at 14 months of follow-up.[171] The Azithromycin and Coronary Events study sought to determine if a longer treatment course of antibiotics would sustain the previously reported protection.[172] In this study, 4,012 patients with established coronary artery disease were randomized to either placebo or 60 mg of Azithromycin weekly for 1 year, with a nearly 4 year follow-up. Here, no decreased risk of cardiovascular disease was reported. Similar results were reported in the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 Trial,[173] and in the Clarithromycin for Patients with Stable Coronary Heart Disease Trial.[174] In the later trial, however, the surprising finding of elevated long-term mortality due to increased cardiovascular disease mortality was reported.[174]

Even though the existing clinical trials failed to provide evidence for a positive effect of antibiotic treatment on cardiovascular disease risk, there are several alternative explanations for the null results. First, *C. pneumoniae*, which is by far the most widely studied pathogen for links to cardiovascular disease is difficult to treat given the intracellular developmental cycle of the pathogen, which often leads to persistence.[175] During persistence, the pathogen is not susceptible to antibiotics, and it has even been demonstrated that certain antibiotics can induce perisistence.[176, 177] Furthermore, study subjects in the previously discussed clinical trials have often had pre-existing, advanced, atherosclerosis. Importantly, the effect of antibiotics on risk of disease among those with early atherosclerosis has yet to be investigated. It is also possible

that pathogen burden may exert an overwhelming influence on vascular risk, such that treating one pathogen in isolation, would be ineffective at reducing the body's inflammatory response to these pathogens.[178] In summary, after replication studies have been conducted to confirm our finding of increased stroke risk among individuals exposed to *H. Pylori*, future antibiotic trials aimed at this particular pathogen, may prove fruitful in reducing stroke risk, despite the previous lack of benefit for cardiovascular disease risk with antibiotics for C. pneumoniae.

Future studies seeking to investigate the effect of pathogen burden on stroke risk could take a different approach to defining pathogen burden, than that used in our study. Although we defined pathogen burden in several ways including: (1) summed sero-positivity to 0-5 pathogens, (2) average IgG antibody levels to 0-5 pathogens and (3) sum of the number of pathogens eliciting a high antibody level, our definition provides no information on which specific combination(s) of persistent pathogen exposures has the greatest impact on stroke risk. We assume that no biologic interaction exists between the pathogens, and that all individuals exposed to a given number of persistent pathogens, have the same stroke risk, which may not be accurate.[179] Future studies should investigate different combinations of pathogen exposures for their effects on stroke risk, as this information may provide information on novel intervention targets for incident stroke.

5.7 Dissertation summary

This dissertation examined the links between five persistent pathogens and incident stroke among elderly MAs at least 60 years old, participating in the SALSA study. We found a significant association between high antibody levels to *H. Pylori* (75th

versus 25th percentile) and incident stroke. Given the possibility that concurrent exposure to several pathogens increases inflammation to a higher degree than exposure to one pathogen at a time, we examined the effect of pathogen burden, defined as (1) summed seropositivity (2) sum of the number of pathogens eliciting a high antibody level (range 0-5), and (3) average antibody level across five pathogens. We found an 18% increase in risk of stroke for each additional pathogen that an individual had high antibody levels to, in unadjusted models. However, upon multivariable adjustment, the effect of the sum of the number of pathogens eliciting a high antibody response on incident stroke was attenuated and no longer significant. Lastly, we examined the mediating effect of the *H. Pylori*-incident stroke association by inflammatory outcomes (CRP, IL-6 and TNF), glucose, systolic and diastolic blood pressure, Vitamin B12, folate and homocysteine. Although we did not find evidence of mediation, we still believe that the proposed mechanisms may exist and that our ability to detect mediation may be hindered by several methodological issues of our study. These findings suggest that future studies examining *H. Pylori*'s effect on incident stroke, as well as the mechanism involved in the association, are warranted. If *H. Pylori* is confirmed to be a risk factor for incident stroke in other studies, interventions aimed at pharmacologic treatment for eradication of the infection could be investigated as a means to reduce the burden of stroke in the United States. Pharmacologic treatment of *H. Pylori*, would at a minimum, reduce human suffering, and decrease medical care expenditures,[180, 181] but may also be fruitful in reducing stroke risk.

References

1. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation.* Jan 3 2012;125(1):e2-e220.
2. United States Census Bureau. 2000; http://www.census.gov. Accessed May 10, 2010.
3. Morgenstern LB, Smith MA, Lisabeth LD, et al. Excess stroke in Mexican Americans compared with non-Hispanic Whites: the Brain Attack Surveillance in Corpus Christi Project. *Am J Epidemiol.* Aug 15 2004;160(4):376-383.
4. Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. *J Am Acad Dermatol.* Nov 2007;57(5):737-763; quiz 764-736.
5. Bennett JM, Glaser R, Malarkey WB, Beversdorf DQ, Peng J, Kiecolt-Glaser JK. Inflammation and reactivation of latent herpesviruses in older adults. *Brain Behav Immun.* Dec 1 2011;(in press).
6. McColl BW, Allan SM, Rothwell NJ. Systemic infection, inflammation and acute ischemic stroke. *Neuroscience.* Feb 6 2009;158(3):1049-1061.
7. Schottker B, Adamu MA, Weck MN, Muller H, Brenner H. Helicobacter pylori infection, chronic atrophic gastritis and major cardiovascular events: a population-based cohort study. *Atherosclerosis.* Feb 2012;220(2):569-574.
8. Elkind MSV, Ramakrishnan P, Moon YP, et al. Infectious Burden and Risk of Stroke: The Northern Manhattan Study. *Arch Neurol.* January 1, 2010 2010;67(1):33-38.
9. Haider AW, Wilson PW, Larson MG, et al. The association of seropositivity to Helicobacter pylori, Chlamydia pneumoniae, and cytomegalovirus with risk of cardiovascular disease: a prospective study. *J Am Coll Cardiol.* Oct 16 2002;40(8):1408-1413.
10. Epstein SE, Zhou YF, Zhu J. Infection and atherosclerosis: emerging mechanistic paradigms. *Circulation.* Jul 27 1999;100(4):e20-28.
11. Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. *Stroke.* Oct 2003;34(10):2518-2532.
12. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. *Clin Infect Dis.* Nov 1 2006;43(9):1143-1151.
13. Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, McQuillan G. Seroprevalence and ethnic differences in Helicobacter pylori infection among adults in the United States. *J Infect Dis.* Apr 2000;181(4):1359-1363.
14. Seroprevalence of herpes simplex virus type 2 among persons aged 14-49 years--United States, 2005-2008. *MMWR Morb Mortal Wkly Rep.* Apr 23 2010;59(15):456-459.
15. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. Toxoplasma gondii infection in the United States, 1999 2004, decline from the prior decade. *Am J Trop Med Hyg.* Sep 2007;77(3):405-410.
16. Aiello AE, Diez-Roux A, Noone AM, et al. Socioeconomic and psychosocial gradients in cardiovascular pathogen burden and immune response: the multi-ethnic study of atherosclerosis. *Brain Behav Immun.* Jul 2009;23(5):663-671.
17. Zajacova A, Dowd JB, Aiello AE. Socioeconomic and race/ethnic patterns in persistent infection burden among U.S. adults. *J Gerontol A Biol Sci Med Sci.* Feb 2009;64(2):272-279.
18. Nazmi A, Diez-Roux AV, Jenny NS, Tsai MY, Szklo M, Aiello AE. The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis. *BMC Public Health.* 2010;10:706.
19. Reeves MJ, Bushnell CD, Howard G, et al. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurology.* Oct 2008;7(10):915-926.

20. Centers for Disease Control and Prevention. *Stroke Drops to Fourth Leading Cause of Death in 2008: Life expectancy declines slightly according to latest CDC deaths report.* Atlanta, Georgia December 9, 2010 2010.

21. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation.* Jan 29 2008;117(4):e25-146.

22. Brown DL, Boden-Albala B, Langa KM, et al. Projected costs of ischemic stroke in the United States. *Neurology.* Oct 24 2006;67(8):1390-1395.

23. U.S. Department of Health and Human Services. Office of Disease Prevention and Health Promotion. Healthy People 2020. Washington, DC.

24. Deb P, Sharma S, Hassan KM. Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. *Pathophysiology.* Jun 2010;17(3):197-218.

25. American Stroke Association. *Atherosclerosis and Stroke* http://www.strokeassociation.org/STROKEORG/LifeAfterStroke/HealthyLivingAfterStroke/UnderstandingRiskyConditions/Atherosclerosis-and-Stroke_UCM_310426_Article.jsp. Accessed July 1, 2012.

26. United States Census 2000. http://quickfacts.census.gov/qfd/states/48/48355.htm. Accessed July 17, 2009.

27. Smith MA, Risser JM, Lisabeth LD, Moye LA, Morgenstern LB. Access to care, acculturation, and risk factors for stroke in Mexican Americans: the Brain Attack Surveillance in Corpus Christi (BASIC) project. *Stroke.* Nov 2003;34(11):2671-2675.

28. Hyre AD, Muntner P, Menke A, Raggi P, He J. Trends in ATP-III-defined high blood cholesterol prevalence, awareness, treatment and control among U.S. adults. *Ann Epidemiol.* Jul 2007;17(7):548-555.

29. Redmond N, Baer HJ, Hicks LS. Health behaviors and racial disparity in blood pressure control in the national health and nutrition examination survey. *Hypertension.* Mar 2011;57(3):383-389.

30. Sundquist J, Winkleby MA, Pudaric S. Cardiovascular disease risk factors among older black, Mexican-American, and white women and men: an analysis of NHANES III, 1988-1994. Third National Health and Nutrition Examination Survey. *J Am Geriatr Soc.* Feb 2001;49(2):109-116.

31. Health Data Interactive. Centers for Disease Control and Prevention, National Center for Health Statistics Website. http://www.cdc.gov/nchs/hdi/htm. Accessed July 19, 2011.

32. Shen AY, Contreras R, Sobnosky S, et al. Racial/ethnic differences in the prevalence of atrial fibrillation among older adults--a cross-sectional study. *J Natl Med Assoc.* Oct 2010;102(10):906-913.

33. Centers for Disease Control and Prevention (CDC). Prevalence of chronic kideny disease and associated risk factors- United States. *MMWR Morb Mortal Wkly Rep.* 2007(56):161-165.

34. Schiller J, Lucas J, Ward B, Peregoy J. Summary health statistics for U. S. adults: National Health Interview Survey, 2010. *Vital Health Stat 10.*252:1-217.

35. Fan T, Koro CE, Fedder DO, Bowlin SJ. Ethnic disparities and trends in glycemic control among adults with type 2 diabetes in the U.S. from 1988 to 2002. *Diabetes Care.* Aug 2006;29(8):1924-1925.

36. Ohana B, Lipson M, Vered N, Srugo I, Ahdut M, Morag A. Novel approach for specific detection of herpes simplex virus type 1 and 2 antibodies and immunoglobulin G and M antibodies. *Clin Diagn Lab Immunol.* Nov 2000;7(6):904-908.

37. Brady RC, Bernstein DI. Treatment of herpes simplex virus infections. *Antiviral Res.* Feb 2004;61(2):73-81.

38. Ribes JA, Steele AD, Seabolt JP, Baker DJ. Six-year study of the incidence of herpes in genital and nongenital cultures in a central Kentucky medical center patient population. *J Clin Microbiol.* Sep 2001;39(9):3321-3325.
39. Centers for Disease Control and Prevention: National Center for Immunization and Respiratory Diseases, Division of Viral Disease2010.
40. Heymann DL, ed *Control of Communicable Diseases Manual.* 18th ed. Washington: American Public Health Association; 2004.
41. Center for Disease Control and Prevention: National Center for Preparedness, Detection, and Control of Infectious Diseases2009.
42. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood.* Jul 15 1993;82(2):513-520.
43. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med.* Jan 14 1999;340(2):115-126.
44. Minick CR, Fabricant CG, Fabricant J, Litrenta MM. Atheroarteriosclerosis induced by infection with a herpes-virus. *Am J Pathol.* 1979;96:673-706.
45. Espinola-Klein C, Rupprecht HJ, Blankenberg S, et al. Are morphological or functional changes in the carotid artery wall associated with Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, or herpes simplex virus infection? *Stroke.* Sep 2000;31(9):2127-2133.
46. Liu R, Moroi M, Yamamoto M, et al. Presence and severity of Chlamydia pneumoniae and Cytomegalovirus infection in coronary plaques are associated with acute coronary syndromes. *Int Heart J.* Jul 2006;47(4):511-519.
47. Evrengul H, Tanriverdi H, Kuru O, et al. Elevated homocysteine levels in patients with slow coronary flow: relationship with Helicobacter pylori infection. *Helicobacter.* Aug 2007;12(4):298-305.
48. Tamura A, Fujioka T, Nasu M. Relation of Helicobacter pylori infection to plasma vitamin B12, folic acid, and homocysteine levels in patients who underwent diagnostic coronary arteriography. *Am J Gastroenterol.* Apr 2002;97(4):861-866.
49. Dierkes J, Ebert M, Malfertheiner P, Luley C. Helicobacter pylori infection, vitamin B12 and homocysteine. A review. *Dig Dis.* 2003;21(3):237-244.
50. Stettin D, Waldmann A, Strohle A, Hahn A. Association between Helicobacter pylori-infection, C-reactive protein and status of B vitamins. *Adv Med Sci.* 2008;53(2):205-213.
51. Itou S, Goto Y, Kondo T, et al. No associations of Helicobacter pylori infection and gastric atrophy with plasma total homocysteine in Japanese. *Int J Med Sci.* 2007;4(2):98-104.
52. Triantafillidis JK, Georgakopoulos D, Gikas A, et al. Relation between Helicobacter pylori infection, thyroid hormone levels and cardiovascular risk factors on blood donors. *Hepatogastroenterology.* Dec 2003;50 Suppl 2:cccxviii-cccxx.
53. Bloemenkamp DG, Mali WP, Tanis BC, et al. The relation between Helicobacter pylori and atherosclerosis cannot be explained by a high homocysteine concentration. *Eur J Clin Invest.* Aug 2002;32(8):549-555.
54. Tufano A, Di Capua M, Coppola A, et al. The infectious burden in atherothrombosis. *Semin Thromb Hemost.* Jul 2012;38(5):515-523.
55. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet.* Aug 9 1997;350(9075):430-436.
56. Koenig W, Rothenbacher D, Hoffmeister A, et al. Infection with Helicobacter pylori is not a major independent risk factor for stable coronary heart disease: lack of a role of cytotoxin-associated protein A-positive strains and absence of a systemic inflammatory response. *Circulation.* Dec 7 1999;100(23):2326-2331.

57. Benagiano M, D'Elios MM, Amedei A, et al. Human 60-kDa heat shock protein is a target autoantigen of T cells derived from atherosclerotic plaques. *J Immunol.* May 15 2005;174(10):6509-6517.
58. Mosorin M, Surcel HM, Laurila A, et al. Detection of Chlamydia pneumoniae-reactive T lymphocytes in human atherosclerotic plaques of carotid artery. *Arterioscler Thromb Vasc Biol.* Apr 2000;20(4):1061-1067.
59. Jackson LA, Campbell LA, Kuo CC, Rodriguez DI, Lee A, Grayston JT. Isolation of Chlamydia pneumoniae from a carotid endarterectomy specimen. *J Infect Dis.* Jul 1997;176(1):292-295.
60. Maass M, Bartels C, Engel PM, Mamat U, Sievers HH. Endovascular presence of viable Chlamydia pneumoniae is a common phenomenon in coronary artery disease. *J Am Coll Cardiol.* Mar 15 1998;31(4):827-832.
61. Jeon CY, Haan MN, Cheng C, et al. Helicobacter pylori Infection Is Associated With an Increased Rate of Diabetes. *Diabetes Care.* Jan 25 2012.
62. Chiang CH, Huang CC, Chan WL, et al. Herpes simplex virus infection and risk of atrial fibrillation: A nationwide study. *Int J Cardiol.* Jul 20 2011.
63. Haarala A, Kahonen M, Lehtimaki T, et al. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: the Cardiovascular Risk in Young Finns Study. *Clin Exp Immunol.* Feb 2012;167(2):309-316.
64. Heuschmann PU, Neureiter D, Gesslein M, et al. Association between infection with Helicobacter pylori and Chlamydia pneumoniae and risk of ischemic stroke subtypes: Results from a population-based case-control study. *Stroke.* Oct 2001;32(10):2253-2258.
65. Muhlestein JB, Anderson JL, Hammond EH, et al. Infection with Chlamydia pneumoniae accelerates the development of atherosclerosis and treatment with azithromycin prevents it in a rabbit model. *Circulation.* Feb 24 1998;97(7):633-636.
66. Laitinen K, Laurila A, Pyhala L, Leinonen M, Saikku P. Chlamydia pneumoniae infection induces inflammatory changes in the aortas of rabbits. *Infect Immun.* Nov 1997;65(11):4832-4835.
67. Ridker PM, Hennekens CH, Stampfer MJ, Wang F. Prospective study of herpes simplex virus, cytomegalovirus, and the risk of future myocardial infarction and stroke. *Circulation.* Dec 22-29 1998;98(25):2796-2799.
68. Elkind MS, Tondella ML, Feikin DR, Fields BS, Homma S, Di Tullio MR. Seropositivity to Chlamydia pneumoniae is associated with risk of first ischemic stroke. *Stroke.* Mar 2006;37(3):790-795.
69. Elkind MS, Lin IF, Grayston JT, Sacco RL. Chlamydia pneumoniae and the risk of first ischemic stroke : The Northern Manhattan Stroke Study. *Stroke.* Jul 2000;31(7):1521-1525.
70. Cook PJ, Honeybourne D, Lip GY, Beevers DG, Wise R, Davies P. Chlamydia pneumoniae antibody titers are significantly associated with acute stroke and transient cerebral ischemia: the West Birmingham Stroke Project. *Stroke; a journal of cerebral circulation.* Feb 1998;29(2):404-410.
71. Wimmer ML, Sandmann-Strupp R, Saikku P, Haberl RL. Association of chlamydial infection with cerebrovascular disease. *Stroke.* Dec 1996;27(12):2207-2210.
72. Kalayoglu MV, Libby P, Byrne GI. Chlamydia pneumoniae as an emerging risk factor in cardiovascular disease. *JAMA.* Dec 4 2002;288(21):2724-2731.
73. Ridker PM, Hennekens CH, Buring JE, Kundsin R, Shih J. Baseline IgG antibody titers to Chlamydia pneumoniae, Helicobacter pylori, herpes simplex virus, and cytomegalovirus and the risk for cardiovascular disease in women. *Ann Intern Med.* Oct 19 1999;131(8):573-577.
74. Fagerberg B, Gnarpe J, Gnarpe H, Agewall S, Wikstrand J. Chlamydia pneumoniae but Not Cytomegalovirus Antibodies Are Associated With Future Risk of Stroke and Cardiovascular Disease : A Prospective Study in Middle-Aged to Elderly Men With Treated Hypertension. *Stroke.* February 1, 1999 1999;30(2):299-305.

75. Pietroiusti A, Diomedi M, Silvestrini M, et al. Cytotoxin-Associated Gene-A-Positive Helicobacter pylori Strains Are Associated With Atherosclerotic Stroke. *Circulation.* July 30, 2002 2002;106(5):580-584.
76. Preusch MR, Grau AJ, Buggle F, et al. Association Between Cerebral Ischemia and Cytotoxin-Associated Gene-A-Bearing Strains of Helicobacter pylori. *Stroke.* August 1, 2004 2004;35(8):1800-1804.
77. Grau AJ, Buggle F, Lichy C, Brandt T, Becher H, Rudi J. Helicobacter pylori infection as an independent risk factor for cerebral ischemia of atherothrombotic origin. *Journal of the Neurological Sciences.* 2001;186(1-2):1-5.
78. Park MH, Min JY, Koh SB, et al. Helicobacter pylori infection and the CD14 C(-260)T gene polymorphism in ischemic stroke. *Thrombosis Research.* 2006;118(6):671-677.
79. De Bastiani R, Gabrielli M, Ubaldi E, et al. High Prevalence of Cag-A Positive *H. Pylori* Strains in Ischemic Stroke: A Primary Care Multicenter Study. *Helicobacter.* 2008;13(4):274-277.
80. Sawayama Y, Ariyama I, Hamada M, et al. Association between chronic Helicobacter pylori infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). *Atherosclerosis.* 2005;178(2):303-309.
81. Corrado E, Rizzo M, Tantillo R, et al. Markers of inflammation and infection influence the outcome of patients with baseline asymptomatic carotid lesions: a 5-year follow-up study. *Stroke.* Feb 2006;37(2):482-486.
82. Elkind MS, Ramakrishnan P, Moon YP, et al. Infectious Burden and Risk of Stroke: The Northern Manhattan Study. *Arch Neurol.* Nov 9 2009.
83. Espinola-Klein C, Rupprecht HJ, Blankenberg S, et al. Impact of infectious burden on progression of carotid atherosclerosis. *Stroke.* Nov 2002;33(11):2581-2586.
84. Espinola-Klein C, Rupprecht HJ, Blankenberg S, et al. Impact of infectious burden on extent and long-term prognosis of atherosclerosis. *Circulation.* Jan 1 2002;105(1):15-21.
85. Kiechl S, Egger G, Mayr M, et al. Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. *Circulation.* Feb 27 2001;103(8):1064-1070.
86. Kis Z, Sas K, Gyulai Z, et al. Chronic infections and genetic factors in the development of ischemic stroke. *New Microbiol.* Jul 2007;30(3):213-220.
87. Liao W. Endotoxin: possible roles in initiation and development of atherosclerosis. *J Lab Clin Med.* Nov 1996;128(5):452-460.
88. Kountouras J, Zavos C, Chatzopoulos D. Helicobacter pylori infection and the risk for coronary artery disease. *Saudi Med J.* May 2004;25(5):679-680, author reply 680-671.
89. Kowalski M, Pawlik M, Konturek JW, Konturek SJ. Helicobacter pylori infection in coronary artery disease. *J Physiol Pharmacol.* Sep 2006;57 Suppl 3:101-111.
90. Sundquist M, Quiding-Jarbrink M. Helicobacter pylori and its effect on innate and adaptive immunity: new insights and vaccination strategies. *Expert Rev Gastroenterol Hepatol.* Dec 2010;4(6):733-744.
91. Mayr M, Kiechl S, Mendall MA, Willeit J, Wick G, Xu Q. Increased risk of atherosclerosis is confined to CagA-positive Helicobacter pylori strains: prospective results from the Bruneck study. *Stroke.* Mar 2003;34(3):610-615.
92. Cremonini F, Gabrielli M, Gasbarrini G, Pola P, Gasbarrini A. The relationship between chronic *H. Pylori* infection, CagA seropositivity and stroke: meta-analysis. *Atherosclerosis.* Apr 2004;173(2):253-259.
93. Teles R, Wang CY. Mechanisms involved in the association between periodontal diseases and cardiovascular disease. *Oral Dis.* Jul 2011;17(5):450-461.
94. Kozarov E, Sweier D, Shelburne C, Progulske-Fox A, Lopatin D. Detection of bacterial DNA in atheromatous plaques by quantitative PCR. *Microbes Infect.* Mar 2006;8(3):687-693.

95. Nakano K, Inaba H, Nomura R, et al. Detection of cariogenic Streptococcus mutans in extirpated heart valve and atheromatous plaque specimens. *J Clin Microbiol.* Sep 2006;44(9):3313-3317.
96. Hsich E, Zhou YF, Paigen B, Johnson TM, Burnett MS, Epstein SE. Cytomegalovirus infection increases development of atherosclerosis in Apolipoprotein-E knockout mice. *Atherosclerosis.* May 2001;156(1):23-28.
97. Vliegen I, Duijvestijn A, Grauls G, Herngreen S, Bruggeman C, Stassen F. Cytomegalovirus infection aggravates atherogenesis in apoE knockout mice by both local and systemic immune activation. *Microbes Infect.* Jan 2004;6(1):17-24.
98. Rodriguez-Yanez M, Castillo J. Role of inflammatory markers in brain ischemia. *Curr Opin Neurol.* Jun 2008;21(3):353-357.
99. Rost NS, Wolf PA, Kase CS, et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke.* Nov 2001;32(11):2575-2579.
100. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* Apr 3 1997;336(14):973-979.
101. Bos MJ, Schipper CM, Koudstaal PJ, Witteman JC, Hofman A, Breteler MM. High serum C-reactive protein level is not an independent predictor for stroke: the Rotterdam Study. *Circulation.* Oct 10 2006;114(15):1591-1598.
102. Cesari M, Penninx BW, Newman AB, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation.* Nov 11 2003;108(19):2317-2322.
103. Everett BM, Kurth T, Buring JE, Ridker PM. The relative strength of C-reactive protein and lipid levels as determinants of ischemic stroke compared with coronary heart disease in women. *J Am Coll Cardiol.* Dec 5 2006;48(11):2235-2242.
104. Sattar N, Murray HM, McConnachie A, et al. C-reactive protein and prediction of coronary heart disease and global vascular events in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). *Circulation.* Feb 27 2007;115(8):981-989.
105. Wu JH, Haan MN, Liang J, Ghosh D, Gonzalez HM, Herman WH. Diabetes as a predictor of change in functional status among older Mexican Americans: a population-based cohort study. *Diabetes Care.* Feb 2003;26(2):314-319.
106. Haan MN, Mungas DM, Gonzalez HM, Ortiz TA, Acharya A, Jagust WJ. Prevalence of dementia in older latinos: the influence of type 2 diabetes mellitus, stroke and genetic factors. *J Am Geriatr Soc.* Feb 2003;51(2):169-177.
107. Rubicz R, Leach CT, Kraig E, et al. Seroprevalence of 13 common pathogens in a rapidly growing U.S. minority population: Mexican Americans from San Antonio, TX. *BMC Res Notes.* 2011;4(1):433.
108. Redeen S, Ryberg A, Petersson F, Eriksson O, Nagga K, Borch K. Homocysteine levels in chronic gastritis and other conditions: relations to incident cardiovascular disease and dementia. *Dig Dis Sci.* Feb 2010;55(2):351-358.
109. Heckbert SR, Kooperberg C, Safford MM, et al. Comparison of self-report, hospital discharge codes, and adjudication of cardiovascular events in the Women's Health Initiative. *Am J Epidemiol.* Dec 15 2004;160(12):1152-1158.
110. Simpson CF, Boyd CM, Carlson MC, Griswold ME, Guralnik JM, Fried LP. Agreement between self-report of disease diagnoses and medical record validation in disabled older women: factors that modify agreement. *J Am Geriatr Soc.* Jan 2004;52(1):123-127.
111. Beck JD, Offenbacher S. Systemic effects of periodontitis: epidemiology of periodontal disease and cardiovascular disease. *J Periodontol.* Nov 2005;76(11 Suppl):2089-2100.

112. Genco R, Offenbacher S, Beck J. Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *J Am Dent Assoc.* Jun 2002;133 Suppl:14S-22S.
113. Pussinen PJ, Alfthan G, Jousilahti P, Paju S, Tuomilehto J. Systemic exposure to Porphyromonas gingivalis predicts incident stroke. *Atherosclerosis.* Jul 2007;193(1):222-228.
114. Pussinen PJ, Alfthan G, Rissanen H, Reunanen A, Asikainen S, Knekt P. Antibodies to periodontal pathogens and stroke risk. *Stroke.* Sep 2004;35(9):2020-2023.
115. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation.* Feb 1 2011;123(4):e18-e209.
116. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. Chronic herpesvirus reactivation occurs in aging. *Exp Gerontol.* Jun 2007;42(6):563-570.
117. Trzonkowski P, Mysliwska J, Szmit E, et al. Association between cytomegalovirus infection, enhanced proinflammatory response and low level of anti-hemagglutinins during the anti-influenza vaccination--an impact of immunosenescence. *Vaccine.* Sep 8 2003;21(25-26):3826-3836.
118. Vecchione C, Frati A, Di Pardo A, et al. Tumor necrosis factor-alpha mediates hemolysis-induced vasoconstriction and the cerebral vasospasm evoked by subarachnoid hemorrhage. *Hypertension.* Jul 2009;54(1):150-156.
119. Moon SK, Cha BY, Kim CH. ERK1/2 mediates TNF-alpha-induced matrix metalloproteinase-9 expression in human vascular smooth muscle cells via the regulation of NF-kappaB and AP-1: Involvement of the ras dependent pathway. *J Cell Physiol.* Mar 2004;198(3):417-427.
120. Fernandez-Real JM, Vayreda M, Richart C, et al. Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. *J Clin Endocrinol Metab.* Mar 2001;86(3):1154-1159.
121. Lip GH, Wise R, Beevers G. Association of Helicobacter pylori infection with coronary heart disease. Study shows association between H pylori infection and hypertension. *BMJ.* Jan 27 1996;312(7025):250-251.
122. Whincup PH, Mendall MA, Perry IJ, Strachan DP, Walker M. Prospective relations between Helicobacter pylori infection, coronary heart disease, and stroke in middle aged men. *Heart.* Jun 1996;75(6):568-572.
123. Barnes RJ, Uff JS, Dent JC, Gear MW, Wilkinson SP. Long-term follow up of patients with gastritis associated with Helicobacter pylori infection. *Br J Gen Pract.* Jul 1991;41(348):286-288.
124. Omura Y. Microbial infection or trauma at cardiovascular representation area of medulla oblongata as some of the possible causes of hypertension or hypotension. *Acupunct Electrother Res.* 1988;13(2-3):131-145.
125. Sacco RL, Benjamin EJ, Broderick JP, et al. American Heart Association Prevention Conference. IV. Prevention and Rehabilitation of Stroke. Risk factors. *Stroke.* Jul 1997;28(7):1507-1517.
126. Liu L, Liu Y, Tong W, et al. Pathogen burden in essential hypertension. *Circ J.* Nov 2007;71(11):1761-1764.
127. Thorand B, Lowel H, Schneider A, et al. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. *Arch Intern Med.* Jan 13 2003;163(1):93-99.
128. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA.* Jul 18 2001;286(3):327-334.
129. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes.* Feb 2002;51(2):455-461.

130. So WY, Tong PC, Ko GT, et al. Low plasma adiponectin level, white blood cell count and Helicobacter pylori titre independently predict abnormal pancreatic beta-cell function. *Diabetes Res Clin Pract.* Nov 2009;86(2):89-95.
131. Gasbarrini A, Ojetti V, Pitocco D, et al. Helicobacter pylori infection in patients affected by insulin-dependent diabetes mellitus. *Eur J Gastroenterol Hepatol.* Jun 1998;10(6):469-472.
132. Ko GT, Chan FK, Chan WB, et al. Helicobacter pylori infection in Chinese subjects with type 2 diabetes. *Endocr Res.* Feb-May 2001;27(1-2):171-177.
133. Lutsey PL, Pankow JS, Bertoni AG, Szklo M, Folsom AR. Serological evidence of infections and Type 2 diabetes: the MultiEthnic Study of Atherosclerosis. *Diabet Med.* Feb 2009;26(2):149-152.
134. Xia HH, Talley NJ, Kam EP, Young LJ, Hammer J, Horowitz M. Helicobacter pylori infection is not associated with diabetes mellitus, nor with upper gastrointestinal symptoms in diabetes mellitus. *Am J Gastroenterol.* Apr 2001;96(4):1039-1046.
135. Migneco A, Ojetti V, Specchia L, et al. Eradication of Helicobacter pylori infection improves blood pressure values in patients affected by hypertension. *Helicobacter.* Dec 2003;8(6):585-589.
136. Harvey R, Lane A, Murray L, Harvey I, Nair P, Donovan J. Effect of Helicobacter pylori infection on blood pressure: a community based cross sectional study. *BMJ.* Aug 4 2001;323(7307):264-265.
137. Weikert C, Dierkes J, Hoffmann K, et al. B vitamin plasma levels and the risk of ischemic stroke and transient ischemic attack in a German cohort. *Stroke.* Nov 2007;38(11):2912-2918.
138. Iso H, Moriyama Y, Sato S, et al. Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. *Circulation.* Jun 8 2004;109(22):2766-2772.
139. Bostom AG, Rosenberg IH, Silbershatz H, et al. Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: the Framingham Study. *Ann Intern Med.* Sep 7 1999;131(5):352-355.
140. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet.* Nov 25 1995;346(8987):1395-1398.
141. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol.* Dec 1986;51(6):1173-1182.
142. Mackinnon D, Dwyer J. Estimating Mediated Effects in Prevention Studies. *Eval Rev.* 1993;17:144-158.
143. Hayes AF. PROCESS: A versatile computational tool for observed variable mediation, moderation, and conditional process modeling [White paper].2012.
144. Hayes A, Preacher KJ. Quantifying and testing indirect effects in simple mediation models when the constituent paths are nonlinear. *Multivariate Behav Res.* 2010;45:627-660.
145. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods.* Aug 2008;40(3):879-891.
146. Tanaka-Shintani M, Watanabe M. Distribution of ghrelin-immunoreactive cells in human gastric mucosa: comparison with that of parietal cells. *J Gastroenterol.* Apr 2005;40(4):345-349.
147. Nweneka CV, Prentice AM. Helicobacter pylori infection and circulating ghrelin levels - a systematic review. *BMC Gastroenterol.* 2011;11:7.
148. Miao Y, Xia Q, Hou Z, Zheng Y, Pan H, Zhu S. Ghrelin protects cortical neuron against focal ischemia/reperfusion in rats. *Biochem Biophys Res Commun.* Aug 3 2007;359(3):795-800.
149. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. *J Clin Invest.* Feb 2007;117(2):289-296.
150. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* Sep 1999;22(9):391-397.

151. Fritz MS, Mackinnon DP. Required sample size to detect the mediated effect. *Psychol Sci.* Mar 2007;18(3):233-239.
152. Allison PD. *Survival Analysis Using SAS® : A Practical Guide*. Second ed. Cary, NC: SAS Institute Inc.; 2010.
153. Engstad T, Bonaa KH, Viitanen M. Validity of self-reported stroke : The Tromso Study. *Stroke.* Jul 2000;31(7):1602-1607.
154. Di Carlo A, Candelise L, Gandolfo C, et al. Influence of different screening procedures on the stroke prevalence estimates: the Italian Longitudinal Study on Aging. *Cerebrovasc Dis.* Jul-Aug 1999;9(4):231-237.
155. Prevalence of chronic diseases in older Italians: comparing self-reported and clinical diagnoses. The Italian Longitudinal Study on Aging Working Group. *Int J Epidemiol.* Oct 1997;26(5):995-1002.
156. Tretli S, Lund-Larsen PG, Foss OP. Reliability of questionnaire information on cardiovascular disease and diabetes: cardiovascular disease study in Finnmark county. *J Epidemiol Community Health.* Dec 1982;36(4):269-273.
157. Sorensen SP, Boysen G, Jensen G, Schnohr P. Prevalence of stroke in a district of Copenhagen the copenhagen city heart study. *Acta Neurol Scand.* Jul 1982;66(1):68-81.
158. O'Mahony PG, Dobson R, Rodgers H, James OF, Thomson RG. Validation of a population screening questionnaire to assess prevalence of stroke. *Stroke.* Aug 1995;26(8):1334-1337.
159. Mittelmark MB, Psaty BM, Rautaharju PM, et al. Prevalence of cardiovascular diseases among older adults. The Cardiovascular Health Study. *Am J Epidemiol.* Feb 1 1993;137(3):311-317.
160. Kriegsman DM, Penninx BW, van Eijk JT, Boeke AJ, Deeg DJ. Self-reports and general practitioner information on the presence of chronic diseases in community dwelling elderly. A study on the accuracy of patients' self-reports and on determinants of inaccuracy. *J Clin Epidemiol.* Dec 1996;49(12):1407-1417.
161. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. *Stroke.* Sep 1996;27(9):1499-1501.
162. Colditz GA, Martin P, Stampfer MJ, et al. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol.* May 1986;123(5):894-900.
163. Reitz C, Schupf N, Luchsinger JA, et al. Validity of self-reported stroke in elderly African Americans, Caribbean Hispanics, and Whites. *Arch Neurol.* Jul 2009;66(7):834-840.
164. Steptoe A, Marmot M. The role of psychobiological pathways in socio-economic inequalities in cardiovascular disease risk. *Eur Heart J.* Jan 2002;23(1):13-25.
165. Phillips AC, Carroll D, Thomas GN, Gale CR, Deary I, Batty GD. The influence of multiple indices of socioeconomic disadvantage across the adult life course on the metabolic syndrome: the Vietnam Experience Study. *Metabolism.* Aug 2010;59(8):1164-1171.
166. Kaplan GA, Keil JE. Socioeconomic factors and cardiovascular disease: a review of the literature. *Circulation.* Oct 1993;88(4 Pt 1):1973-1998.
167. Hagger-Johnson G, Mottus R, Craig LC, Starr JM, Deary IJ. Pathways from childhood intelligence and socioeconomic status to late-life cardiovascular disease risk. *Health Psychol.* Jul 2012;31(4):403-412.
168. Glymour MM, Avendano M, Haas S, Berkman LF. Lifecourse social conditions and racial disparities in incidence of first stroke. *Ann Epidemiol.* Dec 2008;18(12):904-912.
169. Angel JL, Angel RJ, McClellan JL, Markides KS. Nativity, declining health, and preferences in living arrangements among elderly Mexican Americans: implications for long-term care. *Gerontologist.* Aug 1996;36(4):464-473.

170. National Health and Nutrition Examination Survey. Centers for Disease Control and Prevention/National Center for Health Statistics. http://www.cdc.gov/nchs/NHANES.htm. Accessed July 25, 2012.
171. O'Connor CM, Dunne MW, Pfeffer MA, et al. Azithromycin for the secondary prevention of coronary heart disease events: the WIZARD study: a randomized controlled trial. *JAMA.* Sep 17 2003;290(11):1459-1466.
172. Grayston JT, Kronmal RA, Jackson LA, et al. Azithromycin for the secondary prevention of coronary events. *N Engl J Med.* Apr 21 2005;352(16):1637-1645.
173. Cannon CP, Braunwald E, McCabe CH, et al. Antibiotic treatment of Chlamydia pneumoniae after acute coronary syndrome. *N Engl J Med.* Apr 21 2005;352(16):1646-1654.
174. Jespersen CM, Als-Nielsen B, Damgaard M, et al. Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. *BMJ.* Jan 7 2006;332(7532):22-27.
175. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost.* Nov 2011;106(5):858-867.
176. Gieffers J, Fullgraf H, Jahn J, et al. Chlamydia pneumoniae infection in circulating human monocytes is refractory to antibiotic treatment. *Circulation.* Jan 23 2001;103(3):351-356.
177. Beatty WL, Morrison RP, Byrne GI. Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. *Microbiol Rev.* Dec 1994;58(4):686-699.
178. Epstein SE, Zhu J, Najafi AH, Burnett MS. Insights into the role of infection in atherogenesis and in plaque rupture. *Circulation.* Jun 23 2009;119(24):3133-3141.
179. Miggins M, Hasan A, Hohmann S, et al. The potential influence of common viral infections diagnosed during hospitalization among critically ill patients in the United States. *PLoS One.* 2011;6(4):e18890.
180. Rupnow MF, Owens DK, Shachter R, Parsonnet J. Helicobacter pylori vaccine development and use: a cost-effectiveness analysis using the Institute of Medicine Methodology. *Helicobacter.* Dec 1999;4(4):272-280.
181. Azevedo NF, Huntington J, Goodman KJ. The epidemiology of Helicobacter pylori and public health implications. *Helicobacter.* Sep 2009;14 Suppl 1:1-7.