EMERGING, NON-HEREDITARY RISK FACTORS FOR STROKE:

THREE STUDIES

by

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy.

Baltimore, Maryland

April 2014

Abstract

Stroke is a leading cause of death and disability globally. The vast majority of all strokes occur in low- and middle-income countries, where data are least available to guide clinical decision-making and health policy. Many strokes are preventable, and established risk factors for stroke are well-known in the scientific literature. However, some risk factors may be less well-recognized but have a particular influence in specific subpopulations. Here, three risk factors for strokes are studied in three separate populations. Fatal stroke in Bangladesh is studied with an emphasis on the potential role of betel nut as a stroke risk factor. Human Immunodeficiency Virus infection and its relationship to stroke, including use of the predictive Framingham Risk Score, is studied in men who have sex with men in the United States. Finally, groundwater arsenic exposure in American Indians is analyzed as a risk factor for carotid artery disease endpoints which are subclinical measurement that directly relate to stroke risk. The relationship between these risk factors and stroke, including their implications for younger-onset stroke, stroke prevention, and advocacy for vascular diseases on the global health agenda are emphasized.

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Preface

I wish to acknowledge several people who have made this work possible and worthwhile and to whom I am extremely grateful. I thank Robert Black, my doctoral advisor who has provided countless hours of insight, support, and mentorship in this process. Beyond the work seen here, he has also provided ad hoc support on more than twenty other published commentaries, papers, reviews, and grants and sent dozens of letters of support and recommendation. As such, I have a new definition of what an academic leader is and should be. In particular, I recognize his steady, humble, and even-keeled nature. To me, he will remain the quintessential American academic of the type that keeps America's universities truly great.

I have had the good fortune to meet two of my closest friends at Johns Hopkins who ultimately became key collaborators and supports on two of the three main papers seen here and nearly a dozen others. Marco Carone, the "singing statistician" who "performs statistics exclusively after midnight" (now at University of Washington) and Takeshi Shinohara, "very nice, on top of everything, and probably should chair an IRB" (now at University of Pennsylvania), have taught me most of what I know about study design, statistics, and analyses. I am impressed by their selflessness and wish to emulate it.

I thank Ana Navas Acien, closely involved with the second paper, whose good nature and patience have been exceptional.

I thank the staff at Johns Hopkins Bloomberg School of Public Health, in particular Cristina Salazar and Rachel White who work as academic support staff in the Department of International Health and create a culture of excellence for students. I appreciate their help on a regular basis.

Avi Nath of the National Institute of Neurological Disorders and Stroke has been a source of enthusiasm for my work at all times.

I also wish to acknowledge the newer influences in my academic life: Merit Cudkowicz and Lee Schwamm at the Massachusetts General Hospital. I thank Merit for her constant gentleness, kindness, and ease of leadership. She typifies the many benefits of having women in leadership positions without reinforcing any of the painful clichés,

demonstrating power, when used well, can raise the entire profile of women in a specialty. I thank Lee for his wit, insight, and energy as I transition to my first real academic position in Boston. I am very fortunate to have them in my academic life.

I also thank Amartya Sen at Harvard University for his persistent support in spite of a busy schedule and several conversations and meetings on three continents over more than seven years that have helped shape my view of international academia. If only every day involved lunch with Amartya. I thank Steve Hyman, Broad Institute, for his insights into academic life in complex university environments. In general, I am encouraged that nice people make it to the top at big places.

My committee includes several excellent academic leaders: Larry Appel, Department of Epidemiology and the Welch Center; Bob Gilman, Department of International Health; Ciprian Crainiceanu, Department of Biostatistics; and David Sack, Department of International Health.

I have been fortunate to collaborate with several students, residents, and fellows - co-trainees - including Hannah McLane, Elizabeth Sweeney, Aaron Berkowitz, Sarah Wahlster, Kat Moon, Kiran Thakur, Jennifer Lyons, Bryan Smith, Kate Brizzi, Sanjay Menon, Antonio Moya, Hiral Shah, and Tarun Singhal.

I have had an almost innumerable number of foreign and domestic collaborators. Most important of these are Tarun Dua, World Health Organization Geneva; Ed Mills, University of Ottawa; Chencho Dorji, Jigme Dorji Wangchuk National Referral Hospital of Bhutan; Dixon Chibanda Harare Central Hospital Zimbabwe; Paul Spiegel United Nations High Commissioner for Refugees Geneva; Sunil Bahl National Polio Surveillance Unit of India New Delhi; Roland Sutter, World Health Organization Geneva; Nelson Martins National University of Timor-Leste Dili; Yunus Fakir, BRAC (formerly Bangladesh Rural Advancement Cooperative) Dhaka; Claire “Nana” Hugues Center for Tropical Medicine and Filariases in Yaoundé; Wai-Hai Xu Peking Union Medical Center Beijing; Adam Khalifa, United Nations High Commissioner for Refugees Damascus; Nurul Alam at ICDDR, B Matlab; Christopher Varady Caritas Lebanon Beirut; Luca Passerini, Bill & Melinda Gates Foundation; and several others.

Several of my mentors at the Mayo Clinic continue to support me and take an interest in my work, namely Keith Josephs, Eelco Wijdicks, Eric Sorenson, Sean Pittock, Alejandro Rabinstein, and Al Aksamit. Gretchen Birbeck of the University of Rochester (formerly Michigan State University) is an impressive neurologist who trail blazed global health and has taken an interest in me and become a good friend.

I thank my clinical mentors and teachers during fellowship including Justin McArthur, Carlos Pardo, and Peter Calabresi at Johns Hopkins Hospital. In particular, I thank Justin who was an integral part of my earlier development as an academic neurologist and has the impressive ability to always find time to talk to me. His doubt regarding global health research has demanded that I make the work better than I had planned. I thank Peter Calabresi for teaching me about multiple sclerosis in his clinics and showing me what it is.

My "social network" has listened with an unfaltering ear: Ed Leung, Anhar Hassan, Debika Shinohara, RajaNandini Muralidharan, Martha Elias, McHale Newport Berra, and Aleksander Videnovic.

I thank my funding sources including the Sommer Scholars Program at the Bloomberg School of Public Health, the American Academy of Neurology Foundation (now American Brain Foundation), the Canadian Institute of Health Research, the Canadian Federation of University Women, Grand Challenges Canada, the Canadian Stroke Network, Grand Challenges Canada, the Thrasher Research Foundation, private anonymous donations, the Massachusetts General Center for Global Health, and the Johns Hopkins Center for Global Health.

I have had several invitations to present my work at more than 20 locations internationally, and thank the people who decided that it was worth the time and money to invite me and talk about global issues in neurology. I thank Delta for upgrading me occasionally and my rickshaw drivers.

I thank my father who suffered a stroke and died from complications of it long before this idea of a PhD was considered or realized. And my maternal grandfather whose story was the same. If my father was still alive, I would expect him to say: "As far as I am concerned, Farrah-o, another degree is entirely optional."

I thank Marj Benson, a professor of law whose work was very dissimilar to mine - alternative dispute resolution – formerly at the University of Saskatchewan, who provided academic support to me by way of emails and telephone calls for 10 years before her very unusual death related to undiagnosed Celiac Disease in 2010.

I thank my mother who truncated her own educational opportunities so I could pursue mine.

When I began my studies in 2009, I purchased 11 plants from Ikea. Following several trips abroad and living in various locations, only one plant survived my absence. "Hardy" shall remain at Hopkins after my departure.

Occasionally, a process leads to an event. As atherosclerosis is to stroke, a dissertation is to a defense.

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Introduction

Stroke, a sudden, often catastrophic, disruption in cerebral blood supply, is a leading cause of mortality worldwide. Stroke accounts for 5.5 million of the 55 million deaths that occur globally each year (Mathers 2006). In Japan, the country with the longest life expectancy from birth, improvements in life expectancy are partially attributed to the large reduction in stroke mortality rates in the 1960s (Ikeda 2011). In the United States, it is estimated that someone suffers from a stroke every 40 seconds and dies from a stroke every 4 minutes (American Stroke Association 2012). Today, more than 85 percent of all strokes occur in low and middle income countries (Strong 2007). A meta-analysis of stroke incidence in high- versus low- and middle-income countries over a recent four-decade period found a statistically significant difference in stroke incidence with a 42% decrease in high-income countries and a greater than 100% increase in low- and middle-income countries (Feigin 2009). Stroke incidence is now higher in low- and middle-income countries than in high-income countries even though life expectancy is higher in wealthier settings.

A majority of strokes, approximately 80%, are ischemic in nature, due to the sudden interruption in cerebral blood flow causing loss of oxygenation and nutrient availability to the brain tissue. Hemorrhagic stroke accounts for approximately 20% of all strokes, although the proportion may differ depending on the population and its underlying demographic profile and risk factors. For example, in China nearly 30% of strokes are hemorrhagic compared to 10% in Denmark (Zhang 2003, Andersen 2009). Approximately 25 percent of people with stroke die within the first month and half are

dead within one year (Donnan 2008, Hankey 2000). In high income settings, more than 20 percent of people ages 15 to 44 years old die within one month of their first stroke (Groppo 2012). Although stroke is more common in men, women are more likely to experience fatal stroke (Reeves 2008). A majority of people at high risk for stroke are unaware of its major risk factors and its presenting features. This suboptimal awareness of stroke is more common in the highest risk groups, including older women and ethnic minorities (Ferris 2005).

A high number of strokes are preventable in all countries. A recent case-control study of 3000 cases and 3000 controls in 22 countries on 5 continents found that 90 percent of the risk of stroke could be accounted for by 10 risk factors (O'Donnell 2010). Risk factors for stroke may be separated into modifiable and non-modifiable components. Age is the most important non-modifiable risk factor for stroke with the risk of stroke doubling for every decade of life after age 55 years old (American Stroke Association 2012). In 1991, the Framingham Heart Study created a 10-year risk of stroke "health appraisal function" using 9 risk factors. These included age, systolic blood pressure, the use of antihypertensive therapy, diabetes mellitus, cigarette smoking, prior cardiovascular disease (coronary heart disease, cardiac failure, or intermittent claudication), atrial fibrillation, and left ventricular hypertrophy by electrocardiogram (Wolf 1991).

The American Heart Association recognizes age, family history, African American race, sex, and prior stroke, transient ischemic attack, or heart attack as non-modifiable risk factors for stroke. Potentially modifiable risk factors for stroke are common and include

high blood pressure, cigarette smoking, diabetes mellitus, carotid or other artery disease, peripheral artery disease, atrial fibrillation, other heart disease (e.g. dilated cardiomyopathy), sickle cell anemia, high blood cholesterol, poor diet (i.e. high saturated and *trans*-fat diets and cholesterol), and physical inactivity and obesity. As a response to these known risk factors, there is a public health initiative to attack the "simple 7" steps that an individual should take to reduce his or her risk of stroke: (1) get active, (2) eat better, (3) lose weight, (4) stop smoking, (5) control cholesterol, (6) manage blood pressure, and (7) reduce blood sugar (American Stroke Association 2012).

Emerging evidence however suggests that there are environmental and other exogenous risk factors for stroke that may often go unrecognized. These include ambient temperature, season, air and water pollutants, geographic location, infections, medications, and tobaccos among environmental and exogenous risks. Although exogenous and environmental risk factors are less well-documented, they may be pervasive, modifiable, and synergistic with other comorbidities in some populations. The American Heart Association recognizes drug abuse, notably cocaine, alcohol abuse, nondescript socioeconomic factors, and geographic location among the "other" risk factors for stroke in populations. In some cases, these risk factors directly affect an individual's risk of hypertension or heart disease, making the direct impact of the risk factor on the pathophysiology of the cerebrovasculature difficult to separate from important stroke risk factors.

Emerging environmental and exogenous risk factors for stroke may be poorly studied because the populations at risk are marginalized, difficult to engage in research studies, difficult to access geographically, or not routinely studied for vascular disease due to competing priorities in the study of risks for communicable diseases. Stroke registries and stroke units in developing countries remain uncommon (Langhorne 2012). When present, stroke centers are found in urban locations where environmental risks may not be most pronounced and many at-risk populations will be unable to access comprehensive care. In high income countries, epidemiological studies of cardiovascular and cerebrovascular disease may also poorly capture unique stroke risk factors. Major studies including the Framingham study, the Cardiovascular Health Study (CHS), and the Atherosclerosis in Communities (ARIC) study recruited volunteers. Age restriction is also common including the MONICA study by the World Health Organization (limited mostly to <65 years old), Framingham study (recruitment of 30 to 60 year olds), CHS (>65 years old), and ARIC (45 to 64 years old) (Rothwell 2005). Study sites near major academic centers may be convenient to study traditional risk factors in urbanized populations but are less ideal for studying particular environmental risks and populations with the highest degrees of poverty.

Overview of the Non-hereditary, Environmental Risk Factors for Ischemic and Hemorrhagic Stroke

The following section will provide a topical overview of important, non-hereditary, and emerging risk factors for ischemic and hemorrhagic stroke worldwide. Although this is

not an exhaustive review of the entire literature of the putative risk factor for all populations, the aim of this section is to highlight the range, magnitude, and potential impact of these risk factors as well as provide insight into the state of the scientific and epidemiological literature on the risk factor from various locations. All included articles are from peer-reviewed journals and represent original research or reviews of research studies.

Ambient Temperature, Time, and Season

The relationship between stroke and ambient temperature has been debated for several decades. Blood pressure is known to be higher in winter months (Brennan 1982). There is no consensus on the relationship between ischemic and hemorrhagic stroke and ambient temperature, although extremes of temperature may have an important effect on stroke incidence and mortality. Likely, many locations are non-comparable and ecological associations with temperature depend strongly on the underlying risk factors in the population. Notably, locations of more sustained extreme heat are less likely to have population-based stroke registries. In Chinese individuals in Taiwan, a U-shaped relationship between temperature and cerebral infarction was noted with the least number of deaths reported at days with temperatures between 27 to 29 degrees Celsius (Pan 1995). When the temperature was 32 degrees, the risk of ischemic stroke in people over 64 years old was 66 percent higher compared to the 27 to 29 degree Celsius range. Mortality due to hemorrhagic stroke decreased by 3.3% for every 1 degree Celsius increase in temperature. By contrast, a report of the Framingham Study (Kelly-Hayes

1995) found that the incidence of embolic stroke among people aged 30 to 62 years old peaked in winter during a 40-year observation period. In Japan (Shinkawa 1990), stroke incidence varied by season for those with hypertension, normotension, and both elevated cholesterol and low cholesterol levels. High intra-diurnal temperature change and lower temperature predicted increased incidence of both hemorrhagic and ischemic stroke (Shinkawa 1990). In Oxfordshire, England, an increased incidence of ischemic stroke was noted in summer months, but there was an overall non-significant variation of stroke incidence by season (Rothwell 1996). Primary intracerebral hemorrhage occurred more often than expected at temperatures less than 2 degrees Celsius. It was concluded that the excess ischemic strokes seen in winter in other studies was due to excess mortality during wintertime strokes and the use of mortality registries for describing stroke incidence.

The Framingham Study noted increased stroke incidence by day of the week (Monday) and time of day (8 AM to noon) (Kelly-Hayes 1995). The increased incidence of stroke on Mondays was attributed to starting the new work week in the United States. Multiple subsequent studies have related the morning surge in mean blood pressure with a peak risk of stroke during the morning hours of the day, including higher likelihood of fatal stroke (Shimizu 2011).

Geographic Location

Location of birth and residence can impact incidence of stroke. One of the best studied examples of the influence of location is the so-called "stroke belt" in the Southeastern United States. The term "stroke belt" came into use in the 1960s to describe the

nonrandom, extreme magnitude of difference in stroke incidence between this region and the rest of the U.S. The National Heart, Lung, and Blood Institute (NHLBI) described the stroke belt as a 1980 age-adjusted stroke mortality rate of 10% or more above the national average. This included Alabama, Arkansas, Georgia, Indiana, Kentucky, Louisiana, Mississippi, North and South Carolina, Tennessee, and Virginia (Lanska 1995). In the 1920s, stroke incidence was likely highest in the northeast Atlantic states, but this incidence gradually fell, leaving the excess stroke incidence in the southern states. Notably, the stroke belt is not subject to political or state boundaries and affects both sexes and whites and blacks. Both birth and adult residence in the stroke belt predict stroke mortality (Glymour 2009). Chronic disease prevalence, socioeconomic status, dietary intake, and race account for some but not all of the heightened risk of stroke (Lanska 1995, Liao 2009). Geographic variations have also been noted in other countries, including Spain and China (Castilla-Guerra 2010, Chau 2011). High altitude may also be a risk factor for stroke in some countries, but has been studied in a limited manner. In one population-based study in Peru, the risk of stroke was higher in a high altitude town of Cuzco (3380 meters above sea level), which was related to polycythemia vera, but also lifestyle factors such as sedentary lifestyle and alcohol use (Jaillard 1995). A study of high altitude counties in the United States found no significant relationship between the altitude of a county above sea level and changes in stroke mortality rate (Ezzati 2012).

Pollutants

Air pollution is composed of a heterogeneous mixture of particulate matter and gases (e.g. ozone, carbon monoxide). Early studies, including analyses of the Great London Fog of 1952, a renowned pollution episode of the 20th century, found an increased risk of stroke deaths attributable to a short-term extreme rise in air pollution (Logan 1953). This relationship continues to be demonstrated even at lower levels. In most cases, exposure to each pollutant reported, including gases and particles of varying sizes, is associated with an increased risk of cerebrovascular events. Numerous between-study differences exist, including demographic characteristics, measured pollutants, temporal risk associations, and the ascertainment of stroke and stroke outcomes, making direct comparisons difficult. However, the relative risk for stroke-related mortality may reach 2.04 for a 21.3 $\mu g/m^3$-increase in same-day PM_{10} levels (elderly individuals in Seoul, South Korea) (Hong 2002), whereas the incidence rate increase for stroke hospitalizations may reach 13% (95% confidence interval [CI], 4%-22%) when previous-day PM_{10} levels are greater than 30 $\mu g/m^3$ vs. less than 15 $\mu g/m^3$ (in Sweden) (Oudin 2010). In general, the risk of stroke appears to be greater for ischemic stroke compared with hemorrhagic stroke, and the association with PM is at least as strong as with gaseous pollutants.

In a prospective study that evaluated both stroke and heart disease outcomes in women (Miller 2007), long-term exposure to air pollution was associated with an increased risk for a cerebrovascular disease event (odds ratio, 1.35; 95% CI, 1.08-1.68) that was comparable with that for myocardial infarction (odds ratio, 1.06; 95% CI, 0.85-1.34) and all coronary disease events (odds ratio, 1.21; 95% CI, 1.08-1.68).

Multiple heavy metals found in the environment have had inconsistent links to the risk of stroke. Special attention will be given to arsenic since it will be the focus of a part of this thesis proposal. Arsenic has been identified as a potential risk factor for cerebrovascular disease, particularly through its putative role in potentiating atherosclerosis (Engel 1994, Simeonova 2004). In concentrations >500 micrograms/L, arsenic in drinking water has been associated with increased risk of ischemic heart disease and carotid atherosclerosis (Chen 2011), and possibly hypertension (Abhyankar 2012). The relationship between arsenic ingestion and stroke incidence and stroke fatality is not well established. A systematic review of the relationship between arsenic exposure and incident stroke found the relative risk to be 1.19-2.69 in studies from Taiwan, 0.69-1.53 in general populations elsewhere, and 0.30-1.33 for stroke fatality in occupationally exposed populations (Navas Acien 2005). An ecological study of arsenic levels, using population-weighted average arsenic concentrations by county in Michigan found a modestly increased relative risk of stroke hospitalization (1.03, 95% CI 1.01-1.05) per microgram/L increase in arsenic exposure in the county (Lisabeth 2010). However, similar degrees of association were observed between county-level arsenic exposure and duodenal ulcer and hernia, diseases which lack a known relationship with arsenic exposure. In Taiwan, where arsenic levels are generally higher than in the USA, a dose-dependent relationship was found between measured arsenic concentrations in the well water of 8102 participants and the prevalence of cerebral infarction with odds ratios of 1.0, 3.4, 4.5, and 6.9 for well water with arsenic content of <0.1, 0.1-50.0, 50.1-299.9, and >300 micrograms/L, respectively (Chiou 1997). There was a biological gradient between long-term arsenic exposure and carotid

atherosclerosis in one study of endemic arseniasis in Taiwan (Wang 2002). Important limitations of previous research include ecological designs, lack of adjustment for relevant confounders, uncertain outcome ascertainment, and lack of information on stroke subtype.

Cadmium has been less well studied. One report of 50 maintenance hemodialysis patients without known atherosclerotic disease found that cadmium was an independent determinant of carotid intimal-medial thickness (CIMT) compared to age- and sex-matched individuals (Ari 2011). The Atherosclerosis Risk Factors in Female Youngsters study identified cadmium as an independent risk factor for high CIMT as well (Messner 2009). Cadmium is associated with lower aortic pulse wave velocity, lower pulse pressure in multiple arteries, and higher femoral distensibility (Schutte 2007), as well as prevalence of stroke and heart failure using retrospective self-report data (Peters 2010).

Infections

Multiple infections have been associated with later risk of stroke. These can be separated into acute systemic infection, chronic systemic infection, and nervous system-specific infection. There is also recognition that influenza vaccine is associated with stroke, although the relationship remains unclear. Nonspecific systemic infection, most commonly upper respiratory tract infections, may predispose to stroke risk for approximately 1 week following the infectious illness (Grau 2010). Likely, it is the nonspecific procoagulant effects of the infectious illness and an inflammatory response affecting the vasculature that are important to the host rather than a specific microbial

agent. Chronic infectious states that may predispose to stroke include periodontitis, chronic bronchitis, infection with *Helicobacter pylori,* and infection with *Chlamydia pneumoniae*. Specific known agents which increase the risk of stroke in some individuals include *Treponema pallidum* (neurosyphilis), *Mycobacterium tuberculosis* (neurotuberculousis), *Borrelia burgdoferi* (intracranial vasculitis), and rickettsial diseases among bacteria (Grau 2010).

Several studies have suggested an increased risk of stroke in HIV+ adults (Evers 2003, Cole 2004, Corral 2009, Rasmussen 2011, Chow 2012, Vinikoor 2013). One study from Spain found that high alcohol intake, a history of a diagnosis of AIDS, and fewer months on HAART increased the risk of stroke, although only 25 patients with stroke were identified (Corral 2009). HIV infection itself may portend a higher stroke risk, even when accounting for demographic and traditional stroke risk factors. A U.S. study found that HIV remained an independent predictor for stroke when comparing HIV+ versus HIV- individuals from different cohorts even after adjustment for known risk factors. Most others in high income settings suggest that HIV increases stroke risk. In South Africa, however, most studies report similarities in patient characteristics and stroke incidence between HIV+ and HIV- groups (Hoffmann 2000, Mochan 2003, Patel 2005). The reasons for increased rates of stroke among HIV+ men could include such mechanisms as chronic inflammation leading to atherogenic tendency of the endothelium, remodeling of the intracranial and carotid vasculature, and higher frequency of circulating inflammatory markers (Ortiz 2007, Seaberg 2010, Falcone 2011, Sen 2012). In the multinational STACCATO study (Calmy 2009), inflammatory markers associated

with increased cardiovascular risk, such as soluble vascular adhesion molecule-1 (sVCAM-1), were associated with HIV RNA replication and decreased with HAART initiation. In a separate study by the SMART/INSIGHT and D.A.D. working groups (SMART 2008), Abacavir, found in some HAART regimens, was associated with an increased risk of all-cause cardiovascular disease which included stroke. The relationship between HAART and longterm stroke risk in general requires further investigation since typical antiretroviral treatment has changed over time. There may also be important risk factors that confound the relationship between stroke and HIV such as injection drug abuse with cocaine, excessive alcohol use, or poor medication adherence.

Herpes zoster and herpes simplex viruses may increase the risk of stroke, particularly in the immunosuppressed. Hyperviscosity may result from cryoglobulinemia in specific hepatitides (e.g. hepatitis C). Multiple fungal infections may invade the cerebral vasculature with *Aspergillus fumigatus* being an important cause of hemorrhagic stroke in the immunosuppressed. Parasites such as *Trypansoma cruzi* may increase the stroke risk through dilated cardiomyopathy which predisposes to intracardiac thrombosis and embolic phenomena. Schistosomiasis may lead to a vasculitic type of stroke.

Dietary Components

There is a significant association between stroke risk and the number of fast food restaurants in a neighborhood, even after controlling for demographic characteristics and socioeconomic status (Morgenstern 2009). Perhaps the most ubiquitous dietary risk factor

for stroke is salt consumption (Brown 2009). Deliberate and significant reductions in salt intake through efforts in the food industry and public health campaigns over more than 2 decades have been associated with an approximately 60% reduction in stroke mortality in Finland (Puska 1998). In the Northern Manhattan Study (NOMAS), the risk of stroke increased by 17% (95% CI 7-27%) for each 500mg/day increase in mean daily sodium intake (Gardener 2012). A large meta-analysis involving more than 5000 acute vascular events found that a 5-gram daily difference in salt intake represented a 23% difference in risk of fatal and nonfatal stroke (Strazzullo 2009).

In terms of full dietary approaches, the Mediterranean or DASH (Dietary Approaches to Stop Hypertension) diets, diets high in potassium, low-salt and low-added sugar diets, and diets that are at the daily recommended energy requirements may lower stroke risk (Hankey 2012). Multiple nutrients have been studied in detail. Notably, high chocolate, high whole grain, moderate coffee (3-4 cups/day), moderate tea (3 or more cups/day), reduced-fat milk, fish, and high fruit and vegetable (5 or more servings daily) are all associated with lowered stroke risk. By contrast, processed meat, total meat, and sodas are associated with increased stroke risk. There was no recognizable effect of full-strength milk, unprocessed meats, or rice (Hankey 2012). Further work on carotenoids, flavonoids, ω3-fatty acids, and high glycemic index foods is required (Medeiros 2012). Dietary supplementation with vitamin C, vitamin E, beta-carotene, and calcium do not prevent stroke. Vitamin A and E supplementation may increase all-cause mortality, and vitamin D supplementation is being studied. Potassium supplementation may reduce stroke risk (Hankey 2012).

Tobaccos and Medications

Multiple medications have been associated with stroke. The most important of these in recent years have been selective cyclooxygenase-2 inhibitors (*Vioxx,* Rofecoxib), widely prescribed for inflammatory pain, and phenylpropanolamine which was commonly found in over-the-counter cough and cold remedies as well as appetite suppressants in the U.S. (Kernan 2000; Baron 2008; Roumie 2008). Oral hormone replacement therapy, at one time thought to reduce the risk of cardiovascular disease, is associated with an approximately 30-40 percent increase in ischemic stroke. This has been found with both estrogen and combination of estrogen and progesterone hormone replacement (Wassertheil-Smoller 2003, Arana 2006, Sare 2008). The use of transdermal estrogens however does not appear to increase the risk of stroke based on a recent case control study of more than 15,710 cases of stroke including 103 transdermal estrogen patch users (Renoux 2012). Oral contraceptive pill use is also associated with a modest increase in atherothrombotic stroke risk, as very recently reported by observation of more than 1.6 million Danish women on various hormonal combinations (Lidegaard 2012).

Cigarette smoking is a well-recognized risk factor for stroke and has been reviewed in detail elsewhere (Shinton 1989, Ockene 1997). Importantly, passive, chronic exposure to cigarette smoke can also increase the risk of hemorrhagic and ischemic stroke in never-smokers (Zhang 2005, He 2008). There have been few prior studies on the risk of stroke from betel nut consumption. In a prospective cohort study (Wen 2005), *The Survey of Health and Living Status of the Near Elderly and Elderly in Taiwan*, past or current betel

nut chewing increased the risk of stroke death in individuals age 50 years and older (hazard ratio 1.66, 95% CI 1.19-2.30). However, only 14% of the cohort had ever chewed betel nut and they accounted for just 51 stroke deaths. In a separate Taiwanese study in 2001, based on the *National Health Interview Survey*, the relative risk of stroke death among betel chewers was 1.3 (95% CI 0.8-2.2). There were 22 stroke deaths among betel chewers, all of whom also smoked tobacco (Lan 2007).

Life Events and Social Setting

Life events may be a trigger for ischemic strokes. A Canadian study of 24,315 emergency room visits for stroke and 16,088 visits for transient ischemic attacks, and 29,090 for acute myocardial infarction found that all three events occurred with higher than expected frequency on the patient's birthday. A similar association was not observed for the control conditions of asthma, appendicitis, or head trauma. The risk was higher in patients with known hypertension, suggesting that stress associated with birthdays may trigger strokes and TIAs in patients with predisposition to these disorders (Saposnik 2006). In Finland, it was found that young adults (16 to 40 years old) and women have a higher incidence of strokes on weekends and holidays, especially during the evening hours (Haapaniemi 1996). A systematic review (Guiraud 2010) also identified binge alcohol abuse of 40 to 60 grams within the preceding 24 hours or >150 grams within the preceding week to increase the risk of stroke (odds ratio 2.66, 95% CI 1.54-4.61 and 2.47, 95% CI 1.52-4.02). Other putative risk factors for triggering a stroke

event included anger, heavy eating, negative or positive emotions, sudden postural change in response to startle, and psychological distress (Guiraud 2010).

Social circumstances beyond socioeconomic status may also affect risk of stroke. A study of 22,818 individuals over 50 years old in the Health and Retirement Study in the U.S.A. found the highest risk of stroke among widowed men (hazard ratio 1.40, 95% CI 1.12-1.74 compared to married women). Low income and lack of wealth were associated with stroke incidence in married and unmarried women and men, but unmarried women had the highest risk of all groups when affected by low income (Maselko 2009). Parity may also influence the risk of ischemic and hemorrhagic stroke. In the Shanghai Women's Health Study (SWHS), stroke prevalence increased with increasing number of children in both women and men, including after adjustment for socioeconomic status and other potential confounders (Zhang 2009). A separate study (Skilton 2009) found that a higher number of children is associated with increased carotid atherosclerosis in women but not men. The authors concluded that childbearing and not childrearing may be a risk factor for atherosclerosis. In Korea, each additional pregnancy increased the risk of hemorrhagic stroke in women, including intracerebral hemorrhage and subarachnoid hemorrhage when studied separately (Jung 2010). A relationship between stroke mortality and parity was not found after adjustment for socioeconomic status in Norway (Jacobsen 2011). Importantly, risk factors for stroke, such as obesity and smoking, may be shared in social networks beyond the influence of neighborhoods and location (Christakis 2007).

A population-based case–control study of 1250 stroke deaths in rural Bangladesh

Introduction

In 2008, cerebrovascular disease was ranked as the second most common cause of death globally, accounting for 6.2 million of the 57 million deaths that occurred (World Health Organization 2008). Stroke is an increasing burden of disease and leading cause of death in least developed countries, but population studies are lacking. People of Bangladeshi origin who have emigrated to the United Kingdom experience persistent and substantial inequalities in stroke mortality compared with the age matched UK population (Wild 2007). The possibilities for this excess risk are poorly understood and may include higher rates of uncontrolled hypertension, chronic inflammation, vitamin D deficiency, betel nut consumption, and squatting and straining at stool (Bhopal 2005). Studies on the risk of stroke in Bangladeshis in Bangladesh are limited. A study of more than 15 000 people during a door-to-door survey found the prevalence of stroke above the age of 40 years to be three per 1000 adults. There were more strokes in men than in women and in rural compared with urban areas (Mohammad 2011). In a hospital-based study in Chittagong, patients with stroke had high rates of hypertension (59%), ischemic heart disease (19%), and dyslipidemia (11%) (Mollah 2007). In Mymensingh, 66% of hospitalized stroke patients had a known diagnosis of hypertension and 63% were physically inactive prior to onset (Haque 2008).

Although stroke may be a major cause of death in rural Bangladesh, little population-based data on the underlying prevalence of risk factors and attributable mortality are available. A health and demographic surveillance system (HDSS) site has been used to monitor birth, migration, and all-cause mortality in rural Bangladesh since 1966. Over a recent 4-year period, there have been more than 1000 deaths attributed to stroke at this site. Here we analyze data from a population-based mortality surveillance site in rural Bangladesh to (i) determine the burden of fatal stroke in a least developed country and (ii) establish important risk factors for fatal stroke in this population.

Methods

Ethics approval

The institutional review board at ICDDR, B approved the collection of verbal autopsy data at Matlab, Bangladesh. The Johns Hopkins University Bloomberg School of Public Health Institutional Review Board deemed the protocol for reporting the de-identified verbal autopsy data exempt from further review.

Study design and subjects

A HDSS site operates in Matlab, Bangladesh, 55 km southwest of the capital city, Dhaka. Matlab had an average of 223 886 people living in 142 villages in 2008 (ICDDR, B 2009). Verbal autopsy, an interview-based method to determine the cause distribution of

deaths in a population, is performed following every death of a Matlab resident. During a verbal autopsy, family members and close contacts are asked about the symptoms and health status of the deceased prior to the time of death. To participate, the person who reports to the interviewer must be (i) closely related to the deceased, (ii) present during the illness that led to death, and (iii) able to describe the illness symptoms and medical consultations prior to death. A series of standard questions on a validated survey instrument were asked by trained, nonmedical community-level interviewers. The current survey used in Matlab was developed by the World Health Organization and modified by the INDEPTH Network for use in multiple surveillance sites in 2003 (Alam 2010). The 10th edition of the International Disease Classification (ICD) was employed at that time. The cause of death for an individual was assigned by medical officers who reviewed the questionnaire responses. A standard diagnostic code was listed as the primary cause of death, based on the first three digits of the ICD-10 code. All causes of death are monitored in this way, although the traditional use of this data has been for the surveillance of maternal and early childhood deaths. Previous evaluation of the monitoring of deaths by verbal autopsy in Matlab found that 99.9% of all deaths are recorded by verbal autopsy and 90% of all deaths occur at home (Alam 2010).

Amongst deceased persons with an ICD-10 diagnosis of stroke, demographic and clinical information, including sex, education level, occupation, coexistent illnesses including diabetes, hypertension, habitual use of various forms of tobacco (cigarette smoking/biri, pipe smoking/hookah, tobacco powder/gool, and betel), coronary heart disease, location

of death, and month and age at death, were recorded. All information on comorbidities and cause of death were derived from the verbal autopsy questionnaire and narratives. No medical records of the decedents who sought care were reviewed for this study. Only people aged 20 years and above at the time of death were considered adults in this study.

Statistical analysis

All stroke deaths from 1 January 2005 through 31 December 2008 were included in this analysis and compared with all adult injury deaths. Adult injury deaths were used as controls because they represented a large number of adult deaths in this population that could not be attributed to traditional vascular risk factors.

Risk factors for stroke versus injury deaths were assessed using a multivariable logistic regression model, adjusting for age and sex. Confidence intervals for odds ratios were obtained by inverting a profile likelihood ratio test statistic (Venables 2002). The distribution of given characteristics or risk factors amongst several subgroups was compared using a Pearson's chi-square test. An estimate of the proportional mortality because of stroke in the population was obtained using the relationship:

$$Prevalence = [Probability\ of\ positive\ test + Specificity - 1]/[Sensitivity + Specificity - 1]$$

where sensitivity and specificity refer to the verbal autopsy instrument for stroke, prevalence is the proportional mortality of stroke, and probability of a positive test is the proportion of deaths in the population of interest deemed to be because of stroke by this same instrument. Unrecorded measurements were assumed to be missing completely at random. The programming language R (R for Mac OS X GUI 1.35; R Foundation for Statistical Computing, Vienna, Austria) was used to conduct all analyses. All tests of hypotheses were performed under two-sided alternatives at confidence level 0.95.

Results

There were 1250 deaths because of stroke and 246 deaths because of unintentional injury. 1250 stroke deaths occurred of 4870 total adult deaths (population attributable mortality 25.2%) (Table 1). The baseline characteristics of people with stroke were compared with those who died of unintentional injuries (Table 2). Unintentional injury deaths in this population were most often because of falls (25.2% of all injury deaths), drowning (15.5%), train or road accident (12.7%), and acid burns (9.5%).

People who died of stroke had a higher mean age. Likely due to older age, people who died of stroke were also less likely to have received a formal education. More than 90% of stroke deaths occurred at home. Potential risk factors for stroke death were compared between women and men in Table 3. Men who died of stroke were significantly more likely to have diabetes, smoke cigarettes, consume betel, and have attained a higher education than women. There were more strokes in winter (December to March) than summer (June to September) ($P < 0.001$) (Figure 1). The multivariable logistic regression

model for risk of stroke death is presented in Table 4. There were 1140 betel chewers in this study, including 1053 who died of stroke. Overall, there were 403 people who chewed betel and smoked cigarettes (n = 377 stroke deaths, n = 26 injury deaths), 733 who only chewed betel (n = 672 stroke, n = 61 injury), 111 who only smoked cigarettes (n = 98 stroke, n = 13 injury), 132 who used neither betel nor cigarettes (n = 99 stroke, n = 33 injury), and 145 with incomplete data on betel use and/or smoking. Using published sensitivity (81.5%) and specificity (93.6%) of a similar verbal autopsy instrument, validated in Chinese adult deaths (Yang 2006), with the equation depicted earlier, the proportional mortality because of stroke was estimated to be 17.8%.

Discussion

This large, population-based study from a rural, resource-limited setting captured a high number of stroke deaths. Although modifiable risk factors for stroke are generally overlooked in least developed countries, many of which are experiencing the late stages of the epidemiological transition, they represent a significant, remediable, and potentially preventable burden of disease. Approximately 40% of all stroke deaths occurred in persons <60 years old in rural Bangladesh in this study, making stroke in the younger adult a particularly important target for health services intervention. In contrast to the high need, a recent survey (Bleich 2011) found just 11 non-governmental organizations address noncommunicable diseases in Bangladesh, including 2 that include a focus on cardiovascular disease and none on stroke. There is also no emergency thrombolysis for acute ischemic stroke in Bangladesh, even in major cities (Mateen 2010).

Our study emphasizes the potentially high burden of hypertension in the rural Bangladeshi population and its important contribution to fatal stroke death in men and women. In the Bangladeshi population in general, rates of hypertension and risk of diabetes are high (Zaman 1999, Zaman 2001, Sayeed 2002, Bangladesh NCD Risk Factor Survey 2010), portending a high stroke burden in the coming years. The prevalence of systolic hypertension in Bangladesh ranges from 9 to 18% in reported studies (Zaman 1999, Sayeed 2002). One study of 2361 native Bangladeshis in the year 2000 found that 14% of people over 20 years old had systolic hypertension and 9% had diastolic hypertension (Bangladesh NCD Risk Factor Survey 2010). A more recent study in 2009–2010 reported 17.9% of 9275 adults over 25 years old had hypertension (Sayeed 2002). The prevalence of hypertension at this study site and surrounding regions has been previously reported to be 12% amongst adults of age 25 years and older, with 40% of diagnoses made by unqualified medical providers (Khanam 2011). Younger patient age, lower levels of formal education, and diagnosis by an unqualified provider were related to antihypertensive medication non-adherence (Khanam 2011). Although not specifically studied in this population, arsenic exposure has also been proposed as a trigger for hypertension amongst well-water drinkers in rural Bangladesh (Rahman 1999). Diabetes also accounts for a significant burden of disease and was a risk factor for fatal stroke in this study. In rural Mymensingh district, systematic measurements of blood glucose in 4923 subjects over 20 years old revealed that 3.8% had diabetes and 13.0% had impaired fasting glucose (Sayeed 2003). Our findings are likely generalizable to other populations studied in the Indian subcontinent who have a high prevalence of hypertension and

diabetes, including Nepal (34% hypertension, 6% diabetes) (Sharma 2011), Sri Lanka (19% hypertension, 14% diabetes, 14% impaired fasting glucose) (Wijewardene 2005) and India (ranging from 21 to 36% for hypertension, 35% diabetes) (Singh 2011, Gupta 2011, Kaur 2011, Joshi 2012). Seasonality is also a potential contributor to fatal stroke incidence. One study in a referral hospital in Bangladesh found a significantly higher rate of ischemic stroke in summer compared with winter months (Hannan 2001). This study demonstrates the opposite trend: fatal stroke incidence was higher in the winter months. The reasons for this finding are unclear, and literature from other countries (Klimaszewska 2007) in which colder weather is correlated with vascular events cannot be easily generalized to this setting where winter months are still relatively warm.

We have also identified habitual betel nut consumption as a potential independent risk factor for fatal stroke. Betel nut, derived from the palm tree Areca catechu, is regularly chewed by at least 10% of the world's population (Boucher 2002). It is considered the fourth most common addictive substance in the world and consumed in various ways, including raw, baked, or dried, in a preparation that may involve betel leaf, Piper vine, or lime paste, usually combined with tobacco. Betel consumption is common in the Indian subcontinent, China, Southeast Asia, and the South Pacific islands where it is used as a stimulant, to aid digestion, and in social settings (Chu 2002). The International Agency for Research on Cancer (IARC) recognizes betel nut as a carcinogen (World Health Organization International Agency for Research on Cancer 2007). Recently, betel nut has been associated with an increased risk of cardiovascular disease (Hung 1998, Guh 2007,

Lin 2008, Zhang 2010, Tseng 2011), but there are few comparable studies that consider stroke (Wen 2005, Lan 2007).

In a prospective cohort study, The Survey of Health and Living Status of the Near Elderly and Elderly in Taiwan, past or current betel nut chewing increased the risk of stroke death in individuals aged 50 years and older (hazard ratio 1.66, 95% CI 1.19–2.30) (Lan 2007). However, only 14% of the cohort had ever chewed betel nut and they accounted for just 51 stroke deaths. In a separate Taiwanese study in 2001, based on the National Health Interview Survey, the relative risk of stroke death amongst betel chewers was 1.3 (95% CI 0.8–2.2). There were 22 stroke deaths amongst betel chewers, all of whom also smoked tobacco. In the present study, 76% of people chewed betel nut including 91% of women and 77% of men who died of stroke. Possible mechanisms of betel nut include a parasympathetic response from arecoline, the major constituent alkaloid.

Arecoline and guvacoline become hydrolyzed to arecaidine and guvacine when mixed with lime and can inhibit uptake of GABA (Johnston 1975). Betel leaf, used to envelope the nut or paste, contains aromatic phenols that stimulate catecholamine release in vitro. Importantly, the effect of betel nut on stroke may be partially mediated through obesity and dyslipidemia, which were not routinely measured in this population.

This study has limitations. The verbal autopsy instrument used in the diagnosis of stroke at this HDSS site, like many verbal autopsy instruments, has not been validated. There is a possibility of misclassification error in determining stroke deaths. Key responses including sudden onset paralysis, involvement of one half of the body, and lack of fever, for example, were used to make the diagnosis; however, imaging studies were not performed, and most deaths occurred outside of healthcare facilities. The distribution of hemorrhagic versus ischemic strokes is unknown. Even if imaging was available in healthcare facilities, the high number of in-home deaths in Matlab suggests that a population-based assessment for stroke could not justifiably be made using healthcare facility information. Although some of the cases may have been seen at a health facility where they were diagnosed by a physician and returned home before death, this is likely a small number of the total cases. Previous studies of verbal autopsy instrument validation specifically considering adult deaths reported that even though sensitivity for a cause of death in an individual is often modest, the population estimations of cause-specific mortality ultimately exhibit little distortion because of compensatory patterns of misclassification (Yang 2006). In our study, the verbal autopsy method appeared to overestimate the true proportional mortality because of stroke when comparing the population-attributable mortality to the calculated prevalence using the sensitivity and specificity of a similar instrument (25.2% vs. 17.2%). This is possibly because it is more common for other causes of death, such as chronic obstructive pulmonary disease, to be classified as a stroke death than for a stroke death to be classified as another etiology (Yang 2006). Important risk factor data on body mass index, dyslipidemia, and alcohol

use were not completely available in this population, but are important areas of further study and refinement of existing verbal autopsy instruments for chronic disease. Hypertension may have been underestimated in this group, and validation studies are required for future studies.

Compared with published data on the prevalence of hypertension in combined fatal and non-fatal acute stroke, our reported rate of hypertension in fatal stroke (47%) is lower than expected from internationally derived data (66% ischemic stroke, 83% hemorrhagic stroke) (O'Donnell 2010). Family history of vascular deaths is not collected in Matlab, but may be less useful in Bangladeshis whose parents experienced low life expectancy over the past generation (56 years old for men and 54 years old for women in 1978) [8]. The amount and duration of betel consumption was not asked, although other studies of betel chewers have found that a quarter of consumers of betel nut chew the preparation more than 10 times per day (Guh 2007), 85% of chewers chew daily (Yen 2006), and betel cessation is uncommon (Hung 1998). Also, the control group of people who died of unintentional injuries in this study was relatively smaller and younger. Age and sex were adjusted for in all regression analyses; however, assumptions were made that age could be properly adjusted for in a regression model. Although imperfect, the control group of adult injury deaths was chosen because of both the high number of total deaths in the study timeframe and the expectation that it was least likely to have vascular risk factors as a contributing cause of death. There was no more suitable population as other major causes could be affected by the same risk factors. Global estimates on the burden of

stroke are inferred through disease modeling, many times based on verbal autopsy as, worldwide, the vast majority of all deaths are uncounted. Although imperfect, verbal autopsy can provide foundational data in regions where the putative population-attributable mortality from stroke and other neurological diseases is unknown. These otherwise silent deaths can inform policy on the burden of stroke and its important risk factors in some of the world's poorest regions.

Long-term Predictive Value of the Framingham Risk Score for Stroke in HIV-infected versus HIV-uninfected Men: The Multi-Center AIDS Cohort Study

Introduction

The Framingham Risk Score for Stroke (FRS-S) was developed in the early 1990s to identify individuals at substantially increased long term stroke risk. The score provided impetus for risk factor modification and drew attention to individuals who were at risk of stroke due to borderline levels of multiple factors (Wolf 1991). In the early 1990s, survival of people with HIV infection was limited, and the Framingham Heart Study Offspring Cohort, in whom the FRS-S was developed, was not tested for HIV serostatus.

With the advent of highly active antiretroviral therapy (HAART) and longer survival of HIV+ individuals, the relationship between chronic HIV infection, HAART use, and vascular disease has become increasingly important (Friis-Møller 2010, Hasse 2011, Rasmussen 2011, Benjamin 2012, Cruse 2012). The influence of HIV on long-term

stroke risk remains unresolved (Berger 1990, Cole 2004, Corral 2009, Ovbiagele 2011, Mateen 2012) even though more than half of all prevalent HIV cases in the U.S. will be more than 50 years old by the year 2015 (Centers for Disease Control and Prevention 2011). Longitudinal studies that prospectively ascertain the effect of HIV on stroke risk in the HAART era are not reported (Benjamin 2012).

Here we test the long-term predictive value of the FRS-S in a large, prospectively followed cohort of HIV-infected (HIV+) versus uninfected (HIV-) men who have sex with men (MSM) in four U.S. cities. First, we compare the baseline characteristics of the Framingham cohort versus the MACS participants for important stroke risk factor differences. Second, we report the stroke incidence in HIV+ and HIV- men and determine whether the FRS-S, ten years earlier, differed by HIV serostatus, among participants with strokes. Finally, as a separate analysis, we calculate the FRS-S at the beginning of the HAART era for all MACS participants and compare it between HIV+ and HIV- groups.

Methods

The MACS was approved by the Institutional Review Board at each study site. Each participant provided his own informed consent to participate in the MACS. Analysis of data for this study was approved by the Johns Hopkins Institutional Review Board.

Cohort

The MACS began in 1984 to study the natural history of AIDS. The enrolment, recruitment, and goals of the MACS have been reported (Kaslow 1987, Detels 2012). MSM were recruited in Baltimore, Chicago, Los Angeles, and Pittsburgh. Cumulative enrolment is now at >7000 participants. MSM were chosen because they represented a group at high risk for AIDS and could reliably participate in a longitudinal follow up study. The HIV status of the men at enrolment was unknown. MSM who remained HIV- serve as a comparison group in the present study.

MACS participants are followed every 6 months via standardized in-person interviews, clinical assessments, neuropsychological screening tests, and laboratory evaluation, including measurements of HIV viral load and T-cell subsets (Roche ultrasensitive assay, <50 copies/mL), and standardized and quality-controlled flow cytometry) (Kaslow 1987). By late 1996, HAART was initiated by >50% of all participants, which is considered here as the beginning of the HAART era (Jacobson 2002). Stroke events that occurred over a 15-year period of the HAART era (July 1, 1996-June 30, 2011) were used in this analysis.

Definition of Stroke

Stroke was ascertained by either self-report at the study visit, prospective active reporting by participants between visits, review of causes of death, or ad-hoc when reviewing medical records to confirm other diagnoses. At each MACS visit, participants were asked whether they were diagnosed with stroke since their last visit, and, in April 2004 through March 2005, they were asked about lifetime history of stroke. In addition,

reasons for any hospitalization are reviewed as well as other diagnoses found by visits to doctors for vascular or neurologic problems. These reported events are recorded using the International Classification of Disease 9th edition codes. Reported strokes were followed up by each study site's investigators, including neurologists, via correspondence to the participant's physician. Death of MACS participants is continuously monitored. For deaths that were reported by participant contact (e.g., next-of-kin, partner, physician), death certificates were ascertained and systematically searched for the diagnosis of stroke. Deaths in the MACS are also captured and verified via the National Death Index, Social Security Death Index, credit databases, and scanning of obituaries. Medical records prior to death were requested. Fatal stroke was defined as a stroke with a reported death within 30 days after the date of first presentation of stroke. Death events were provided on the death certificate of the participant.

Any event that fit the definition of "silent stroke," defined as imaging findings representative of stroke that were not correlated with clinical symptoms of stroke, were excluded. Self-reported stroke events that could not be confirmed were included, and sensitivity analyses were performed (Table 5).

Framingham Risk Score for Stroke

The original FRS-S score was sex-specific and based on 36 calendar years of follow up of 2372 men and 3362 women aged 55 to 84 years old (Wolf 1991). There were 213 strokes in men and 259 strokes in women in the Framingham Heart Study's original cohort from whom the FRS-S was derived. The distribution of events diagnosed in men

was *atherothrombotic brain infarction* (46%), *transient ischemic attack only* (24%), *cerebral embolus* (19%), *intracerebral hemorrhage* (5%), *subarachnoid hemorrhage* (4%), and *other* (2%). Points for sex-specific FRS-S were ascribed based on eight baseline risk factors, identified via Cox proportional hazards regression models: age, systolic blood pressure (mmHg), antihypertensive therapy, diabetes mellitus, cigarette smoking, atrial fibrillation on electrocardiogram, left ventricular hypertrophy on electrocardiogram, and history of previously diagnosed cardiovascular disease (Table 6). Cardiovascular disease in the original score included coronary heart disease, history of myocardial infarction, angina pectoris, coronary insufficiency, cardiac failure, and intermittent claudication.

Vascular Risk Factor Definitions

In the MACS, risk factors for stroke were defined according to the Framingham Study whenever possible. For participants who were less than 55 years old, the number of points ascribed for age as a risk factor was zero. Diabetes mellitus was defined here, based on updated recommendations, and previous reports from the MACS as either (1) measured fasting glucose levels ≥126mg/dL, (2) measured nonfasting glucose levels ≥200 mg/dL when fasting samples are unavailable, or (3) self-reported diagnosis of diabetes treated with medications. Diabetes was ascertained at study entry and during regular study visits after April 1, 1999. Cigarette smokers are defined here as current smokers. Consistent with the FRS-S, former cigarette smokers were not given risk points in the risk assessment. Specific cardiac conditions used in the original FRS, were not

regularly evaluated clinically by MACS investigators. Electrocardiograms were also not performed as part of the MACS. It was assumed here that no MACS participants had atrial fibrillation or left ventricular hypertrophy; therefore, no MACS participants received points for these conditions on the FRS-S in this analysis. However, within the MACS, participants were asked to report their lifetime history between April 2004 and March 2005 - and follow up information on subsequent visits - on whether or not they had experienced: (1) myocardial infarction, (2) angina or chest pain caused by the heart, and (3) congestive heart failure. Participants who responded affirmatively to these questions were ascribed the 3 points for cardiovascular disease defined in the FRS-S (Table 6). All other participants were ascribed zero points. The FRS-S risk score conversion to the 10-year predicted risk of stroke is based on the percentage points derived from the original Framingham cohort (Wolf 1991).

Statistical Analysis

Participants with prevalent stroke at the time of study entry or stroke in the pre-HAART era were excluded. Among participants with a first-ever stroke during the HAART era, FRS was calculated using risk factors and characteristics from the study visit closest to 10 years prior to the date of the stroke event. The algorithm provided by the FRS cohort was employed to ascribe points (Table 6). Ten-year predicted probability of stroke was based on the original Framingham study (Wolf 1991). Participants in whom risk factor information was missing were considered not to have the risk factor of interest.

As a second measure of stroke risk, calculation of the baseline FRS-S among the entire MACS cohort was performed and compared between HIV+ and HIV- participants. This was done on all MACS participants, whether they had a stroke event or not, and calculated at the beginning of HAART era (1 July 1996) or, if the participant entered the MACS afterwards, at the participant's first date of study entry in the HAART era.

Comparison between groups was performed using the Wilcoxon-Rank sum tests and two-sample binomial tests for proportions. To reconstruct the FRS-S in the MACS, Cox proportional hazards models were both employed, stratified by serostatus and combined using serostatus as a binary predictor. The date of data censoring was death of the participant, study drop-out by the participant, or the last follow up visit on or before June 30, 2011.

Results

There were 3945 participants (1776 HIV+, 2169 HIV-) in MACS during the study timeframe. The baseline characteristics of this cohort and incidence of all neurological disease have been reported[7] and are provided by stroke and HIV serostatus in Table 7. There were 114 strokes in MACS participants recorded, including 19 fatal strokes among HIV+ participants (n=12) and HIV- participants (n=7). The stroke events occurred throughout the study observation period (Figure 2).

There were 94 first-ever strokes among 57 HIV+ and 37 HIV- participants with an incidence rate of first-ever stroke of 1.7/1000 person-years in HIV- and 3.3/1000 person-years in HIV+ participants (Table 7). The median age at the time of first stroke diagnosis

was 55.6 years (range 34.5-8.5 years old). Among HIV+ participants, the median age of first stroke was 51.4 compared to 61.8 years in HIV- participants ($p<0.0001$). A total of 70 (74%) were enrolled in the MACS for 10 years or more prior to their first stroke event.

Among HIV+ participants with first-ever stroke, 52 (91%) occurred while the participant was taking HAART. CD4 count within 60 days prior to the stroke event was available for 42 of the 57 HIV+ participants with stroke (mean 484 cells/mL, range 17-1256). Among this group, 8 had a CD4 count <200 cells/mL of whom 7 reported being on HAART.

For HIV+ participants with stroke, the FRS-S 10 years prior to stroke event, for 10-year prediction of stroke, averaged 4.9% (range 0-15%) compared to HIV- participants with stroke whose baseline FRS-S averaged 6.6% (range 3-26%) ($p<0.04$). When considering only the subgroup of MACS participants who were enrolled in the cohort study for 10 years or longer, the difference in mean FRS-S between HIV+ and HIV- participants was still significant (4.0% vs. 5.9%, $p<0.02$). Notably, three HIV+ participants with strokes and more than 10 years of follow up had a FRS-S predicted risk of zero.

The distribution of risk factors for HIV+ and HIV- men with stroke events is reported in Figure 3. Points are given according to the original FRS. Overall, HIV+ participants were more likely to be younger and smoke cigarettes. HIV- participants were marginally more likely to have hypertension. The distribution of history of diabetes and history of myocardial infarction was similar between HIV+ and HIV- men with strokes.

In the entire cohort of HIV+ men who did not experience a stroke (median and mean age at first visit during the HAART era 41 years), the mean 10-year predicted risk of stroke was 4.11% (range 0-26%) versus 4.05% (0-22%) in HIV- men (median and mean age 43) ($p<0.02$). In participants without known stroke events, hypertension and cigarette smoking were more likely among HIV+ men (Figure 4).

In the analysis of baseline stroke prediction, the association between stroke and traditionally measured risk factors in the FRS-S was similar between the Framingham cohort participants and the MACS participants but there was a significant added risk of HIV status among HIV+ MACS participants ($p<0.0001$, Table 8).

Discussion

Several studies have suggested an increased risk of stroke in HIV+ adults (Evers 2003, Cole 2004, Corral 2009, Rasmussen 2011, Chow 2012, Vinikoor 2013), but prospectively followed HIV+ individuals over 10 years or more with incident stroke have not previously been available. Many studies of HIV+ adults are limited to small numbers of stroke events (Hoffmann 2000, Mochan 2003, Evers 2003, Cole 2004, Corral 2009) and none have had the opportunity to compare baseline risk with long-term incidence of stroke. The comparison of baseline risk and long-term outcome remains of critical importance to HIV+ individuals. Traditional scores for risk prediction appear to be insufficient to accurately inform long-term cerebrovascular health status in chronic HIV infection. We observed a higher incidence of first-ever strokes in HIV+ versus HIV- participants (3.3 versus 1.7/1000 person years); however, the average 10-year FRS-S

predicted risk at baseline was lower in HIV+ versus HIV- subjects (4.9% versus 6.6%). Remodeling of the baseline stroke risk in the MACS demonstrates that HIV adds a significant risk of stroke.

There are several reasons that the FRS-S may have underestimated stroke risk in HIV+ men in this study. Risk difference using the FRS-S between HIV+ and HIV- men may be partly accounted for by the younger onset of stroke in HIV+ men therefore leading to lower FRS-S point calculation at 10 years prior to the event. The original FRS-S was designed in men aged 55 to 84 years old and was not modeled for young-onset stroke prediction. In the current study, the median age for stroke was in the fifth and sixth decades of life for both HIV+ and HIV- MSM.

HIV+ individuals may have unique risk factors for stroke that are measurable and can therefore lead to adjustment in their FRS-S. One study from Spain found that high alcohol intake, a history of a diagnosis of AIDS, and fewer months on HAART increased the risk of stroke, although only 25 patients with stroke were identified (Corral 2009). HIV infection itself may portend a higher stroke risk, even when accounting for demographic and traditional stroke risk factors. A U.S. study found that HIV remained an independent predictor for stroke when comparing HIV+ versus HIV- individuals from different cohorts even after adjustment for known risk factors. Most others in high income settings suggest that HIV increases stroke risk (Table 9) (Hoffmann 2000, Evers 2003, Mochan 2003, Cole 2004, Patel 2005, Ortiz 2007, Tipping 2007, Corral 2009, Lifson 2010, Rasmussen 2011, Chow 2012, Vinikoor 2013). In South Africa, however,

most studies report similarities in patient characteristics and stroke incidence between HIV+ and HIV- groups (Hoffman 2000, Mochan 2003, Patel 2005).

The reasons for increased rates of stroke among HIV+ men could include such mechanisms as chronic inflammation leading to atherogenic tendency of the endothelium, remodeling of the intracranial and carotid vasculature, and higher frequency of circulating inflammatory markers (Ortiz 2007, Seaberg 2010, Falcone 2011, Sen 2012). In the multinational STACCATO study (Calmy 2009), inflammatory markers associated with increased cardiovascular risk, such as soluble vascular adhesion molecule-1 (sVCAM-1), were associated with HIV RNA replication and decreased with HAART initiation. In a separate study by the SMART/INSIGHT and D.A.D. working groups (Calmy 2009), Abacavir, found in some HAART regimens, was associated with an increased risk of all-cause cardiovascular disease which included stroke. The relationship between HAART and longterm stroke risk in general requires further investigation since typical antiretroviral treatment has changed over time.

There may also be important risk factors that confound the relationship between stroke and HIV such as injection drug abuse with cocaine, excessive alcohol use, or poor medication adherence. Given that the majority of the HIV+ cohort with strokes was HAART-treated, it is unlikely that the stroke were caused by opportunistic infections; however, a fraction of the HIV+ participants with stroke had lower than expected CD4 counts and should be considered immunosuppressed. The MACS represents mostly

HAART-treated adults, a situation that almost certainly mimics the clinical scenario in most high-income settings.

Our study had several limitations. Stroke ascertainment in this cohort was partially dependent on self-reported events that were later verified by physicians and study investigators. In some cases, medical records from the patient could not be verified by the study site and diagnosis could only be based on the participant's provided information. Although a majority of strokes received an ICD-9 designation by the treating physician, there were several events that were not sub-classified by mechanism or stroke type. We are unable to report the number of strokes by subtype and compare them to the original Framingham Heart Study cohort; nonetheless, the FRS-S is useful for general risk of stroke and not specific to subtype. Assessments related to stroke severity, functional outcome, and neuroimaging confirmation were not consistently available in the MACS. This is true in most study cohorts of HIV+ individuals in whom vascular disease, particularly stroke, was not a priority condition to study. Since MACS began studying self-reported strokes in detail in 2006, it is possible that strokes that occurred earlier in the HAART era were not all ascertained. However, given our definition of the HAART era as 1996 and beyond, the inclusion of stroke in 2006 on interview forms is however still appropriate for assessment of risk at the required time point of 10 years earlier.

Some MACS participants had a stroke event prior to 2006, and pre-HAART era years were included in their risk period. This occurred in a minority of cases. Diabetes

mellitus was ascertained regularly from April 1999 onwards and at the time of the participant's study entry into the MACS. Notably, a participant who developed diabetes between his study entry and April 1999 may not have the date of diabetes diagnosis recorded with the MACS cohort until 1999. This would lead to under-estimation of his FRS-S, since points would not have been ascribed for diabetes in that individual in that timeframe.

Our study was limited to adult MSM. It is possible that our findings are not generalizable to other HIV+ populations such as women, ethnic minorities, or persons with other high-risk behaviors for stroke such as injection drug abuse. There are more African Americans in the MACS compared to the Framingham Heart Study which may lead to a differing baseline risk of stroke. Race is not part of the FRS-S but has been studied in cardiovascular disease prediction (D'Agostino 2001). Also, access to care may be better among MACS participants compared to other populations at risk.

This study had several strengths. The MACS has the advantage of enrollment of both HIV+ and HIV- MSM in the same prospective cohort study and does not derive its control group from other databases or registries. Many other cohorts lack an HIV-control group, making it difficult to discern the relative contributions of age, other HIV-related risk factors, and HIV infection. The number of strokes in the MACS is amongst the highest of any HIV+ cohort with well-described cardiovascular risk factor variables and HIV status information. In spite of the high number of reports suggesting HIV is an important risk factor for stroke, the number of reported patients with both HIV and stroke

is small. Other studies suffer from survival bias, such that an increased number of HIV+ individuals experiencing stroke in the HAART era may be inaccurately equated with HIV as a risk factor for stroke. In the MACS, we had the unique opportunity to retrospectively calculate the FRS-S in participants 10 years prior to stroke events. Since events were ascertained prospectively, there is no recall bias. Also, our finding that some individuals with stroke had an FRS-S of zero suggests that there are particular individuals at high risk which is difficult to predict using standard scores.

Adjustment and calibration of the FRS-S may be of high future utility in the HIV+ adult population. Accurate information on stroke risk is especially important to the growing number of HIV+ adults with multiple comorbidities (Bergersen 2004, Kaplan 2007, Kim 2012, Guiterrez 2013). Follow up of existing cohorts, with dedicated attention to vascular disease outcomes, is imperative to understand the accuracy of stroke prediction in HIV+ populations with various baseline risks.

Arsenic Exposure and Carotid Artery Atherosclerosis: Prospective data from the Strong Heart Study

Introduction

Arsenic is one of the ten chemicals of major public health concern listed by the World Health Organization (WHO) (No authors listed. World Health Organization International Program on Chemical Safety 2014). An established carcinogen, inorganic arsenic is most commonly ingested through drinking naturally contaminated groundwater, although exposure may also occur via food (rice and other grains), air pollution, smelting

operations and other occupational settings (Falk 2011, Jackson 2012, No authors listed. World Health Organization 2014). Increasing evidence supports the role of inorganic arsenic in a broad range of vascular diseases, particularly among populations exposed to levels above the WHO's recommended upper limit in drinking water (10 μg/liter) (Chen 2011, Moon 2013).

Arsenic exposure has been postulated to be a risk factor for cardiovascular disease through its putative role in potentiating atherosclerosis (Engel 1994, Simeonova 2004). High-level arsenic exposures have also been linked to stroke (Moon 2012, Moon 2013), but the mechanism of stroke remains poorly understood. Likely, a proximal source of thromboembolism is important for ischemic stroke occurrence in the setting of chronic arsenic exposure.

Carotid intimal-medial thickness (CIMT) is an important direct risk factor for stroke (Bots 1997, Ebrahim 1999, O'Leary 1999). CIMT is subclinical and increases progressively, serving as a biomarker of *in vivo* atherosclerosis. Moreover, traditional vascular risk factors, such as hypertension and age, explain only a small degree of the risk of carotid atherosclerosis in reported studies (Rundek 2013). This suggests that other risk factors may have an important etiological role in atherosclerotic plaque formation and progression in some populations, but these alternative risk factors remain mostly unexplored.

One study in high-arsenic containing areas in Taiwan found a dose-dependent relationship between the number of years exposed to arsenic-containing well water and

later development of carotid atherosclerosis (Wang 2002). After adjustment for recognized cardiovascular risk factors, the odds ratio of carotid atherosclerosis (≥50% of intimal medial thickness) was 3.1 (95% CI 1.3 to 7.4) for people with a cumulative arsenic exposure of ≥20mg/L-years versus those without arsenic exposure from drinking well water. However, individuated arsenic exposures were not available, and arsenic exposure times were estimated by history and duration of residence. More recently, in Mexico, a study of 199 children found a positive association between higher concentrations of urinary inorganic arsenic and CIMT (0.058 mm per 1ng/mL in those with >70-ng/mL exposures) (Osorio-Yáñez 2013).

In the Southwestern and Central United States, inorganic arsenic exposure through consumption of naturally contaminated groundwater has been a long-term concern and remains a problem in some rural communities, especially for families with private water wells. Here, we examine the relationship between arsenic exposure and atherosclerotic plaque and CIMT in a large cohort of American Indians from the Southwest and Midwest of the United States. We hypothesized that inorganic arsenic exposure is a risk factor for subclinical atherosclerosis of the common carotid arteries.

Methods

Ethics Approval

The Indian Health Service, institutional review boards, and the participating tribes approved the study protocol. Each participant provided his or her own consent to

participate in the Strong Heart Study. Data analysis for this study was also approved by the Johns Hopkins University Institutional Review Board.

Study Population

The Strong Heart Study is a prospective cohort study designed in the 1980s, funded by the National Heart, Lung, and Blood Institute (NHLBI) to estimate cardiovascular morbidity and mortality in American Indians. The Strong Heart Study was specifically designed to address a recognized lack of information on important risk factors for vascular diseases among American Indians in whom vascular diseases are a leading cause of death. Overall, 12 tribes in three geographic areas – Arizona, Oklahoma, and North and South Dakota, were represented in the study (Lee 1990). Targeted enrolment was 1500 participants ages 45 to 74 years old in each of Oklahoma, Arizona, and North and South Dakota (Lee 1990, Weltry 1995). Cluster sampling was used for participant enrolment in North and South Dakota, whereas all tribal members in the selected communities in Oklahoma and Arizona were invited, either by telephone or letter. Final enrolment in the Strong Heart Study was 4549 participants and the participation rate was 62% (Stoddart 2009).

Each participant underwent a structured interview, physical examination, anthropometric measurements, and collection of blood and urine specimens. The study observation period began at the date of the participant's baseline study examination between 1989 and 1991 in which urinary arsenic and other vascular risk factors were measured. For this study, the follow up was until study visit three (1998-1999) when 88% of all surviving

cohort participants were re-examined, including carotid ultrasound measurements. We used data from 3,973 participants with sufficient urine available for arsenic measurements. We then excluded 1495 participants who did not participate in the carotid ultrasound measurement. Subjects who did not undergo carotid ultrasound measurements were not different in terms of baseline risk of atherosclerosis compared to those who had the procedure performed (data not shown).

Arsenic Measurements

Arsenic exposure is through consumption of groundwater and well water contaminated by arsenic. Spot urine arsenic level was measured on a single occasion and is used as a proxy for arsenic exposure and arsenic internal dose. Arsenic measurements were performed on spot urine samples provided by participants at the baseline study visit (1989-1991). Urine was collected in polypropylene tubes, frozen within 1 to 2 hours of collection, and transported on dry ice to long term storage at -70 degrees Celsius at the Penn Medical Laboratory (MedStar Research Institute, Washington DC, USA). Urine arsenic measurements were performed on thawed samples, using up to 1 mL of urine in 2007 (Trace Element Laboratory, Graz University, Austria). Total arsenic concentration was measured using high-performance liquid chromatography/vapor generation inductively coupled plasma/mass spectrometry (Agilent 1100 HPLC and Agilent 7700x ICP-MS, Agilent Technologies, Santa Clara, California) (Scheer 2012). In 2009, inorganic arsenic (arsenite, arsenate), monomethylarsonate (MMA) and dimethylarseninate (DMA) were measured in the participants' baseline urine samples.

The concentration of arsenobetaine – a measure of seafood arsenicals - was very low, confirming that seafood consumption was low in this population, as expected (Moon 2013). As previously reported from a random sample of 380 adults in the Strong Heart Study, the interclass coefficient of combined inorganic and methylated arsenic species was 0.64 (95% confidence interval (CI) 0.60, 0.69), and the average change in urine arsenic concentration between study visits spanning ten years was -0.8 µg/g urine creatinine. In general, DMA, MMA, and inorganic arsenic have half-lives of approximately 2, 9, and 38 days respectively (Pomroy 1980, Cullen 1989). To assess inorganic arsenic exposure in the present study, we used the sum of inorganic and methylated arsenic species, hereafter referred to as arsenic.

Carotid Ultrasonography

Imaging of the extracranial carotid arteries was performed using standardized protocols with centralized training of field sonographers. The participant was placed in the supine position with mild neck hyperextension in order to visualize the carotid bulb and extracranial internal and external carotid arteries (Roman 2012). Both carotid arteries were imaged using the Acuson 128 systems equipped with a 7 MHZ linear array arterial imaging transducer (The Strong Heart Study Operations Manual 2001). Two-dimensionally assisted M-mode tracings were obtained in tandem with electrocardiogram to achieve end-diastolic (maximal diameter) measurements of the common carotid arteries, approximately 1 centimeter caudal to the carotid bulb (The Strong Heart Study Operations Manual 2001, Roman 2012). Images were recorded on VHS videotape and

read at the Weill Cornell Medical Center's Reading Center by an experienced cardiologist masked to the study question of interest. Carotid measurements were made on stored images chosen from real-time videotapes using a frame-grabber computer software program (Imaging Technology, Woburn, MA) and high resolution (640 x 640-pixel) video monitor (The Strong Heart Study Operations Manual 2001, Roman 2012).

Three outcomes were measured on the carotid arteries. The presence of atherosclerotic plaque was made based on carotid arterial wall thickening >50% compared to the thickness of the surrounding wall (Salonen 1988). A carotid plaque score was calculated based on the number of segments containing plaque, combining left and right common carotid, carotid bulb, external and internal carotid artery zones (Hollander 2002). Carotid plaque score ranged from zero (no plaque in any segment in either artery) to eight. Wall thickness was measured in the standard way, using electronic calipers, using the far carotid arterial wall at end-diastole, using several cycles on the taped video and averaged (Roman 2012). Wall thickness was never measured at the level of a plaque. Left and right wall thicknesses were averaged and the mean thickness of the two (in millimeters) is used here.

Other variables

The laboratory and anthropometric measurements used in the Strong Heart Study have been previously reported in detail (Lee 1990, Weltry 1995). The seventh report of the Joint National Committee on Prevention, Detection, and Evaluation, and Treatment of Hypertension definition of hypertension (Alberti 1998) was employed for hypertension:

systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, and/or the use of antihypertensive medications to treat blood pressure. Participants with values <140/90 mmHg were deemed non-hypertensive. Diabetes was based on the definition of the Provisional World Health Organization Report as fasting glucose ≥7.0mmol/L (126mg/dL), post-oral glucose challenge glucose measurement of ≥11.1 mmol/L (200mg/dL), and/or the use of oral hypoglycemic medications or insulin to treat diabetes (The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure 2004). Serum high- and low-density lipoproteins and triglycerides were measured in the fasting state at study baseline and treated as continuous variables (National Cholesterol Education Program 2002).

Smoking was defined as current, past, or never in one's lifetime. Past smoking was defined as having smoked at least 100 cigarettes but not currently smoking. Current smoking includes smoking currently and having smoked at least 100 cigarettes. Current alcohol use was defined as drinking regularly and having 12 alcoholic drinks or more in a lifetime. Past alcohol use was defined as drinking regularly in the past, drinking 12 or more alcoholic drinks in the past, and not drinking alcohol within the previous year. Body mass index was defined in the standard way (kg/m^2) (Garrow 1985).

Statistical Analysis

Continuous variables were described by their mean, standard deviation, median, range, and/or interquartile range. Categorical variables were summarized with frequencies and percentages. Urine arsenic was reported as the ratio of urine arsenic to urinary creatinine

to account for dilution in the spot samples. Arsenic was right-skewed and log-transformed for the analyses. Then, this creatinine-corrected arsenic variable was divided by the natural logarithm of two so that each one-unit increase in the transformed arsenic variable represented a doubling of the urinary arsenic per creatinine value.

After graphical display, mean CIMT was considered to be approximately normally distributed. The association between mean CIMT and the variables of interest was analyzed using multiple linear regression. The association between the presence or absence of atherosclerotic plaque and the variables of interest was analyzed using logistic regression. In the case of plaque score, the relationship between urinary arsenic and each score was assessed using ordinal logistic regression. Potential confounders of interest in all models were determined *a priori* based on previous knowledge and included smoking status (current, former, never), alcohol use (current, former, never), sex, and presence or absence of diabetes, as defined above. Continuous variables included systolic blood pressure (mmHg), waist-to-hip ratio, serum high density lipoprotein (HDL) (mg/dL), total cholesterol (mg/dL), and triglyceride level (mg/dL). Analyses were also performed using the "atherogenic index" which is the $\log_{10}$ value of serum HDL divided by serum triglycerides but yielded no significant association on the outcomes of interest compared to using HDL and triglycerides as separate adjustment variables. We also adjusted for geographic location (North and South Dakota, Arizona, or Oklahoma). All analyses were performed with Stata (version 11.2, StataCorp, Austin, TX).

Results

There were 2478 participants with measurements of both urine arsenic concentration and carotid artery endpoints. The carotid artery measurements from the total cohort are presented in Table 1, including CIMT of the left and right common carotid arteries, the average CIMT, presence of carotid artery plaque, and distribution of carotid plaque score. The distribution of characteristics of participants, by the presence or absence of atherosclerotic plaque in the common carotid artery, is provided in Table 2. The prevalence of traditional cardiovascular risk factors was high: diabetes 45% and hypertension 31%, with a high average BMI 31.0 kg/m^2 and waist-to-hip ratio was 0.948. Only 33% of participants never smoked cigarettes and 16% never drank alcohol.

The mean arsenic concentration (μg per microgram creatinine) was 12.1 (SD 17.2) and median was 9.23 (25^{th} and 75^{th} percentiles of 5.62, 14.7). The relationship between the risk factors of interest, including the sum of arsenic metabolites, and the mean difference in CIMT is found in Table 3. In the complete model, adjusted for both behavioral and biological risk factors, older age, male sex, higher systolic blood pressure, lower high density lipoprotein (HDL), former vs. never smoking, current vs. never drinking alcohol, and higher waist-to-hip ratio as well as higher urine arsenic concentration were all independently associated with a statistically significant increase in the average CIMT. After adjusting for study center (model C in tables), the relationship between arsenic and CIMT and currently drinking alcohol both became non-statistically significant.

The relationship between these same risk factors and the absolute presence of atherosclerotic plaque is given in Table 13. In this separate analysis, higher age, male versus female sex, higher systolic blood pressure, higher fasting blood glucose, presence of diabetes, current versus never smoking, current versus never drinking alcohol, higher total cholesterol, higher HDL, and higher BMI were all independently associated with the presence of atherosclerotic plaque. After adjustment for other recognized risk factors, there was no observed increased risk of carotid artery atherosclerotic plaque with increased levels of excreted creatinine-corrected urine arsenic.

Carotid artery plaque score was grouped into the following categories: 0, 1, 2, 3, and 4 and above given the smaller numbers of cases in the higher plaque score categories (as seen in Table 10). The relationship between carotid artery plaque score and the risk factors of interest are provided in Table 5. The relationship between score 0 and score 1 is given and is approximately similar among the various strata as compared to score 0. In this analysis of risk factors for higher carotid artery plaque score, higher age, male versus female sex, higher systolic blood pressure, higher fasting blood glucose, higher HDL, higher total cholesterol, higher atherogenic index, higher BMI, and current versus never smoking were all associated with a higher carotid plaque score. As in the case of atherosclerotic plaque presence, there was no statistically significant association between creatinine-corrected arsenic levels in the urine and a higher carotid artery plaque score.

Discussion

In this prospective cohort of nearly 2,500 adults with low-to-moderate arsenic chronic exposure, an association between creatinine-corrected arsenic concentration and average CIMT was found; however, an association between arsenic and the presence and degree of common carotid artery plaque was not observed after adjustment for important behavioral and biological risk factors for vascular disease. Although other cohort studies have reported an association between CIMT and arsenic exposure, only one other study (Wang 2002) has reported on arsenic and carotid artery plaque score (see summary Table 6). The adjusted odds ratio for arsenic and the risk of atherosclerotic plaque was 1.2 (0.4-3.4) for 0.1-19.9 years of arsenic-containing groundwater exposure and 2.3 (0.8-6.4) for ≥20.0 years exposure among 463 Taiwanese adults (test for trend p-value=0.045) (Wang 2002). In that study, however, the individuated measurements of arsenic were not available and the relationship between plaque and each stratum of arsenic exposure was not shown.

CIMT increase and presence of plaque are correlated and are both potentially useful in predicting the long-term risk of stroke. However, CIMT and plaque represent different steps in the biological development and progression of carotid artery atherosclerosis. In general, carotid plaque score is a marker of atherosclerotic disease due to the deposition of cholesterol, activated macrophages, and other inflammatory cells on the endothelial layer. In spite of plaque being rare in the common carotid artery, a fairly high number of participants in this study had atherosclerotic plaque, confirming that the participants in

this study were at high risk for vascular disease events that later developed throughout the study period (Moon 2013). By contrast, CIMT thickening represents medial hypertrophy of the carotid artery due to smooth muscle proliferation from shear and tensile stress. Thickening of the medial layer is more representative of chronic high stress on the smooth muscle wall and more representative of hypertension. As such CIMT represents an earlier process in the development of atherosclerotic disease than plaque score. Given the observation of less than 10 years between baseline visit and carotid imaging, the development of early changes in the setting of arsenic exposure, but not later changes, is biologically plausible.

This study differs from other reports on the relationship between arsenic and carotid artery disease in important methodological, environmental, and clinical ways. We have been able to estimate the individual's actual arsenic exposure using creatinine-adjusted urinary arsenic concentration. Most studies in low-income populations have depended upon ecological understanding of arsenic exposure, including random arsenic levels in the groundwater and years of residence. In a recent systematic review of arsenic and vascular disease, nine studies specifically reported the relationship between arsenic exposure and stroke, all of which lacked individuated arsenic measurements (Chiou 1997, Yuan 2007, Meliker 2007, Yoshikawa 2008, Wade 2009, Xia 2009, Lisabeth 2010, Cheng 2010, Medrano 2010, Chen 2011). Of the previous four studies on carotid vascular disease and arsenic, only the most recent studies, of Mexican children and Bangladeshi adults, measured urinary arsenic concentrations in individuals (Table 15). Neither of these included plaque score. Although groundwater estimates provide detail on the

potential environmental exposure, they may not as accurately reflect the actual inorganic arsenic intake in individuals. The potential for unmeasured confounders is high in ecological studies (Greenland 1989), and retrospectively recalling duration of residence is subject to recall bias. The Strong Heart Study cohort by contrast followed participants prospectively over time, allowing for the consideration of a large number of potential confounders at study baseline.

Arsenic levels in other studied locations are higher compared to the Central and Southwestern United States. Elsewhere, such as in parts of southwestern Taiwan and Bangladesh, arsenic is found in a range considered to be toxic by the WHO and European Environmental Protection Agency. Relatively little is known about arsenic ingestion at the low- to moderate level that occurs in the groundwater in the U.S. and to which a larger population is almost certainly exposed globally. It is possible that arsenic in low- to moderate levels has a less potent or less immediate effect on the development of carotid atherosclerosis and a critical threshold exists for arsenic to etiologically relate to both atherosclerosis and thickening of the carotid vascular wall.

In the present population, there was a strikingly high prevalence of diabetes mellitus, current or previous smoking, alcohol use, obesity, and hypertension. This population is at high risk for carotid atherosclerosis in the absence of arsenic exposure and it is possible that the effect of arsenic is stronger in populations that lack these more important risk factors. In other reported studies (Ebrahim 1999), the average CIMT was as low as 0.60 mm in women and 0.66 mm in men (vascular disease free participants, mean age 57

years, in the U.S. Atherosclerosis Risk in Communities) (Heiss 1991) and 0.65 mm in women and 0.69 mm in men in the Vascular Aging Study (mean age 65 years) (Bonithon-Kopp 1996). The highest CIMTs are generally reported in the oldest age groups, including the Seven Countries Study (mean 1.5 mm, age range 70 to 89 years) (Salonen 1994) and Cardiovascular Health Study (age 65 years and above: women 1.35 mm and men 1.57mm) (O'Leary 1999). Plaque prevalence is also high in other studies including a range of 24% in the MONICA Project in Germany (ages 25-65 years) (Gostomzyk 1988) to 93% in the Seven Countries Study in Finland (Salonen 1994). The combined assessment of multiple carotid outcome measurements here helps confirm that the findings are indeed pertinent to range of ways in which the carotid artery can be usually noninvasively studied.

This study had several strengths. The large, well-characterized cohort is ideally suited to study carotid atherosclerosis given its initial design to understand the vascular disease prevalence in American Indians at a time when such risk was poorly described. To date, no other study that we are aware of has reported on the risk factors for CIMT in a large population of this ethnic background. The results herein demonstrate that the traditional risk factors for higher CIMT and carotid atherosclerosis, including age, sex, systolic blood pressure, diabetes, smoking, and waist-to-hip ratio, are also important risk factors in American Indians. While confirming these recognized risk factors, we identified a dose dependent risk between arsenic and CIMT. Our study is also the first to report on the risk of carotid disease in the setting of lower than toxic levels of arsenic, a setting much more likely to occur than the levels reported in the available, published studies.

Our study also had several limitations. It would be ideal to study the risk of carotid atherosclerosis in a longer period of follow up that occurred in the Strong Heart Study. Our follow up period ranged from 6 to 9 years overall. Not all participants participated in the carotid testing and participants who did not complete carotid measurements were necessarily excluded. Although there is no medical reason that separates participants who did and did not undergo carotid ultrasonography, we cannot fully exclude the possibility that people who did not have carotid artery measurements were in some way different in their carotid artery endpoint measurements. For instance, participants who died before follow up could not get CIMT assessed. There may also be several risk factors related to absolute low income that include arsenic exposure and confound the relationship between arsenic, low socioeconomic status, and carotid artery disease. While the number of educational years attained is used here as a proxy for socioeconomic status, it is an imperfect measure for addressing all of the various health behaviors that accompany low income. In such cases, arsenic exposure itself may simply be a proxy for health behaviors that are imperfect and do not maximize health in various ways. At the same time, adjustment for location or education could result in over adjustment, as arsenic exposure tracks with location and socioeconomic status. Adjustment for these location and education can result in over adjustment. In order to display this relationship as clearly as possible, we have shown models that include and exclude adjustment for study center. Since arsenic exposure can never be ethically studied prospectively or randomized, the level of evidence for arsenic exposure and health risk necessarily rests upon data such as available here.

In summary, we observed a significant, independent risk of low- to moderate exposure to inorganic arsenic on carotid intimal medial thickness but not on the presence of atherosclerotic plaque or the atherosclerotic plaque score in this large cohort of American Indians in the United States. Although this study employs the carotid arteries, atherosclerosis is a diffuse, generalized process in the body and the imaging findings in the carotid arteries may also be considered as an *in vivo* biomarker of general vascular health. Our results improve the quality of evidence for the relationship between CIMT and arsenic and confirm that arsenic exposure at levels deemed to be low- to-moderate may still be harmful to long-term vascular health. Such data were previously lacking. Meanwhile, several details regarding the genetic susceptibility, degree and chronicity of exposure, and relationship to traditional vascular risk factors remain to be more fully explored.

Concluding Remarks

In the preceding three studies, I have discussed the putative risk of three separate risk factors for stroke that are justifiably considered non-hereditary and more often a result of an individual's setting rather than any clear genetic predisposition. It can be argued that the combination of location and behavior are key to environmental risk factors. For instance, the mere growth of palm trees with betel nut, local prevalence of HIV infection, or presence of arsenic in groundwater does not lead to necessary uptake or exposure to that risk factor. In this case, as in many cases of stroke risk, individual behavior is crucial. Given that human behaviors are necessary to acquire environmental risk in these three cases, there is a valuable role for education-based interventions. Unlike other

environmental risk factors for stroke, such as air pollution, ambient temperature, or latitude, behavioral change does not necessarily require removing the individual from his or her environment in order to mitigate risk.

Most of the risk seen in stroke can be attributed to known risk factors, those that the American Heart Association, major epidemiological cohort studies, and guidelines-setting organizations have already identified as major stroke risk factors. These risks most notably include systolic hypertension and age. If all other risk factors were ignored and only systolic hypertension was uniformly addressed, the global burden of stroke would be significantly reduced. Systolic hypertension has a magnitude of effect well beyond the risk conferred by any of the three risk factors above, and indeed, beyond all three risk factors combined. Appropriately, the global burden of hypertension is starting to garner attention. It is likely that 1 in every 4 adults has hypertension globally. Stroke is largely an end-organ manifestation of hypertension. Addressing the large minority of adults who have hypertension in a meaningful and sustained way would be a major global public health success.

What are the barriers to stroke research in low-income settings?

In spite of a large number of published papers reporting stroke is a concern in developing countries, there are limited data from the lower-income settings. When considering the number of reports compared to the number of likely stroke deaths and disability-adjusted life years, the number of data-driven reports on stroke in vulnerable population remains surprisingly small. Data quality is also limited in many published reports and several

countries still lack reportable information and data to inform policy. The accuracy of the Global Burden of Disease (Kim & Johnston 2011, Murray et al. 2012, Feigin et al 2014), its broad inferences and lack of inputted data, make all estimates questionable, particularly in low-income settings. Most literature totters on just a handful of available studies that are repeatedly cited versus implementing new efforts to study stroke in new settings in a standardized fashion.

Based on my experience, I would suggest the following ten reasons why stroke remains unstudied and under-studied in a variety of resource-limited regions globally.

1) Lack of recognition of stroke as a "public health" problem
2) Lack of clarity and awareness that stroke is a "human development" problem
3) Conflation of stroke as a lifestyle issue rather than a scientific challenge
4) No major organization advocating for neurological disorders globally
5) Limited numbers of trained health professionals in the neurosciences to advocate for stroke care
6) Reduced patient self-advocacy due to stigma and disability with the brain disorders, e.g. stroke causes aphasia making patient advocacy for that sequela particularly difficult
7) Absence of reliable biomarkers and inadequate case definitions for stroke
8) Limited access to neuroimaging globally, limiting sub classification and separation from competing diagnoses such as meningitis, cerebral malaria, and tuberculosis involving the central nervous system

9) Limited baseline data on stroke in populations and paucity of stroke registries
10) Lack of stroke treatment options including an absence of affordable technologies and confirmed efficacious interventions

Stroke in low- and middle-income countries

Population-based approaches to stroke include stroke prevention, emergent management, rehabilitation, and secondary prevention, all of which are severely lacking in most low- and middle-income countries (LMIC). Stroke incidence should largely be considered to result from increases in the prevalence of recognized cerebrovascular disease risk factors in LMIC. However, genetic predisposition and health behaviors that predispose to stroke may be exacerbated by weak health systems, unregulated industries, and limited public health messaging for chronic disease risk factors. The widespread uptake of cigarette smoking and alcohol; limited diagnosis and access to treatment for diabetes and hypertension; physical inactivity due to increasing urbanization and motorized transportation; dyslipidemia and poor intake of fruit and vegetables due to "Westernized" diets; and poor awareness of family history of vascular disease due to premature deaths of older relatives from infectious diseases may all increase the risk of stroke in LMIC.

The growing burden of young-onset-stroke in low- and middle-income countries

Stroke is not a disease exclusively of the elderly. Stroke in young adults – defined roughly as onset between ages 18 to 50 years old - accounts for approximately 5% of all strokes in Western countries, but, where studied, between 19% and 30% of strokes in

LMIC (Marini et al 2011). There is almost certainly an increasing burden of young-onset stroke in LMIC in contrast to the "graying" of other epidemics such as HIV/AIDS.

Large, clinical studies of stroke epidemiology in young adults have been performed almost exclusively in high-income settings, most notably including Scandinavia, East Asia, and the United States. In high-income settings, more than 20% of people ages 15 to 44 years old die within one month of their first stroke (Groppo et al 2012). In LMIC, the few studies reported are retrospective and lack uniformity in clinical evaluation of young stroke patients (Nayak et al 1997, Zhang et al 2012). However, young-onset stroke is an emerging public health issue for countries of all income levels. Among the young, there are longer periods at risk for repeat vascular events, more years of healthy life sacrificed, and an unexpected loss of productive working years with limited employment and rehabilitation prospects. For survivors, young-adult-onset stroke may lead to levels of disability that are financially untenable for families and health care systems in LMIC.

Youth and young adults are increasingly called to provide care to their parents with vascular disease, and prematurely so compared to the more late-onset stroke that occurs in predominantly older populations in high-income settings with relatively established support networks. In this way, addressing stroke also prevents financial demise and missed opportunities for the next generation who could be alleviated of the burden of long-term parental care.

The Neglected Stroke Epidemic in Sub-Saharan Africa

There is particularly a neglected and growing epidemic of stroke in Sub-Saharan Africa (SSA). In spite of the high number of people living in the region, very limited data on the burden of stroke in this region exist (Sokrab et al 2002, Damasceno et al 2010, Akubaka et al 2010, de Villiers et al 2011, Damorou et al 2011, Mudzi et al 2012, Kengne et al 2012, Heikinheimo et al 2012). The published reports suggest a high stroke prevalence with disproportionate mortality and morbidity compared to higher-income settings. SSA is now among the only places in the world where vascular disease is not the established leading cause of death (Lozanos et al 2012). As the HIV-infected community survives longer, the burden of vascular diseases will also grow. Recent studies of blood pressure distribution in SSA reveal a high burden of hypertension and its sequelae, including among HIV-infected groups (Bloomfield 2011 et al, Kaddumukasa et al 2012, Damasceno et al 2012, Mateen et al 2013). Meanwhile, HIV-infected individuals in SSA often receive care through vertical programs that are specific to HIV. These clinics may lack resources or skilled personnel to address other important disease risk factors such as hypertension or diabetes. While countries in SSA undergo the last stages of the epidemiological transition, the relationship between chronic HIV infection, antiretroviral use, and stroke will become increasingly important.

A prospective study of betel nut and betel nut cessation?

The first paper reports the potential role of betel chewing in the development of stroke. However, many questions remain unanswered. Betel nut may be chewed by disproportionately older people in society or those with more detrimental lifestyle habits

such as immobility, high-fat diets, and cigarette smoking. The prospective study of betel nut chewing versus not with matched subjects on age and sex would be of interest. The use of a specific amount and type of betel that can be quantified and recorded would improve this area of work. Given the widespread use of betel nut, there are many potential participants in the betel chewing group but perhaps fewer willing participants in the non-betel chewing group! The roll-out of betel cessation campaigns, particularly in pregnant women, and the regulation of pre-packaged quid advertising would be of interest in future studies.

Studies of betel nut may be difficult to tease apart from the way of life that betel represents. In several locations in the Western Pacific and Indian Subcontinent, betel is chewed with a regularity and heterogeneity that makes it difficult to study. Future studies of betel nut and stroke would best employ a prospective design that provides the substance in a standardized fashion; prospectively ascertains, measures, and records comorbidites and risk factors; and follow in vivo biomarkers such as C-reactive protein, cholesterol, and betel nut metabolites over time. There is also a strong impetus to improve pre-human studies of alternative tobaccos such as betel nut including animal studies that would help lead to the study of vasoreactivity, and soluble inflammatory marker release as well as teratogenicity. Basic biochemical analyses of what constitutes betel nut and its active metabolites would also be of value given the wide distribution of its use.

Stroke: Another nail in the coffin for arsenic ingestion

The study on arsenic as an emerging risk factor for stroke and subclinical atherosclerotic disease is yet another negative impact of arsenic. The World Health Organization's listing of arsenic as a chemical of major public health concern is based on its carcinogenicity more than its long-term potential to influence vascular health. This is also true of betel which is much better known to cause oral cancers than it is to change vascular disease risk. The recommendations by the Environmental Protection Agency, including a cutoff point for arsenic in water, may over time require re-review. The safest comment is likely that there is no definite safe ingestion of arsenic in groundwater and food and the long-term consequences of arsenic on health may include vascular disease at levels previously considered acceptable and non-carcinogenic.

Future directions for stroke research and care: what is truly innovative?

Although stroke incidence is increasing substantially in LMIC (Feigin et al 2009) and has a broad range of etiologies, the impact of disease can be mitigated. Improved care may be achieved in several ways specific to stroke as well as expand upon other services that are already established for acute care, such as expansion of acute care currently in place for cardiovascular disease. Technology can enhance the efforts for stroke in LMIC, particularly in rural and resource-limited locations.

Services for stroke in LMIC and vulnerable populations would benefit from a combination of programmatic approaches and should not simply model the inpatient-predominant and older adult focus of stroke treatment in high-income settings. Diagonal approaches that capitalize on existing services and treatments could incorporate stroke

into usual care. Stroke in the peri-partum period, environmentally contaminated regions, neonatal pre-term hemorrhage, and the adolescent with coagulopathy such as sickle cell anemia, must all be included within the broad rubric of stroke care in LMIC. Introduction of comprehensive stroke units and their scale up in the lowest-resourced settings is required - alongside the more generally recognized need for interventions for HIV/AIDS, tuberculosis, and malaria. Widespread screening for hypertension and education on antihypertensive treatments is currently the most necessary, available, and affordable opportunity to broadly reduce lifetime risk of stroke.

New drug development for acute stroke intervention is severely needed. Emergent treatments for stroke, such as intravenous thrombolysis, are expensive, require expert providers, and difficult to access even in wealthier settings. Perhaps most neglected to date, the incorporation of stroke rehabilitation and secondary prevention of vascular diseases into health systems is necessary. Stroke rehabilitation is evidence-based and effective in reducing long-term disability and reestablishing survivors' core activities of daily living. Several randomized trials attest to the value of stroke rehabilitation, including such measures as intensive physiotherapy, speech therapy, dietary modifications, occupational therapy, and environmental alterations. Stroke rehabilitation improves mobility, promotes independence, and maintains human dignity.

From epidemiology to action: two interventions for stroke in resource-limited settings

1) *An African Surveillance Network and Clinical Trials Network for Stroke?*

The next steps in the study of stroke in SSA almost certainly require coordination and planning of multiple, predominantly urban centers. Through my thesis and non-thesis work, I have established several collaborators throughout southeast Africa and hope to work with the American Heart Association and World Stroke Organization to create a "Get with the Guidelines Africa" program. This can be modeled after the popularized Get with the Guidelines in the U.S. and relate to resource-limited areas more specifically. It is my hope to include collaborators who range from psychiatrists, nurses, physiotherapists, and cardiologists to participate in Rwanda, Tanzania, Malawi, Zambia, Botswana, Zimbabwe, and Uganda. This, in my opinion, would be a major step forwards in understand stroke care. With the rising use of cell phones internationally, the opportunity to "crowd source" information on stroke would be a welcome accomplishment in building upon nearly non-existent data in this region. Get with the Guidelines in the U.S.A. currently reaches 2.7 million people and Get with the Guidelines Africa has the potential to reach millions more.

2) *Does fluoxetine have a role in post-stroke recovery in resource-limited settings?*

Currently, there are no pharmacological treatment options for motor recovery after stroke. One recent phase III clinical trial of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), showed improved motor recovery among 118 ischemic stroke survivors in France (FLAME Study) (Chollet et al. 2011). Fluoxetine, and potentially other SSRIs, may be neuroprotective by reducing inflammation within the central nervous system and promoting neuroplasticity. A Cochrane review (Mead et al 2012) reported 52 clinical trials, including 4059 patients: those on fluoxetine post-stroke were less likely to be

depressed, anxious, neurologically impaired, dependent, or disabled. However, an excess of adverse events was noted in the treated group, making the beneficial use of fluoxetine in all recovering stroke patients unproven. Larger studies are needed, particularly in resource-limited settings and among HIV-infected stroke populations.

Part of my work following completion of the dissertation is to test the use of fluoxetine in Harare Central Hospital's stroke unit, a location funded by the Medical Education Partnership Initiative (MEPI). This study will join a larger, established network of clinical trials alongside investigators in Australia (Dr. Graeme Hankey), the United Kingdom (Professors Gillian Mead, Martin Dennis), and Sweden (Dr. Veronica Murray). These centers also see the need for large randomized controlled trials of fluoxetine for stroke recovery, and have initiated the Assessment oF FluoxetINe In sTroke recoverY (AFFINITY) trial in Australia (Australian New Zealand Clinical Trials Registry: ACTRN12611000774921), the Fluoxetine Or Control Under Supervision (FOCUS) trial in the United Kingdom (ISRCTN83290762). This network is also planning the EFFECTS trial in Sweden. In that proposal, currently under review, and with additional confirmed funding support, I propose to study fluoxetine as a treatment for post-stroke depression and accelerated motor recovery with collaborators in Zimbabwe. The proposed hypothesis is: Daily administration of fluoxetine 40 mg by mouth daily within 7 days of onset of acute ischemic stroke will lead to a clinically relevant improvement on the Fugl-Meyer motor score (FMSS) (≥12 points) at 90 days among Zimbabwean patients aged 18 to 80 years old.

Final thoughts

There is almost certainly synergy between traditional and emerging risk factors in stroke. These risks together raise complex policy questions around such issues as the legality of alternative forms of tobacco, environmental justice for marginalized populations, and re-financing programs for health to move beyond infectious and communicable diseases to the more common non-communicable disorders that adults in LMIC more often acquire. Organization, coordination, and training remain major barriers to improving the incidence and mortality of stroke in many countries. As these papers have shown, even in an established health care system, stroke continues to heavily burden populations. Stroke prevention and care now require a much needed cadre of experts and organized initiatives to avert the current march towards unnecessary loss of life worldwide.

References

Abhyankar LN, Jones MR, Guallar E, Navas Acien A. Arsenic exposure and hypertension: a systematic review. Environ Health Perspect 2002;120:494-500.

Abubaka SA, Yunusa GH, Isezuo SA. Predictors of 30 days outcome of patients with acute stroke in Sokoto. Sahel Medical Journal 2010;13:68-73.

Adams HP Jr., Bendixen BH, Kappelle J, et al. Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial. Stroke 1993;24:35-41.

Alam N, Chowdhury HR, Bhuiyan MA, Streatfield PK. Causes of death of adults and elderly and healthcare-seeking before death in rural Bangladesh. J Health Popul Nutr 2010; 28:520-8.

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.

Ali WM, Zubaid M, Al-Motarreb A, et al. Association of khat chewing with increased risk of stroke and death in patients presenting with acute coronary syndrome. Mayo Clin Proc 2010;85:974-80.

American Stroke Association. Last accessed July 12, 2012 at: http://www.strokeassociation.org/STROKEORG/AboutStroke/UnderstandingRisk/Understanding-Stroke-Risk_UCM_308539_SubHomePage.jsp

Andersen KK, Olsen TS, Dehlendorff C, Kammersgaard P. Hemorrhagic and ischemic strokes compared: stroke severity, mortality, and risk factors. Stroke 2009;40:2068-72.

Arana A, Varas C, González-Pérez A, Gutiérrez L, Bjerrum L, Garcia Rodriguez LA. Hormone therapy and cerebrovascular events: a population-based nested case-control study. Menopause 2006;13:730-6.

Ari E, Kaya Y, Demir H, Asicioglu E, Keskin S. The correlation of serum trace elements and heavy metals with carotid artery atherosclerosis in maintenance hemodialysis patients. Biol Trace Elem Res 2011;144:351-9.

Baron JA, Sandler RS, Bresalier RS, et al. Cardiovascular events associated with rofecoxib: final analysis of the APPROVe trial. Lancet 2008;372:1756-64.

Benjamin LA, Bryer A, Emsley HC, Solomon T, Connor MD. HIV infection and stroke: current perspectives and future directions. Lancet Neurol 2012;11:878-90.

Berger JR, Harris JO, Gregorios J, Norenberg M. Cerebrovascular disease in AIDS: a case-control study. AIDS 1990;4:239-44.

Bergersen BM, Sandvik L, Bruun JN, Tonstad S. Elevated Framingham risk score in HIV-positive patients on highly active antiretroviral therapy: results from a Norwegian study of 721 subjects. Eur J Clin Microbiol Dis 2004;23:625-30.

Bernstein AM, de Koning L, Flint AJ, Rexrode KM, Willett WC. Soda consumption and the risk of stroke in men and women. Am J Clin Nutr 2012;95:1190-9.

Bhopal R, Rahemtulla T, Sheikh A. Persistent high stroke mortality in Bangladeshi populations. BMJ 2005;331:1096-7.

Bleich SN, Koehlmoos TL, Rashid M, Peters DH, Anderson G. Noncommunicable chronic disease in Bangladesh: overview of existing programs and priorities going forward. Health Policy 2011;100:282-9.

Bloomfield GS, Hogan JW, Keter A, et al. Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in Western Kenya. PLos One 2011;6:e22288.

Bonithon-Kopp C, Touboul PJ, Berr C, Leroux C, Mainard F, Courbon D, Ducimetiere P. Relation of intima-media thickness to atherosclerotic plaques in carotid arteries: the Vascular Aging (EVA) Study. Arterioscler Thromb Vasc Biol 1996;16:310-6.

Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96:1432-37.

Boucher BJ, Mannan N. Metabolic effects of the consumption of *Areca catechu*. Addict Biol 2002;7:103-10.

Brennan PJ, Greenberg G, Miall WE, Thompson SG. Seasonal variation in arterial blood pressure. BMJ 1982;285:919-23.

Brook RD, Weder AB, Rajagopalan S. “Environmental Hypertensionology” the effects of environmental factors on blood pressure in clinical practice and research. J Clin Hypertension 2011;13:836-42.

Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. Int J Epidemiol 2009;38:791-83.

Calmy A, Gayet-Ageron A, Montecucco F, et al. on behalf of the STACCATO Study Group. AIDS 2009;23:929-39.

Cappuccio FP, Ji C. Less salt and less risk of stroke: further support to action. Stroke 2012;43:1195-6.

Castilla-Guerra L, del Carmen Fernández-Moreno M, Marín J. Stroke 2010;41:e114.

Centers for Disease Control and Prevention (CDC). HIV testing among men who have sex with men: 21 cities, United States, 2008. MMWR Morb Mort Wkly Rep 2011;60:694–9.

Chau PH, Woo J, Goggins WB, Wong M, Chan KC, Ho SC. Analysis of spatio-temporal variations in stroke incidence and case-fatality in Hong Kong. Geospat Health 2011;6:13-20.

Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, Islam T, et al. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. BMJ 2011;342:d2431.

Chen Y, Wu F, Graziano JH, Parvez F, Liu M, Paul R, et al. Arsenic exposure from drinking water, arsenic methylation capacity, and carotid intima-media thickness in Bangladesh. Am J Epidemiol 2013;178:372-81.

Cheng TJ, Ke DS, Guo HR. The association between arsenic exposure from drinking water and cerebrovascular disease mortality in Taiwan. Water Res 2010;44:5770-6.

Chiou H, Huang W, Su C, Chang S, Hsu Y, Chen C. Dose-response relationship prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 1997;28:1717-23.

Chollet F, Tardy J, Albucher J, et al. Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomized placebo-controlled trial. Lancet Neurol 2011;10:123-30.

Chow FC, Regan S, Feske S, Meigs JB, Grinspoon SK, Triant VA. Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US health care system. J Acquir Immune Defic Syndr 2012;60:351-8.

Christakis NA, Fowler JH. The spread of obesity in a large social network over 32 years. N Engl J Med 2007;357:370-9.

Chu N. Neurological aspects of areca and betel nut chewing. Addict Biol 2002;7:111-4.

Cole JW, Pinto AN, Hebel JR, et al. Acquired immunodeficiency syndrome and the risk of stroke. Stroke 2004;35:51-6.

Corral I, Quereda C, Moreno A, et al. Cerebrovascular ischemic events in HIV-1-infected patients receiving highly active antiretroviral therapy: incidence and risk factors. Cerebrovasc Dis 2009;27:559-63.

Cruse B, Cysique LA, Markus R, Brew BJ. Cerebrovascular disease in HIV-infected individuals in the era of highly active antiretroviral therapy. J Neurovirol 2012;18:264-76.

Cullen WR, Reimer KJ. Arsenic speciation in the environment. Chemical Reviews 1989;89:713-64.

D'Agostino RB, Grundy S, Sullivan LM, Wilson P for the CHD Risk Prediction Group. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA 2001;286:180-7.

Damasceno A, Gomes J, Azevedo A, et al. An epidemiological study of stroke hospitalizations in Maputo, Mozambique: A high burden of disease in a resource-poor country. Stroke 2010;41:2463-9.

Damasceno A, Mayosi BM, Sani M, et al. The causes, treatment, and outcome of acute heart failure in 1006 Africans from 9 countries. Arch Intern Med 2012; 172:1386–1394.

Damorou F, Yayehd K, Pessinaba S, Tcherou T, Amonu J. Mortality associated with cardiovascular diseases in three hospitals of Lomé-city. Pan Afr Med J 2011;10:40.

de Villiers L, Badri M, Ferreira M, Bryer A. Stroke outcomes in a socio-economically disadvantaged urban community. S Afr Med J 2011;101:345-8.

Detels R, Jacobson L, Margolick J, et al. The multicenter AIDS cohort study, 1983 to . . . . Public Health 2012;126:196-8.

Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet 2008;371:1612-23.

Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. Stroke 1999;30:841-50.

Engel RR, Hopenhayn-Rich C, Receveur O, Smith AH. Vascular effects of chronic arsenic exposure: a review. Epidemiol Rev 1994;16:184-209.

Evers S, Nabavi D, Rahmann A, Heese C, Reichelt D, Husstedt IW. Ischaemic cerebrovascular events in HIV infection. Cerebrovasc Dis 2003;15:199-205.

Ezzati M, Horwitz ME, Thomas DS, et al. Altitude, life expectancy and mortality from ischaemic heart disease, stroke, COPD and cancers: national population-based analysis of US counties. J Epidemiol Community Health 2012;66:e17.

Falcone EL, Mangili A, Skinner S, Alam A, Polak JF, Wanke CA. Framingham risk score and early markers of atherosclerosis in a cohort of adults infected with HIV. Antivir Ther 2011;16:1-8.

Falk H, Briss P. Environmental- and injury-related epidemic-assistance investigations, 1946-2005. Am J Epidemiol 2011;174(11Suppl):S65-79.

Feigin VL, Lawes CM, Bennett DA, Barker-Collo S, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol 2009;8:355-69.

Feigin VL, Forouzanfar MH, Krishnamurthi R, et al.; Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010) and the GBD Stroke Experts Group. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet 2014;383:245-54.

Ferris A, Robertson RM, Fabunmi R, Mosca L. American Heart Association and American Stroke Association national survey of stroke risk awareness among women. Circulation 2005;111:1321-26.

Friis-Møller N, Thiébaut R, Reiss P, et al.; DAD study group. Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. Eur J Cardiovasc Prev Rehabil 2010;17:491-501.

Gardener H, Rundek T, Wright CB, Elkind MS, Sacco RL. Dietary sodium and risk of stroke in the Northern Manhattan Study. Stroke 2012;43:1200-5.

Garrow JS and Webster J. Quetelet's index (W/H2) as a *measure of fatness.* International Journal of Obesity 1985;9:147–153.

Glymour MM, Kosheleva A, Boden-Albala B. Birth and adult residence in the Stroke Belt independently predict stroke mortality. Neurology 2009;73:1858-65.

Gordis L. Epidemiology. 4th ed. Philadelphia: Saunders Elsevier, 2009.

Gostomzyk JG, Heller WD, Gerehardt P, Lee PN, Keil U. B-scan ultrasound examination of the carotid arteries with a representative population (MONICA Projet Augsburg). Klin Wochenschr 1988;66:58-65.

Groppo E, De Gennaro R, Granieri G, Fazio P, Cesnik E, Granieri E, Casetta I. Incidence and prognosis of stroke in young adults: a population-based study in Ferrara, Italy. Neurol Sci 2012;33:53-8.

Guiraud V, Ben Amor M, Mas J, Touzé E. Triggers of ischemic stroke a systematic review. Stroke 2010;41:2669-77.

Grau AJ, Urbanek C, Palm F. Common infections and the risk of stroke. Nat Rev Neurol 2010;6:681-94.

Greenland S, Morgenstern H. Ecological bias, confounding, and effect modification. Int J Epidemiol 1989;18:269-74.

Groppo E, De Gennaro R, Granieri G, Fazio P, Cesnik E, Granieri E, Casetta I. Incidence and prognosis of stroke in young adults: a population-based study in Ferrara, Italy. Neurol Sci 2012;33:53-8.

Guh J, Chen H, Tsai J, Chuang L. Betel-quid use is associated with heart disease in women. Am J Clin Nutr 2007;85:1229-35.

Gutierrez J, Elkind MS, Marshall RS. Cardiovascular profile and events of US adults 20-49 years with HIV: results from the NHANES 1999-2008. AIDS Care 2013;25:1385-91.

Gupta R, Pandey RM, Misra A, et al. High prevalence and low awareness, treatment and control of hypertension in Asian Indian women. J Hum Hypertens 2012;26:585-93.

Haapaniemi H, Hillbom M, Juvela S. Weekend and holiday increase in the onset of ischemic stroke in young women 1996;27:1023-7.

Hannan MA, Rahman MM, Haque A, Ahmed HU. Stroke: seasonal variation and association with hypertension. Bangladesh Med Res Counc Bull 2001;27:69-78. Hankey GJ, Jamrozik K, Broadhurst RJ, et al. Five-year survival after first-ever stroke and related prognostic factors in the Perth Community Stroke Study. Stroke 2000;31:2080-6.

Hankey GJ. Nutrition and the risk of stroke. Lancet Neurol 2012;11:66-81.

Haque MM, Nasreen SA. Epidemiological study of risk factors of stroke and its immediate consequence. Mymensingh Med J 2008;17:124-8.

Hasse B, Ledergerber B, Furrer H, et al. Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. Clin Infect Dis 2011;53:1130-9.

He Y, Lam TH, Jiang B, et al. Passive smoking and risk of peripheral arterial disease and ischemic stroke in Chinese women who never smoked. Circulation 2008;118:1535-40.

Heikinheimo T, Chimbayo D, Kumwenda JJ, Kampondeni S, Allain TJ. Stroke outcomes in Malawi, a country with high prevalence of HIV: A prospective follow-up study. Plos One 2012;7:e33765.

Heiss G, Sharrett AR, Barnes R, Chambless L, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associatins with cardiovascular risk factors in the ARIC Study. Am J Epidemiol 1991;134:250-6

Hoffmann M, Berger JR, Nath A, Rayens M. Cerebrovascular disease in young, HIV-infected, black Africans in the KwaZulu Natal Province of South Africa. J Neurovirol 2000;6:229-36.

Hollander M, Bots ML, Iglesias del Sol A, Koudstaal PJ, Witteman JC, Grobbee DE, et al. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. Circulation 2002;105:2872-7.

Hong YC, Lee JT, Kim H, Ha EH, Schwartz J, Christiani DC. Effects of air pollutants on acute stroke mortality. Environ health Perspect 2002;110:187-91.

Hsieh Y, Lien L, Chung W, et al. Significantly increased risk of carotid atherosclerosis with arsenic exposure and polymorphisms in arsenic metabolism genes. Environ Res 2011;111:804-10.

Hung DZ, Deng JF. Acute myocardial infarction temporarily related to betel nut chewing. Vet Hum Toxicol 1998;40:25-8.

ICDDR,B (2010) Health and Demographic Surveillance System–Matlab, v. 42. Registration of health and demographic events 2008, Scientific Report No. 109. Dhaka: ICDDR,B.

Ikeda N, Saito E, Kondo N, et al. What has made the population of Japan healthy? Lancet 2011;378:1094-105.

International Statistical Classification of Diseases and Related Health Problems. 10th Revision. http://apps.who.int/classifications/apps/icd/icd10online/ Accessed September 25, 2011.

Jackson BP, Taylor VF, Karagas MR, et al. Environ Health Perspect 2012;120:623-6.

Jacobsen BK, Knutsen SF, Oda K, Fraser GE. Parity and total, ischemic heart disease and stroke mortality. The Adventist Health Study, 1976-1988. Eur J Epidemiol 2011;711-8.

Jacobson LP, Li R, Phair J, Margolick JB, Rinaldo CR, Detels R, Munoz A. Evaluation of the effectiveness of highly active antiretroviral therapy in persons with Human Immunodeficiency Virus using biomarker-based equivalence of disease progression. Am J Epidemiol 2002;155:760-70.

Jaillard AS, Hommel M, Mazetti P. Prevalence of stroke at high altitude (3380 m) in Cuzco, a town of Peru. A population-based study. Stroke 1995;26:562-8.

Johnston GA, Krogsgaard-Larsen P, Stephanson A. Betel nut constituents as inhibitors of gamma-aminobutyric acid uptake. Nature 1975;258:627-8.

Joshi SR, Saboo B, Vadivale M, et al. on behalf of the SITE investigators. Prevalence of diagnosed and undiagnosed diabetes and hypertension in India – results from the screening India's twini epidemic (SITE) study. Diabetes Tech Therapeutics 2012;14:1-8.

Jung SY, Bae HJ, Park BJ, Yoon BW for the Acute Brain Bleeding Analysis Study Group. Parity and risk of hemorrhagic strokes. Neurology 2010;74:1424-9.

Kaddumukasa M, Ddumba E, Duncan P, Goldstein LB. Poststroke hypertension in Africa. Stroke 2012;43:3402-4.

Kaplan RC, Kingsley LA, Sharrett R, et al. Ten-year predicted coronary heart disease risk in HIV-infected men and women. Clin Infect Dis 2007;45:1074-81.

Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am J Epidemiol 1987;126:310-8.

Kaur P, Rao SR, Radhakrishnan E, Rajasekar D, Gupte MD. Prevalence, awareness, treatment, control and risk factors for hypertension in a rural population in South India. Int J Public Health 2012;57:87-94.

Kengne AP, Ntyintyane LM, Mayosi BM. A systematic overview of prospective cohort studies of cardiovascular disease in sub-Saharan Africa. Cardiovas J Afr 2012;23:103-12.

Kelly-Hayes M, Wolf PA, Kase CS, Brand FN, McGuirk JM, D'Agostino RB. Temporal patterns of stroke onset: the Framingham study. Stroke 1995;26:1343-7.

Kernan WN, viscoli CM, Brass LM, et al. Phenylpropanolamine and the risk of hemorrhagic stroke. N Engl J Med 2000;343:1826-32.

Khanam MA, Lindeboom W, Koehlmoos TP. Hypertension: adherence to treatment in rural Bangladesh – findings from a population-based study. [Abstract 008(117)]. 13th ASCON, 2011. LaCroix AZ, Chlebowski RT, Manson JE, et al. for the WHI Investigators. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: a randomized controlled trial. JAMA 2011;305:1305-14.

Kim AS, Claiborne Johnston S. Global variation in the relative burden of stroke and ischemic heart disease. Circulation 2011;124:314-323.

Kim DJ, Westfall AO, Chamot E, et al. Multimorbidity patterns in HIV-infected patients: the role of obesity in chronic disease clustering. J Acquir Immune Defic Syndr 2012;61:600-5.

Klimaszewska K, Kulak W, Jankowiak B, Kowalczuk K, Knodzior D, Baranowska A. Seasonal variation in ischaemic stroke frequency in Podlaskie Province by season. Adv Med Sci 2007;52:Suppl 1:112-4.

Lan T, Chang W, Tsai Y, Chuang YL, Lin HS, Tai TY. Areca nut chewing and mortality in an elderly cohort study. Am J Epidemiol 2007;165:677-83.

Langhorne P, de Villiers L, Pandian JD. Applicability of stroke-unit care to low-income and middle-income countries. Lancet Neurol 2012;11:341-8.

Lanska DJ, Kuller LH. The geography of stroke mortality in the United States and the concept of a stroke belt. Stroke 1995;26:1145-9.

Lee ET, Welty TK, Fabsitz R, et al. The Strong Heart Study: A study of cardiovascular disease in American Indians: design and methods. Am J Epidemiol 1990;132:1141-55.

Li W, Sun C, Cheng T, Chang K, Chen C, Wang S. Risk of carotid atherosclerosis is associated with low serum paraxonase (PON1) activity among arsenic exposed residents in Southwestern Taiwan. Toxicology Applied Pharmacol 2009;236:246-53.

Liao Y, Greenlund KJ, Croft JB, Keenan NL, Giles WH. Factors explaining excess stroke prevalence in the US Stroke Belt. Stroke 2009;40:336-41.

Lidegaard Ø, Løkkegaard E, Jensen A, Skovlund CW, Keiding N. Thrombotic stroke and myocardial infarction with hormonal contraception. N Engl J Med 2012;366:2257-66.

Lifson AR; INSIGHT Endpoint Review Committee Writing Group, Belloso WH, Davey RT, Duprez D, et al., INSIGHT Study Group. Development of diagnostic criteria for serious non-AIDS events in HIV clinical trials. HIV Clin Trials 2010;11:205-19.

Lin W, Chiu T, Lee L, Lin CC, Huang CY, Huang KC. Betel nut chewing is associated with increased risk of cardiovascular disease and all-cause mortality in Taiwanese men. Am J Clin Nutr 2008;87:1204-11.

Lisabeth LD, Ahn H, Chen JJ, Sealy-Jefferson S, Burke JF, Meliker JR. Arsenic in drinking water and stroke hospitalizations in Michigan. Stroke 2010;41:2499-504.

Logan WP. Mortality in the London fog incident, 1952. Lancet 1953;1:336-8.

Lozanos R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095-128.

Marini C, Russo T, Felzani G. Incidence of stroke in young adults: a review. Stroke Res Treat 2011;article ID 535672.

Maselko J, Bates LM, Avendano M, Glymour MM. The intersection of sex, marital status, and cardiovascular risk factors in shaping stroke incidence: results from the health and retirement study. J Am Geriatr Soc 2009;57:2293-9.

Mateen FJ. International issues: neurology in Bangladesh. Neurology 2010;74:e5-8.

Mateen FJ, Cascino GD. Khat chewing: a smokeless gun? Mayo Clin Proc 2010;85:971-3.

Mateen FJ, Brook RD. Air pollution as an emerging global risk factor for stroke. JAMA 2011;305:1240-1.

Mateen FJ, Shinohara RT, Carone M, et al. for the Multicenter AIDS Cohort Study (MACS) Investigators. Neurologic disorders incidence in HIV+ vs HIV- men: Multicenter AIDS Cohort Study, 1996-2011. Neurology 2012;79:1873-80.

Mateen FJ, Kanters S, Kalyesubula R, et al. Hypertension prevalence and Framingham risk score stratification in a large HIV-positive cohort in Uganda. J Hypertens 2013; 31:1372-78.

Mathers CD, Lopez AD, Murray CJ. The burden of disease and mortality by condition: data, methods, and results for 2001. In: Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJL, eds. Global Burden of Disease and Risk Factors. Washington, DC: World Bank, 2006.

Mathiesen EB, Johnsen SH. Ultrasonographic measurements of subclinical carotid atherosclerosis in prediction of ischemic stroke. Acta Neurol Scand Suppl 2009;189:68-72.

Mead GE, Hsieh C, Lee R, Kutlubaev MA, Claxton A, Hankey GJ, Hackett ML. Selective serotonin reuptake inhibitors (SSRIs) for stroke recovery. Cochrane Database of Systematic Reviews 2012:CD009286.

Medeiros F, de Abreu Casanova M, Fraulob JC, Trindale M. How can diet influence the risk of stroke? Int J Hyper 2012;763507.

Medrano MA, Boix R, Pastor-Barriuso R, et al. Arsenic in public water supplies and cardiovascular mortality in Spain. Environ Res 2010;110:448-54.

Meliker JR, Wahl RL, Cameron LL, Nriagu JO. Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. Environ Health 2007;6:4.

Messner B, Knoflach M, Seubert A, et al. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. Arterioscler Thromb Vasc Biol 2009;29:1392-8.

Miller KA, Siscovick DS, Sheppard L, et al. Long-term exposure to air pollution and incidence of cardiovascular events in women. N Engl J Med 2007;356:447-58.

Mochan A, Modi M, Modi G. Stroke in Black South African HIV-positive patients: a prospective analysis. Stroke 2003;34:10-15.

Mohammad QD, Habib M, Hoque A, et al. Prevalence of stroke above forty years. Mymensingh Med J 2011;20:640-4.

Mollah AS, Rahman SW, Das KK, Hassanuzzaman M. Characteristics of patients admitted with stroke. Mymensingh Med J 2007;16:20-4.

Moon K, Guallar E, Navas Acien A. Arsenic exposure and cardiovascular disease: an updated systematic review. Curr Atheroscler Rep 2012;542-55.

Moon KA, Guallar E, Umans JG, et al. Association between exposure to low to moderate arsenic levels and incident cardiovascular disease. Ann Intern Med 2013;159:649-59.

Morgenstern LB, Escobar JD, Sánchez BN, et al. Fast food and neighborhood stroke risk. Ann Neurol 2009;66:165-70.

Mudzi W, Stewart A, Musenge E. Case fatality of patients with stroke over a 12-month period post stroke. S Afr Med J 2012;102:765-7.

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation 2002;106:3143-421.

Navas-Acien A, Sharrett AR, Silbergeld EK, Schwartz BS, Nachman KE, Burke TA, Guallar E. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. Am J Epidemiol 2005;162:1037-49.

Navas-Acien A, Umans JG, Howard BV, et al. Urine arsenic concentrations and species excretion patterns in American Indian communities over a 10-year period: the Strong Heart Study. Environ Health Perspect 2009;117:1428-33.

Nayak SD, Nair M, Radhakrishnan K, Sarma PS. Ischaemic stroke in the young adult: clinical features, risk factors and outcome. Natl Med J India 1997;10:107-12.

No authors listed. Bangladesh NCD Risk Factor Survey 2010. www.whoban.org/LinkFiles/Publication_NCD_FACT_SHEET.pdf.pdf

No authors listed. World Health Organization. International Program on Chemical Safety. Ten chemicals of major public health concern. Last accessed 10 Feb 2014 at http://www.who.int/ipcs/assessment/public_health/chemicals_phc/en/

No authors listed. World Health Organization. “Preventing disease through healthy environments: arsenic.” Last accessed online 10 February 2014 at http://www.who.int/ipcs/features/arsenic.pdf

O’Donnell MJ, Xavier D, Liu L, et al. on behalf of the INTERSTROKE Investigators. Risk factors for ischaemic and intracerebral hemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet 2010;376:112-23.

O’Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, JR. for the Cardiovascular Health Study Collaborative Research Group. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. N Engl J Med 1999;340:14-22.

Ockene IS, Miller NH. Cigarette smoking, cardiovascular disease, and stroke: a statement for healthcare professionals from the American Heart Association. American Heart Association Task Force on Risk Reduction. Circulation 1997;96:3243-7.

Ortiz G, Koch S, Romano JG, Forteza AM, Rabinstein AA. Mechanisms of ischemic stroke in HIV-infected patients. Neurology 2007;68:1257-61.

Osorio-Yáñez C, Ayllon-Vergara JC, Aguillar-Madrid G, et al. Carotid intima-media thickness and plasma asymmetric dimethylarginine in Mexican children exposed to inorganic arsenic. Environ Health Perspect 2013;121:1090-96.

Oudin A, Strömberg U, Jakobsson K, Stroh E, Björk J. Estimation of short-term effects of air pollution on stroke hospital admissions in southern Sweden. Neuroepidemiology 2010;34:131-42.

Ovbiagele B, Nath A. Increasing incidence of ischemic stroke in patients with HIV infection. Neurology 2011;76:444-50.

Patel VB, Sacoor Z, Francis P, Bhigjee AI, Connolly C. Ischemic stroke in young HIV-positive patients in Kwazulu-Natal, South Africa. Neurology 2005;65:759-61.

Pan W, Li L, Tsai M. Temperature extremes and mortality from coronary heart disease and cerebral infarction in elderly Chinese. Lancet 1995;345:353-5.

Peters JL, Perlstein S, Perry MJ, McNeely E, Weuve J. Cadmium exposure in association with history of stroke and heart failure. Environ Res 2010;110:199-206.

Pomroy C, Charbonneau SM, McCollough RS, Tam GK. Human retention studies with 74As. Toxicol Appl Pharmacol 1980;53:550-6.

Puska P, Vartiainen E, Tuomilehto J, Salomaa V, Nissinen A. Changes in premature deaths in Finland: successful long-term prevention of cardiovascular diseases. Bull World health Organ 1998;76:419-25.

Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH, Axelson O. Hypertension and arsenic exposure in Bangladesh. Hypertension 1999;33:74-78.

Rasmussen LD, Engsig FN, Christensen H, Gerstoft J, Kronborg G, Pedersen C, Obel N. Risk of cerebrovascular events in persons with and without HIV: a Danish nationwide population-based cohort study. AIDS 2011;25:1637-46.

Reeves MJ, Bushnell CD, Howard G, et al. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. Lancet Neurol 2008;7:915-26.

Renoux C, Dell'Aniello S, Garbe E, Suissa S. Transdermal and oral hormone replacement therapy and the risk of stroke: a nested case-control study. BMJ 2010;340:c2519.

Roman MJ, Kizer JR, Best LG, Lee ET, Howard BV, Shara NM, Devereux RB. Vascular biomarkers in the prediction of clinical cardiovascular disease: the Strong Heart Study. Hypertension 2012;59:29-35.

Rothwell PM, Wroe SJ, Slattery J, Warlow CP, on behalf of the Oxfordshire Community Stroke Project. Is stroke incidence related to season or temperature? Lancet 1996;347:934-6.

Rothwell PM, Coull AJ, Silver LE, et al. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). Lancet 2005;366:1773-83.

Roumie CL, Mitchel EF Jr., Kaltenbach L, Arbogast PG, Gideon P, Griffin MR. Nonaspirin NSAIDs, cyclooxygenase 2 inhibitors, and the risk for stroke. Stroke 2008;39:2037-45.

Rundek T, Blanton SH, Bartels S, et al. Traditional risk factors are not major contributors to the variance in carotid intima-media thickness. Stroke 2013;44:2101-8.

Salonen R, Seppanen K, Ravramara R, Salonen JT. Prevalence of carotid atherosclerosis and serum cholesterol levels in eastern Finland. Arteriosclerosis 1988;8:788-92.

Salonen R, Tervahauta M, Salonen JT, Pekkanen J, Nissinen A, Karvonen MJ. Ultrasonographic manifestations of common carotid atherosclerosis in elderly eastern Finnish men: prevalence and associations with cardiovascular diseases and risk factors. Arterioscler Thromb 1994;141631-40.

Saposnik G, Baibergenova A, Dang J, Hachinski V. Does a birthday predispose to vascular events? Neurology 2006;67:300-4.

Sare GM, Gray LJ, Bath PM. Association between hormone replacement therapy and subsequent arterial and venous vascular events: a meta-analysis. Eur Heart J 2008;29:2031-41.

Sayeed MA, Banu A, Hag JA, Khanam PA, Mahtab H, Azad Khan AK. Prevalence of hypertension in Bangladesh: effect of socioeconomic risk factor on difference between rural and urban community. Bangladesh Med Res Counc Bull 2002;28:7-18.

Sayeed MA, Mahtab H, Akter Khanam P, Latif ZA, Kermat SM, Banu A, et al. Diabetes and impaired fasting glycemia in a rural population in Bangladesh. Diabetes Care 2003;1034-9.

Scheer J, Findenig S, Goessler W, Francesconi KA, Howard B, Umans JG, et al. Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study. Anal Methods 2012;4:406-13.

Schlesselman JJ. Case-control Studies: design, conduct, analysis. New York: Oxford University Press, 1982.

Schutte R, Nawrot T, Richart T, et al. Arterial structure and function and environmental exposure to cadmium. Occup Environ Med 2008;65:412-9.

Seaberg EC, Benning L, Sharrett AR, et al. Association between Human Immunodeficiency Virus infection and stiffness of the common carotid artery. Stroke 2010;41:2163-70.

Sen S, Rabinstein AA, Elkind MS, Powers WJ. Recent developments regarding human immunodeficiency virus infection and stroke. Cerebrovasc Dis 2012;33:209-18.

Serradj Jaillard A, Hommel M, Mazetti P. Prevalence of stroke at high altitude (3380 m) in Cuzco, a town of Peru. Stroke 1995;26:562-8.

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure - Complete Report. 2004. Last accessed 4 April 2014 at http://www.nhlbi.nih.gov/guidelines/hypertension/jnc7full.pdf

Sharma SK, Ghimire A, Radhakrishnan J, et al. Prevalence of hypertension, obesity, diabetes, and metabolic syndrome in Nepal. Int J Hypertens 2011;821971.

Shimizu M, Ishikawa J, Yano Y, Hoshide S, Shimada K, Kario K. The relationship between the morning blood pressure surge and low-grade inflammation on silent cerebral infarct and clinical stroke events. Atherosclerosis 2011;219:316-21.

Shinkawa A, Ueda K, Hasuo Y, Kiyohara Y, Fujishima M. Seasonal variation in stroke incidence in Hisayama, Japan. Stroke 1990;21:1262-7.

Shinton R, Beevers G. Meta-analysis of relation between cigarette smoking and stroke. BMJ 1989;298:789-94.

Simeonova PP, Luster MI. Arsenic and atherosclerosis. Toxicology Applied Pharmacol 2004;198:444-9.

Singh RB, Fedacko J, Pella D, et al. Prevalence and risk factors for prehypertension and hypertension in five Indian cities. Acta Cardiol 2011;66:29-37.

Skilton MR, Sérusclat A, Begg LM, Moulin P, Bonnet F. Parity and carotid atherosclerosis in men and women: insights into the roles of childbearing and child-rearing. Stroke 2009;40:1152-7.

Sokrab TO, Sid-Ahmed FM, Idris MN. Acute stroke type, risk factors, and early outcome in a developing country: a view from Sudan using a hospital-based sample. J Stroke Cerebrovasc Dis 2002;11:63-5.

Sørensen M, Hvidberg M, Andersen ZJ, et al. Road traffic noise and stroke: a prospective cohort study. Eur Heart J 2011;32:737-42.

Stoddart ML, Jarvis B, Blake B, et al. Recruitment of American Indians in epidemiologic research: the Strong Heart Study. Am Indian Alsk Native Ment Health Res 2009;9:20-37.

Strategies for Management of Anti-Retroviral Therapy/INSIGHT; DAD Study Groups. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients. AIDS 2008;22:F17-24.

Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ 2009;339:b4567.

The Strong Heart Study. Cardiovascular Disease in American Indians (Phase IV). Operations Manual vol. 5: Special studies – carotid ultrasound, tonometry, and echocardiography. June 1, 2001. Oklahoma City, OK. Last accessed 16 Februrary 2014 at http://strongheart.ouhsc.edu/manual/phaseiv/volume5.pdf

Strong K, Mathers C, Bonita R. Preventing stroke: saving lives around the world. Lancet 2007;182-87.

Sudlow CLM, Warlow CP. Comparing stroke incidence worldwide: what makes studies comparable? Stroke 1996;27:550-8.

Tipping B, de Villiers L, Wainwright H, Candy S, Bryer A. Stroke in patients with human immunodeficiency virus infection. J Neurol Neurosurg Psychiatry 2007;78:1320-4.

Tseng C. Betel nut chewing and subclinical ischemic heart disease in diabetic patients. Cardiology Research and Practice 2011;1-5.Turin TC, Kita Y, Rumana N, et al. Stroke

case fatality shows seasonal variation regardless of risk factor status in a Japanese population: 15-year results from the Takashima Stroke Registry. Neuroepidemiology 2009;32:53-60.

Venables WN, Ripley BD. Modern Applied Statistics with *S*, 4th edn. London: Springer, 2002.

Vinikoor MJ, Napravnik S, Floris-Moore M, Wilson S, Huang DY, Eron JJ. Incidence and clinical features of cerebrovascular disease among HIV-infected adults in the Southeastern United States. AIDS Res Human Retro 2013;29:1068-74.

Wade TJ, Xia Y, Wu K, et al. Increased mortality associated with well-water arsenic exposure in Inner Mongolia, China. Int J Environ Res Public Health 2009;6:1107-23.

Wang C, Jeng J, Yip P, et al. Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 2002;105:1804-9.

Wassertheil-Smoller S, Hendrix S, Limacher M, et al. for the WHI Investigators. Effect of estrogen plus progestin on stroke in postmenopausal women: The Women's Health Initiative: a randomized trial. JAMA 2003;289:2673-84.

Wang C, Jeng J, Yip P, Chen C, Hsu L, Hsueh Y, et al. Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 2002;105:1804-9.

Weltry TK, Lee ET, Yeh J, Cowan LD, Go O, Fabsitz RR, et al. Cardiovascular disease risk factors among American Indians The Strong Heart Study. Am J Epidemiol 1995;142:269-87.

Wen CP, Tsai SP, Cheng TY, Chen CJ, Levy DT, Yang HG, et al. Uncovering the relation between betel quid chewing and cigarette smoking in Taiwan. Tob Control 2005;14 Suppl 1:i16-22.

Wijewardene K, Mohideen MR, Mendis S, et al. Prevalence of hypertension, diabetes and obesity: baseline findings of a population based survey in four provinces in Sri Lanka. Ceylon Med J 2005;50:62-70.

Wild SH, Fischbacher C, Brock A, Griffiths C, Bhopal R. Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. J Publ Health 2007;29:191-8.

Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: a risk profile from the Framingham Study. Stroke 1991;22:312-18.

World Health Organization International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 89: Smokeless tobacco and some tobacco-specific *N*-nitrosamines. Lyon, France, 2007.

Xia Y, Wade TJ, Wu K, et al. Well water arsenic exposure, arsenic induced skin-lesions and self-reported morbidity in Inner Mongolia. Int J Environ Res Public Health 2009;6:1010-25.

Yang G, Rao C, Ma J, et al. Validation of verbal autopsy procedures for adult deaths in China. Int J Epidemiol 2006;35:741–48.

Yen A, Chiu Y, Chen L, Wu HM, Huang CC, Boucher BJ, Chen TH. A population-based study of the association between betel-quid chewing and the metabolic syndrome in men. Am J Clin Nutr 2006;83:1153-60.

Yoshikawa M, Aoki K, Ebine N, Kusunoki M, Okamoto A. Correlation between the arsenic concentrations in the air and the SMR of lung cancer. Environ Health Prev Med 2008;13:207-18.

Yuan Y, Marshall G, Ferreccio C, et al. Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. Am J Epidemiol 2007;166:1381-91.

Zaman MM, Rouf MA. Prevalence of hypertension in a Bangladeshi adult population. J Human Hypertension 1999;13:547-9.

Zaman MM, Yoshiike N, Rouf MA, et al. Cardiovascular risk factors: distribution and prevalence in a rural population of Bangladesh. J Cardiovasc Risk 2001;8:103-8.

Zhang B, Gao C, Hou Q, et al. The potent different risk factors for cerebral infarction in young patients with and without type 2 diabetes: subanalysis of the young cerebral infarction study (YCIS). Atherosclerosis 2012;221:215-20.

Zhang L, Yang J, Hong Z, et al. Proportion of different subtypes of stroke in China. Stroke 2003;34:2091-96.

Zhang L, Yang Y, Xu Z, Gui Q, Hu Q. Chewing substances with or without tobacco and risk of cardiovascular disease in Asia: a meta-analysis. Biomed & Biotechnol 2010;11:681-9.

Zhang X, Shu XO, Yang G, et al. Association of passive smoking by husbands with prevalence of stroke among Chinese women nonsmokers. Am J Epidemiol 2005;161:213-18.

Zhang X, Shu XO, Gao YT, Yang G, Li H, Zheng W. Pregnancy, childrearing, and risk of stroke in Chinese women. Stroke 2009;40:2680-4.

Zhang Y, Galloway JM, Welty TK, Wiebers DO, Whisnant JP, Devereux RB, et al. Incidence and risk factors for stroke in American Indians: The Strong Heart Study. Stroke 2008;118:1577-84.

Tables

Table 1. Population-Based Mortality from Stroke by Year for persons 20 years and older in Matlab, 2005 to 2008

	Population			Deaths			Stroke Deaths		
Year	Male	Female	Total	Male	Female	Total	Male	Female	Total
2005	107057	117693	224750	691	520	1211	193	171	364
2006	106512	118026	224538	653	482	1135	172	171	343
2007	105626	118413	224039	678	589	1265	143	195	338
2008	103579	118639	222218	669	590	1259	110	95	205
Total	422774	472771	895545	2691	2179	4870	616	632	1250

Table 2. Baseline Characteristics of Adults who died of Stroke versus Unintentional Injury

Characteristic	Stroke Deaths (n=1250)	Injury Deaths (n=246)	p-value
Female (%)	50.9% (636/1250)	47.4% (130/274)	0.336
Age (years) (mean, range) 20-29 years (%) 30-59 years (%) 60-69 years (%) 70-79 years (%) 80-89 years (%) 90 years and older (%)	72.3 (20.6-101.1) 1.0% (7/1250) 10.0% (133/1250) 21.0% (299/1250) 36.9% (516/1250) 18.2% (246/1250) 3.9% (49/1250)	55.8 (20.2-100.1) 17.5% (48/274) 35.0% (96/274) 13.9% (38/274) 17.9% (49/274) 13.1% (36/274) 2.6% (7/274)	<0.001
Level of Education None (%) Primary School (%) Secondary School (%) College or More (%)	 60.6% (757/1250) 24.9% (311/1250) 13.0% (163/1250) 1.5% (19/1250)	 53.6% (147/274) 25.9% (71/274) 15.3% (42/274) 5.1% (14/274)	0.001
Death at Home (%)	91.0% (1138/1250)	42.2% (113/268)	<0.001
Most common occupations (%)	1. disabled physically or mentally 44.3% (554/1250) 2. housewife / housework 22.5% (281/1250) 3. farmer (landowner) 9.5% (119/1250) 4. retired 6.1% (76/1250) 5. working proprietors 4.9% (61/1250)	1. housewife / housework 29.6% (81/274) 2. disabled physically or mentally 20.4% (56/274) 3. farmer / landowner 10.2% (28/274) 4. farm worker / daily laborer (landless) 5.1% (14/274) 5. no occupation 5.1% (14/274)	---
Hypertension (%)	47.4% (549/1158)	5.8% (16/274)	<0.001
Diabetes Mellitus (%)	18.0% (211/1173)	3.3% (9/270)	<0.001
Coronary Artery Disease (%)	6.1% (73/1188)	1.5% (4/270)	0.003

Cigarette smoking/biri (%)	38.1% (475/1246)	29.3% (39/133)	0.046
Pipe smoking/hookah (%)	9.3% (115/1243)	6.8% (9/133)	0.342
Betel consumption (%)	83.9% (1049/1250)	65.4% (87/133)	<0.001
Tobacco powder/gool (%)	1.8% (23/1246)	1.5% (2/133)	1

Table 3. Distribution of Potential Risk Factors of People who died of Stroke by Sex

	Women (n=632)	Men (n=618)	
Age (years) (mean, range)	72.6 (20.6-101.1)	71.9 (21.7-96.9)	0.316
Hypertension	47.7% (281/589)	47.1% (268/569)	0.836
Diabetes	15.2% (90/592)	20.8% (121/581)	0.013
Coronary Heart Disease	5.7% (34/601)	6.6% (39/587)	0.479
Level of Education			
None (%)	81.2% (513/632)	39.5% (244/618)	<0.001
Primary School (%)	15.2% (96/632)	34.8% (215/618)	
Secondary School (%)	4.9% (31/632)	22.8% (141/618)	
College or More (%)	0.2% (1/632)	2.9% (18/618)	
Cigarette smoking/biri	5.9% (37/631)	71.2% (438/615)	<0.001
Pipe smoking/hookah	1.3% (8/631)	17.5% (107/612)	<0.001
Betel consumption	90.8% (574/632)	77.5% (479/618)	<0.001
Tobacco powder/gool	0.8% (5/631)	2.9% (18/615)	0.009

Table 4. Risk Factors for Stroke compared to Unintentional Injury Deaths

Potential Risk Factor	Odds Ratio*	95% Confidence Interval	p-value
Hypertension	7.94	4.44-15.54	<0.001
Diabetes	2.54	1.21-6.21	0.02
Betel nut	2.36	1.45-3.80	<0.001
Heart disease	1.37	0.45-5.95	0.62
Cigarette smoking	1.41	0.82-2.45	0.22
Tobacco powder	1.15	0.30-7.64	0.86
Cigar/hookah pipe smoking	0.94	0.45-2.18	0.88

*Adjusted for age and sex in a multivariable logistic regression model

Table 5. Sensitivity analysis for regression coefficients and relative risks for significant risk factors in cox proportional hazards regressions for stroke profiles in male Framingham subjects ages 55-84 years old and MACS participants

	Framingham Heart Study (n=2,372)		Total MACS Cohort (n=3945)	
	Coefficient	Relative Risk**	Relative Risk (95% CI)	p-value
Risk Factor				
Age (per 10 year increase)	0.049	1.63 (1.33-1.99)**	2.37 (1.71-3.30)	<0.001
Systolic BP (per 10mmHg increase)	0.015	1.16 (1.10-1.24)**	0.97 (0.77-1.22)	0.80
Antihypertensive Therapy/SBP term	0.00019	-	$3.9x10^{-4}$*** ($-3.4x10^{-4}$, $1.1x10^{-3}$)	0.29
Diabetes mellitus	0.34	1.41 (0.97-2.04)	2.05 (0.42-10.0)	0.37
Cigarette smoking	0.52	1.69 (1.27-2.23)	1.35 (0.72-2.55)	0.36
Cardiovascular Disease*	0.55	1.73 (1.68-1.78)	2.47 (0.70-8.69)	0.16
Atrial fibrillation	0.60	1.82 (1.01-3.29)	-	-
Left ventricular hypertrophy	0.79	2.20 (1.26-3.84)	-	-
HIV	-	-	1.72 (0.90-3.31)	0.10

Table 6. Point System of the Framingham Study for probability of stroke within 10 years for men aged 55-84 years and free of previous stroke (Wolf 1991)

	Points										
	0	1	2	3	4	5	6	7	8	9	10
Risk Factor											
Age (years)	54-56	57-59	60-62	63-65	66-68	69-71	72-74	75-77	78-80	81-83	84-86
Systolic BP (mmHg)	95-105	106-116	117-126	127-137	138-148	149-159	160-170	171-181	182-191	192-202	203-213
Hypertensive Medications	No		Yes								
Diabetes	No		Yes								
Cigarettes	No			Yes							
Cardiovascular Disease	No			Yes							
Atrial Fibrillation	No				Yes						
Left Ventricular Hypertrophy	No						Yes				

Note: To assess the sensitivity of our findings to errors in the reporting of stroke events, we reran our analyses using only confirmed events by medical records review in the MACS (45 strokes among 40 subjects), as described in the methods section. When excluding all unconfirmed events, we found the estimated baseline FRS-S for HIV+ patients who experienced a stroke to average 4.8% (range 0-11%) compared to HIV- participants with stroke whose baseline FRS-S averaged 6.5% (range 0.3%-26%) (p=0.11). In addition, for the second analysis we refit a Cox proportional hazards model as in Table 9.

Table 7. Comparison of Baseline Demographic and Clinical Features related to Stroke Risk in the Framingham Heart Study (FHS) and Multicenter AIDS Cohort Study (MACS)

Risk Factor	**Framingham Heart Study Original Cohort (men only), 1954-1990**	**MACS, HIV+, 1996-2011**		**MACS, HIV-, 1996-2011**		**Comparison of HIV+ and HIV- MACS participants**
		Entire Cohort (n=1776)	**Stroke Subjects (n=57)**	**Entire Cohort (n=2169)**	**Stroke Subjects (n=37)**	
Variables in FRS-S						
Mean Age, Range (y)*	65.4, 55-84	41.0, 17-69	45.4, 30-66	43.4, 18-82	53.4, 38-72	<0.001
Systolic BP (mean mmHg)	139.3	121.7, 83-180	122.5, 94-150	122.6, 80-201	128.2, 100-178	0.06
Antihypertensive therapy (%)	16.1	8.9	21.4	5.2	13.2	0.41
Diabetes mellitus (%)	10.6	1.5	1.8	1.5	2.6	0.98
Cigarette smoking (%)	33.8	39.5	42.9	33.2	36.8	<0.001
Cardiovascular disease** (%)	22.2	2.7	8.9	1.5	7.9	1
Atrial fibrillation (%)	2.8	Unknown	Unknown	Unknown	Unknown	-
Left ventricular hypertrophy (%)	3.5	Unknown	Unknown	Unknown	Unknown	-
Other Variables, not in FRS-S						
HIV-Related Variables						
HIV (%)	Unknown	100	100	0	0	<0.001
HIV Viral Load at Baseline Mean (IQR) (copies/mL)†	N/A	24,130 (7770)	7,194 (340)	N/A	N/A	N/A
CD4 Count at Baseline (cells/mL) Mean (IQR)	N/A	688 (401)	760 (481)	957 (450)	947 (381)	<0.001
No. of Years since HIV diagnosis, Mean (IQR)	N/A	5.9 (11.6)	6.4 (11.6)	N/A	N/A	N/A
Duration on HAART (years), Mean (IQR)	N/A	0.9 (0.3)	1.0 (0.2)	N/A	N/A	N/A
Other Variables						

Race (% Caucasian-Black-Other)	Predominantly Caucasian	65-29-7	75-20-5	79-18-4	82-16-3	<0.001
Body Mass Index (kg/m^2) Mean (IQR)	Unknown	24.2 (4.0)	24.4 (5.1)	24.6 (4.4)	25.2 (3.5)	0.05
Alcohol, Average in no. drinks per week, Mean (IQR)	N/A	7.0 (10.0)	7.1 (10.1)	7.4 (9.6)	6.6 (8.4)	<0.001
Use of Cocaine or Crack in the past 2 years or the previous study visit (%)	N/A	35	20	29	29	<0.001
Any Other Drug Abuse by History, including Poppers, Marijuana, Hash, "Uppers" or other drugs‡	N/A	68	71	69	74	0.37

Legend: *At the time of study entry in the HAART era, **As stated in methods section in the MACS †More than 50% of participants had missing values. ‡Includes crystal meth, meth, speed, and ice. N/A=not applicable

Table 8. Regression coefficients and relative risks for significant risk factors in cox proportional hazards regressions for stroke profiles in male Framingham participants ages 55-84 years old and MACS participants

	Framingham Heart Study (n=2,372)		MACS HIV+ (n=2042)	MACS HIV- (n=1531)	Total MACS Cohort (n=3945)	
	Coefficient	Relative Risk**	Relative Risk (95% CI)	Relative Risk (95% CI)	Relative Risk (95% CI)	p-value
Risk Factor						
Age (per 10 years)	0.049	1.63 (1.33-1.99)**	1.80 (1.26-2.55)	2.55 (1.89-3.46)	2.19 (1.74-2.75)	<0.001
Systolic BP	0.015	1.16 (1.10-1.24)**	0.97 (0.78-1.20)	1.10 (0.76-1.18)	1.03 (0.89-1.20)	0.77
Antihypertensive Therapy/SBP term (per 10 mmHg increase)	0.00019	-	$3.3x10^{-4}$*** ($1.5x10^{-4}$, $1.4x10^{-3}$)	$8.0x10^{-4}$*** ($1.6x10^{-4}$, $1.4x10^{-3}$)	$4.5x10^{-4}$*** ($-8.0x10^{-6}$, $9.1x10^{-4}$)	0.05
Diabetes mellitus	0.34	1.41 (0.97-2.04)	0.83 (0.11-6.28)	0.38 (0.04-3.62)	0.75 (0.17,3.25)	0.66
Cigarette smoking	0.52	1.69 (1.27-2.23)	1.20 (0.69-2.10)	1.29 (0.66-2.55)	1.24 (0.80-1.90)	0.33
Cardiovascular Disease*	0.55	1.73 (1.68-1.78)	3.11 (1.09-8.9)	3.78 (0.98-14.6)	3.12 (1.38-7.04)	<0.01
Atrial fibrillation	0.60	1.82 (1.01-3.29)	-	-	-	-
Left ventricular hypertrophy	0.79	2.20 (1.26-3.84)	-	-	-	-
HIV	-	-	-	-	2.16 (1.39-3.31)	<0.001

*In MACS, limited to self-reported myocardial infarction ascertainment.

**Given for 10-unit changes, as per the original Framingham study; all other variables reported dichotomously. In FHS, all variables were significant at a $p<0.05$ level.

***Coefficient from predictive model, as opposed to relative risk of stroke, due to nonlinearity

Table 9. Selected Clinical Studies of Stroke in HIV-Infected Adult Patients Performed in the HAART era with n≥10 HIV-infected stroke patients (July 1996 - June 2013)

Primary Author Last Name (Year of Publication)	**Study Design**	**Study Location**	**No. of Stroke Events in HIV+ Individuals**	**Conclusions**
Hoffmann (2000)	Retrospective case control	KwaZulu Natal, South Africa	22	Incidence rate similar between HIV+ and HIV- young Africans but higher incidence of large vessel cryptogenic strokes in HIV+
Evers (2003)	Prospective academic medical center-based cohort	Münster, Germany	15	Ischemic stroke more common in HIV+ individuals
Mochan (2003)	Prospective hospital-based study	Johannesburg, South Africa	35	Similar patient characteristics between HIV+ and HIV- patients
Cole (2004)	Retrospective medical records review of 46 hospitals	Central Maryland and Washington, DC, U.S.A.	12	AIDS is strongly associated with both ischemic and hemorrhagic stroke
Patel (2005)	Hospital-based retrospective chart review	KwaZulu Natal, South Africa	56	No difference in angiographic, cardiac, or serologic tests between HIV+ and HIV- groups
Ortiz (2007)	Hospital-based retrospective chart review	Miami, U.S.A.	82	High incidence of vasculopathy and hypercoaguability but mostly immunocompromised patients

Tipping (2007)	Prospective, hospital-based study	Cape Town, South Africa	67	HIV vasculopathy occurred in 20% of patients
Corral (2009)	Retrospective hospital-based cohort study	Madrid, Spain	25	Stroke incidence is increased in HIV treated with HAART
Lifson (2010)	Events recorded in a clinical trial of HIV+ participants	U.S.A., multi-center	38	55% of strokes were confirmed based on defined criteria vs. 5% probable and 39% unconfirmed
Rasmussen (2011)	Population-based cohort using active register	Denmark	140	Increased risk of ischemic stroke in HIV, with and without proven risk factors
Ovbiagele (2011)	Retrospective, hospital-based national registry	Nationwide Inpatient Sample, U.S.A.	10,944	Increased number of admitted stroke patients with HIV in recent years
Chow (2012)	Cohort study	Boston, U.S.A.	132	Ischemic stroke rates increased in HIV, especially younger patients and women
Vinikoor (2013)	Retrospective cohort study with scheduled chart reviews	North Carolina, U.S.A.	53	Ischemic stroke incidence ~1.5 times higher than a population-based cohort; risk not associated with antiretroviral therapy

Table 10. Common Carotid Artery (CCA) Measurements in the Strong Heart Study

Carotid Measurement	*Measure*	*Value*
Presence of CCA Plaque	Yes No	1605 (64.8) 873 (35.2)
Number of Effected CCA Segments with Plaque (n, %)	0 1 2 3 4 5 6 7 8	877 (34.1) 544 (21.0) 520 (20.0) 299 (11.5) 174 (6.7) 88 (3.4) 47 (1.8) 25 (1.0) 12 (0.5)
Atherosclerosis Score, Left CCA (n, %)	0 1 2 3	1203 (48.6) 1233 (49.8) 27 (1.1) 15 (0.6)
Atherosclerosis Score, Right CCA (n, %)	0 1 2 3	1161 (46.9) 1278 (51.6) 31 (1.3) 8 (0.3)
Far Wall Thickness of CCA, Left, millimeters	mean, (25^{th}, 75^{th} percentiles)	0.75 (0.60, 0.87)
Far Wall Thickness of CCA, Right, millimeters	mean, (25^{th}, 75^{th} percentiles)	0.74 (0.60, 0.85)
Far Wall Thickness of CAA, mean Left and Right, millimeters	mean, 25^{th}, 75^{th} percentiles	0.75 (0.63, 0.84)

Table 11. Characteristics of the 2478 Study Participants at Study Visit including Carotid Ultrasound Measurements

Characteristic	*Measure*	*Value*	*No Carotid Plaque*	*Carotid Plaque*
Mean age (standard deviation ((SD))	years	55.3 (7.6)	52.6 (6.5)	56.8 (7.8)
Female sex	n (%)	1563 (63)	613 (70.2)	950 (59.2)
Inorganic plus methylated arsenic species (25^{th}, 75^{th} percentile)	(μg/g creatinine)	2.22 (1.73, 2.69)	2.20 (1.71, 2.67)	2.24 (1.74, 2.70)
Systolic blood pressure (SD)	mmHg	125.5 (18.4)	123 (16.1)	127.1 (19.4)
Diastolic blood pressure (SD)	mmHg	76.6 (10.0)	77.0 (9.5)	76.4 (10.3)
Hypertension, [U.S. Definition]*	present diagnosis n (%)	853 (34.5)	257 (29.5)	596 (37.2)
Hypertension medication treatment	yes (%)	522 (21.1)	162 (18.6)	360 (22.4)
Fasting blood glucose (SD)	ng/mL(check)	145 (72.4)	137 (67.2)	150 (74.7)
Hemoglobin A1c (SD)	%	6.6 (2.32)	6.3 (2.24)	6.7 (2.35)
Diabetesł	%	1109 (44.8)	328 (37.6)	781 (48.7)
Diabetes medication treatment	oral, insulin, both, none n (%)	433 (17), 215 (9), 9 (0), 1821 (73)	117 (13), 54 (6), 1 (0), 701 (80)	316 (20), 161 (10), 8 (1), 1120 (70)
Serum triglycerides (SD)	mg/dL	147 (123)	137 (121)	152 (124)
Serum total cholesterol (SD)	mg/dL (check)	191 (37.5)	183 (36.2)	196 (37.4)
Serum high density lipoprotein (SD)	mg/dL	46.0 (13.0)	46.4 (12.7)	45.8 (13.3)
Atherogenic index (SD)	mg/dL	1.02 (0.71)	0.951 (0.679)	1.06 (-3.35, 4.34)
Serum fibrinogen (SD)	mmol/L (check)	299 (75.7)	300 (74.5)	298 (76.4)
Estimated glomerular filtration rate >60 mL/min/1.73m^2	n (%)	2258 (92.7)	826 (95.9)	1432 (90.9)
Waist-to-hip ratio (SD)	Cm	0.948 (0.07)	0.941 (0.07)	0.951 (0.07)
Body mass index (SD)	kg/m^2	31.0 (6.1)	32.5 (6.7)	30.2 (5.6)
Height (SD)	Cm	165.1 (9.0)	164.2 (8.8)	165.6 (9.1)
Weight (SD)	Kg	84.6 (17.8)	87.7 (19.4)	83.0 (16.7)
Cigarette smoking	current, previous, never n (%)	790 (32), 861 (35), 824 (33)	221 (25), 317 (36), 335 (38)	569 (36), 544 (34), 489 (31)
Smoking pack-years (SD)	years	15.4 (19.4)	12.0 (16.9)	17.0 (20.3)

Alcohol use	current, previous, never n (%)	1026 (41), 1043 (42), 405 (16)	358 (41), 369 (42), 146 (17)	668 (42), 674 (42), 259 (16)
Educational attainment level (SD)	years	11.3 (3.0)	11.6 (2.9)	11.2 (3.0)
Study location	Oklahoma, Arizona, North and South Dakota n (%)	857 (35), 809 (33), 812 (33)	316 (36), 352 (40), 205 (23)	541 (33), 457 (28), 607 (38)

Note: ŧDefinition of Diabetes: fasting glucose, medication for diabetes, or HBA1c

Table 12. Relationship between traditional cardiovascular risk factors, urinary arsenic levels, and increase in common carotid arterial intimal-medial thickness (per micrometer)

Charac-teristic	Mean Difference (95% CI)	p-value	Mean Difference, Adjusted for Age and Sex (95% CI)	p-value	Mean Difference, Adjusted, Model A	p-value Model A	Mean Difference, Adjusted, Model B	p-value, Model B	Mean Difference, Adjusted, Model C	p-value Model C
Age (y)	6.92 (6.18, 7.66)	<0.001	7.00 (6.26, 7.73)	<0.001	6.70 (5.93, 7.47)	<0.001	6.48 (5.68, 7.27)	<0.001	6.73 (5.92, 7.53)	<0.001
Sex	36.4 (24.0, 48.8)	<0.001	39.9 (28.3, 51.5)	<0.001	41.1 (28.8, 53.4)	<0.001	32.4 (19.6, 45.3)	<0.001	34.2 (21.4, 47.1)	<0.001
Arsenicł *	7.47 (1.51, 13.4)	0.01	10.4 (4.86, 16.0)	<0.001	-318 (-622, -15.3)	0.04	9.35 (3.35, 15.4)	<0.01	3.29 (-3.60, 10.2)	0.35
Systolic blood pressure (mmHg)	1.40 (1.08, 1.73)	<0.001	0.663 (0.349, 0.977)	<0.001	93.4 (78.0, 109)	<0.001	0.562 (0.239, 0.886)	<0.01	0.580 (0.254, 0.906)	<0.001
Fasting blood glucose* (mmol/L)	0.133 (0.050, 0.216)	<0.01	0.187 (0.110, 0.265)	<0.001	-8.67 (-12.7, -4.62)	<0.001	0.053 (-0.053, 0.159)	0.33	0.027 (-0.080, 0.134)	0.62
HDL (mg/dL)	-0.896 (-1.36, -0.435))	<0.001	-0.952 (-1.39, -0.516)	<0.001	51.9 (29.4, 74.5)	<0.001	-0.764 (-1.24, -0.287)	<0.01	-0.711 (-1.19, -0.235)	<0.01
Total Cholesterol (mg/dL)	-0.053 (-0.215, 0.108)	0.52	-0.050 (-0.201, 0.100)	0.51	7.15 (-0.565, 14.9)	0.07	0.079 (-0.090, 1.14)	0.36	0.121 (-0.052, 0.295)	0.17
Triglycerides (mg/dL)	-2.90×10^{-3} (-0.052, 0.046)	0.91	0.016 (-0.030)	0.49	-3.09 (-5.41, -0.758)	<0.01	-0.039 (-0.087, 0)	0.11	-0.032 (-0.080, 0.016)	0.19
Atherogenic index*	7.08 (-1.4, 15.6)	0.10	9.19 (1.24, 17.1)	0.02	-614 (-1020, -207)	<0.01	-14.3 (-33.2, 4.71)	0.14	-7.94 (-26.1, 10.3)	0.39
Smoking Former vs. Never	17.0 (2.37, 31.5)	0.02	14.2 (0.218, 28.2)	0.047	16.3 (1.75, 30.8)	0.03	16.7 (2.22, 31.2)	0.02	18.6 (4.07, 33.1)	0.01
Current vs. Never	-7.04 (-21.9, 7.87)	0.36	2.09 (-12.2, 16.4)	0.78	6.30 (-8.63, 21.2)	0.41	12.4 (-2.67, 27.5)	0.11	15.8 (0.359, 31.1)	0.045

Alcohol Former vs. Never Current vs. Never	-9.18 (-26.7, 8.31) -34.8 (-52.4, -17.3)	0.30 <0.001	-1.60 (-18.3, 15.1) -15.3 (-32.8, 2.11)	0.85 0.09	-5.25 (-22.8, 12.3) -18.3 (-36.8, -0.052)	0.56 0.049	-3.22 (-20.7, 1.24) -12.1 (-30.4, 6.23)	0.48 <0.001	-4.49 (-21.9, 12.9) -15.7 (-34.0, 2.63)	0.61 0.09
BMI* (kg/m^2)	0.131 (-0.855, 1.17)	0.79	1.54 (0.606, 2.47)	<0.01	-185 (-232, -137)	<0.001	-0.067 (-1.11, 9.72)	0.89	-0.120 (-1.16, 0.922)	0.82
Waist-to-hip ratio	0.345 (0.255, 0.434)	<0.001	213 (126, 301)	<0.001	6946 (2412, 11480)	<0.01	120 (27.4, 21.2)	0.01	100 (7.92, 193)	0.03
Education (y)	-4.91 (-6.91, -2.90)	<0.001	-1.63 (-3.54, 0.271)	0.09	-1.77 (-3.69, 0.151)	0.07	-0.710 (-2.66, 1.24)	0.48	0.730 (-1.31, 2.77)	0.48
Center ref=AZ, OK DK	-26.6 (-41.2, -11.9) -10.3 (-25.2, 4.50)	<0.001 0.17	-39.5 (-53.1, -25.8) -19.5 (-33.3, -5.71)	<0.001 <0.01	2836 (2103, 3569) 2139 (1.413, 2865)	<0.001 <0.001	-33.1 (-47.9, -18.2) -9.24 (-24.1, 5.60)	<0.001 0.22	-	-

ł sum of inorganic and methylated arsenic species, natural logarithm (µg/L creatinine) (ln As/ln2)

*For these variables, the model also includes the variable of interest in each model.

Model A includes age, sex, education, ethanol intake, smoking.

Model B includes age, sex, education, ethanol intake, smoking, waist-to-hip ratio, diabetes mellitus, systolic blood pressure, serum triglycerides, and HDL.

Model C includes Model B + study center.

Table 13. Odds Ratio for Risk Factors for the Presence of Carotid Atherosclerotic Plaque

Charac-teristic	Crude Odds Ratio (OR) (95% CI)	p-value	OR, Adjusted for Age and Sex (95% CI)	p-value	OR, Adjusted, Model A	p-value, Model A	OR, Adjusted Model B	p-value Model B	OR, Adjusted, Model C	p-value, Model C
Age (y)	1.08 (1.07, 1.10)	<0.001	1.09 (1.07, 1.10)	<0.001	1.09 (1.08, 1.11)	<0.001	1.09 (1.08, 1.11)	<0.001	1.08 (1.07, 1.10)	<0.001
Sex	1.63 (1.36, 1.94)	<0.001	1.75 (1.46, 2.11)	<0.001	1.58 (1.31, 1.92)	<0.001	1.64 (1.33, 2.02)	<0.001	1.55 (1.27, 1.91)	<0.001
Arsenicł*	1.07 (0.986, 1.16)	0.10	1.12 (1.03, 1.22)	0.01	1.10 (1.00, 1.21)	0.04	1.05 (0.957, 1.16)	0.29	1.10 (0.982, 1.23)	0.10
Systolic Blood Pressure	1.01 (1.009, 1.018)	<0.001	1.01 (1.001, 1.011)	0.03	1.01 (1.00, 1.01)	<0.01	1.01 (1.00, 1.01)	0.02	1.01 (1.00, 1.02)	<0.001
Fasting blood glucose*	1.00 (1.001, 1.004)	<0.001	1.00 (1.002, 1.004)	<0.001	1.00 (1.00, 1.01)	<0.001	1000 (1001, 1004)	<0.01	1.00 (1.00, 1.01)	<0.001
Diabetes (yes vs. no)	1.57 (1.33, 1.86)	<0.001	1.59 (1.34, 1.90)	<0.001	1.77 (1.47, 2.12)	<0.001	1.69 (1.39, 2.05)	<0.001	2.28 (1.86, 2.80)	<0.001
Smoking Former vs. Never Current vs. Never	 1.18 (0.967, 1.43) 1.76 (1.43, 2.17)	 0.11 <0.001	 1.16 (0.940, 1.44) 2.07 (1.65, 2.59)	 0.16 <0.001	 1.16 (0.926, 1.44) 2.02 (1.60, 2.56)	 0.20 <0.001	 1.18 (0.938, 1.47) 2.27 (1.78, 2.90)	 0.16 <0.001	1.13 (0.894, 1.43) 1.90 (1.47, 2.46)	0.30 <0.001
Alcohol Former vs. Never Current vs. Never	 1.03 (0.811, 1.31) 1.05 (0.827, 1.34)	 0.81 0.68	 1.17 (0.908, 1.52) 1.38 (1.06, 1.81)	 0.22 0.02	 1.06 (0.811, 1.40) 1.14 (0.858, 1.51)	 0.65 0.37	 1.08 (0.816, 1.42) 1.17 (0.878, 1.57)	 0.61 0.28	1.14 (0.854, 1.51) 1.11 (0.822, 1.49)	0.30 <0.001
Education	0.948 (0.922, 0.975)	<0.001	0.980 (0.952, 1.01)	0.20	0.984 (0.954, 1.01)	0.28	0.997 (0.966, 1.03)	0.83	0.985 (0.951, 1.02)	0.37

Total cholesterol *	1.01 (1.01, 1.01)	<0.001	1.01 (1.00, 1.01)	<0.001	1.01 (1.01, 1.01)	<0.001	1.01 (1.01, 1.01)	<0.001	1.01 (1.01, 1.01)	<0.001
HDL	0.997 (0.990, 1.00)	0.29	0.996 (0.990, 1.00)	0.30	0.995 (0.989, 1.02)	0.19	1.00 (0.995, 1.01)	0.56	0.988 (0.980, 1.00)	<0.01
Triglycerides	1.001 (1.000, 1.002)	<0.01	1.00 (1.000, 1.002)	<0.01	1.00 (1.00, 1.00)	<0.01	1.00 (1.00, 1.00)	0.02	1.00 (1.00, 1.00)	0.86
BMI*	0.941 (0.928, 0.954)	<0.001	0.951 (0.934, 0.965)	<0.001	0.957 (0.943, 0.971)	<0.001	0.934 (0.919, 0.950)	<0.001	0.952 (0.937, 0.968)	<0.001
Waist-to-hip ratio	12.8 (3.64, 45.3)	<0.001	2.38 (0.615, 9.23)	0.21	3.01 (0.758, 11.9)	0.12	0.843 (0.193, 3.69)	0.82	3.58 (0.713, 17.9)	0.12
Center ref=AZ, OK DK	1.32 (1.08, 1.60) 2.28 (1.85, 2.82)	<0.01 <0.001	1.16 (0.948, 1.43) 2.20 (1.76, 2.74)	0.15 <0.001	1.11 (0.906, 1.36) 1.97 (1.60, 2.41)	0.32 <0.001	1.100 (0862, 1.40) 1.90 (1.47, 2.46)	0.45 <0.001	-	-

Model 1 includes age, sex, education, ethanol intake, smoking.

Model 2 includes age, sex, education, ethanol intake, smoking, waist-to-hip ratio, diabetes mellitus, systolic blood pressure, serum triglycerides, and HDL.

Model 3 is Model 2 + Study Center.

*For these variables, the model also includes the variable of interest in each model.

Table 14: Relationship between traditional risk factors, arsenic, and carotid atherosclerotic plaque score (score 0 through 4+; n=870, 35%; n=528, 21%; n=502, 20%; n=278 11%; n=300, 12%)

Characteristic	Crude Odds Ratio (OR) (95% CI)	OR, Adjusted for Age and Sex (95% CI)	OR, Adjusted, Model B	p-value, Model B	OR Adjusted, Model C	OR Adjusted, Model C
Age (y)	1.08 (1.07, 1.09)	1.08 (1.07, 1.09)	1.09 (1.07, 1.10)	<0.001	1.08 (1.07, 1.09)	<0.001
Sex	1.46 (1.27, 1.69)	1.57 (1.35, 1.82)	1.46 (1.24, 1.73)	<0.001	1.44 (1.22, 1.69)	<0.001
Arsenic*	1.11 (1.03, 1.19)	1.12 (1.04, 1.20)	1.05 (0.976, 1.14)	0.18	1.06 (0.969, 1.16)	0.20
Systolic Blood Pressure	1.02 (1.01, 1.02)	1.01 (1.00, 1.01)	1.01 (1.00, 1.01)	<0.001	1.01 (1.01, 1.02)	<0.001
Fasting blood glucose	1.00 (1.00, 1.00	1.67 (1.43, 1.94)	1.00 (1.00, 1.00)	0.001	1.00 (1.00, 1.00)	<0.001
Diabetes	1.65 (1.43, 1.91)	1.65 (1.42, 1.91)	1.73 (1.48, 2.02)	<0.001	2.33 (1.98, 2.75)	<0.001
HDL	1.00 (0.99, 1.00)	1.00 (1.00, 1.00)	1.00 (0.996, 1.01)	0.48	0.99 (0.98, 1.00)	<0.01
Total cholesterol	1.01 (1.01, 1.01)	1.01 (1.01, 1.01)	1.01 (1.01, 1.01)	<0.001	1.01 (1.01, 1.01)	<0.001
Triglycerides	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	<0.01	1.00 (1.00, 1.00)	0.76
Atherogenic Index	1.22 (1.10, 1.35)	1.28 (1.16, 1.42)	1.45 (1.15, 1.84)	<0.01	1.46 (1.15, 1.84)	<0.01
Smoking Former vs. Never Current vs. Never	 1.16 (0.976, 1.38) 1.73 (1.45, 2.06)	 1.20 (1.00, 1.44) 2.09 (1.74, 2.51)	 1.19 (0.982, 1.44) 2.30 (1.88, 2.80)	 0.08 <0.001	 1.15 (0.948, 1.40) 1.92 (1.56, 2.36)	 0.15 <0.001
Alcohol Former vs. Never Current vs. Never	 1.07 (0.875, 1.32) 1.03 (0.841, 1.27)	 1.26 (1.01, 1.56) 1.41 (1.12, 1.76)	 1.17 (0.927, 1.47) 1.20 (0.939, 1.52)	 0.19 0.15	 1.21 (0.958, 1.52) 1.11 (0.872, 1.42)	 0.11 0.39
Body-mass index	0.945 (0.934, 0.956)	0.955 (0.943, 0.967)	0.937 (0.923, 0.951)	<0.001	0.955 (0.942, 0.968)	<0.001

Waist-to-hip ratio	12.4 (4.22, 36.5)	3.012 (0.977, 9.33)	0.909 (0.270, 3.06)	0.89	3.60 (0.997, 13.0)	0.05
Education (y)	0.944 (0.922, 0.967)	0.978 (0.954, 1.001)	0.993 (0.968, 1.02)	0.59	0.984 (0.968, 1.011)	0.24
Center ref=AZ, OK DK	 1.29 (1.09, 1.54) 2.15 (1.80, 2.57)	 1.13 (0.947, 1.35) 2.09 (1.74, 2.50)	 1.30 (1.06, 1.58) 2.47 (2.03, 3.01)	 0.01 <0.001	-	-

Model B includes age, sex, education, ethanol intake, smoking, waist-to-hip ratio, diabetes mellitus, systolic blood pressure, serum triglycerides, and HDL.

Model C includes Model A + Study Center.

Table 15. Risk of CIMT Increase and Plaque Presence in American Indians

	CIMT	Plaque
Age	√	√
Male	√	√
Systolic blood pressure	√	√
HDL	√ (lower)	√ (higher)
Total Cholesterol		√
Smoking Cigarettes	√ (former vs. never)	√ (current vs. never)
Waist-to-Hip Ratio	√	
BMI		√
Diabetes		√
Fasting Blood Glucose		√
Arsenic	√	

*Also adjusted for EtOH, Education, and not adjusted for region

Figures

Figure 1. Incidence of fatal stroke in Matlab, Bangladesh by month of year (2005-2008)

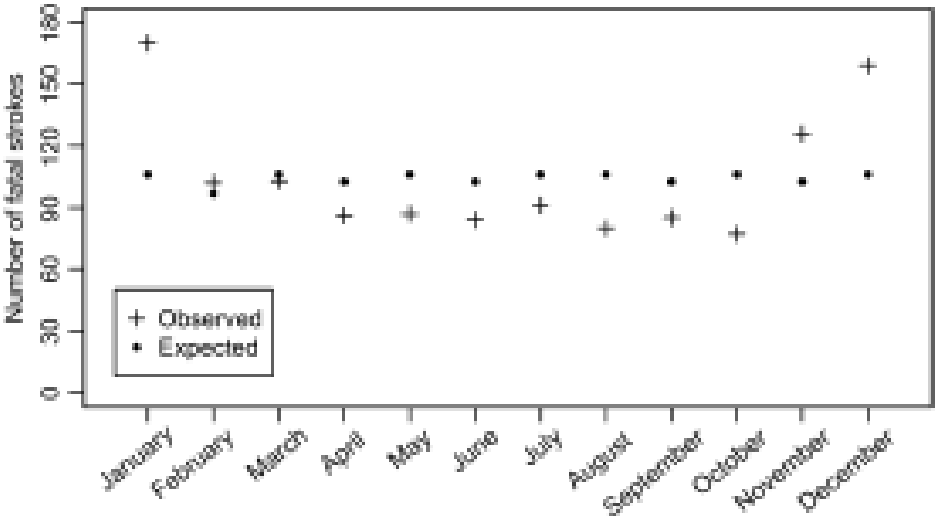


Figure 2. Distribution of stroke events by calendar year in the MACS

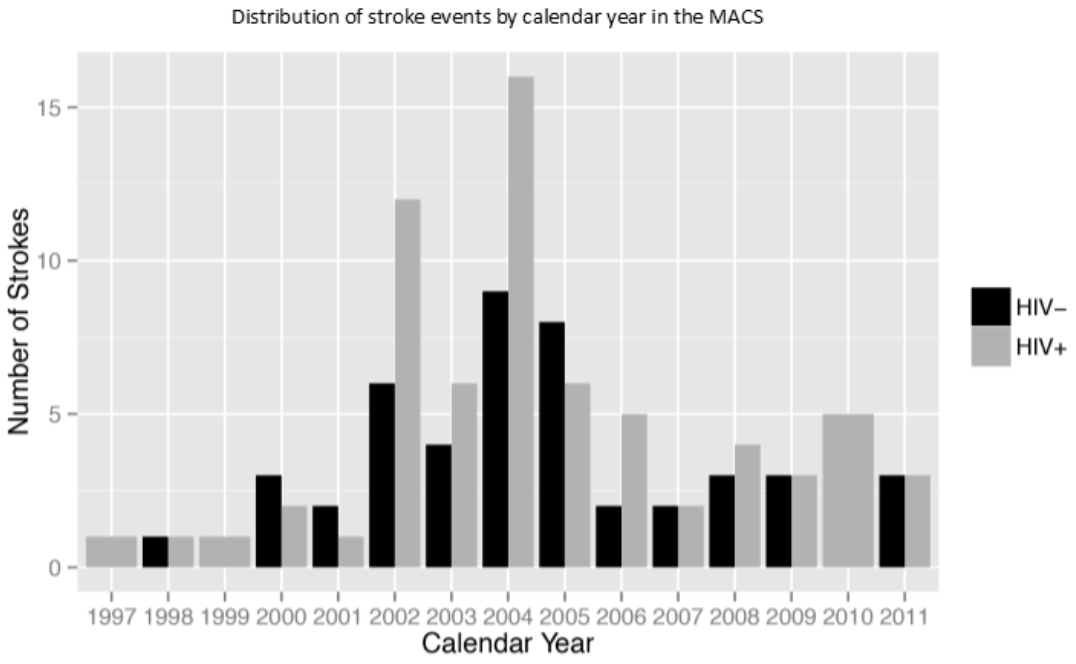


Figure 3. Histograms of the Framingham Risk Score for Stroke point composition of the entire MACS cohort at the beginning of the HAART era.

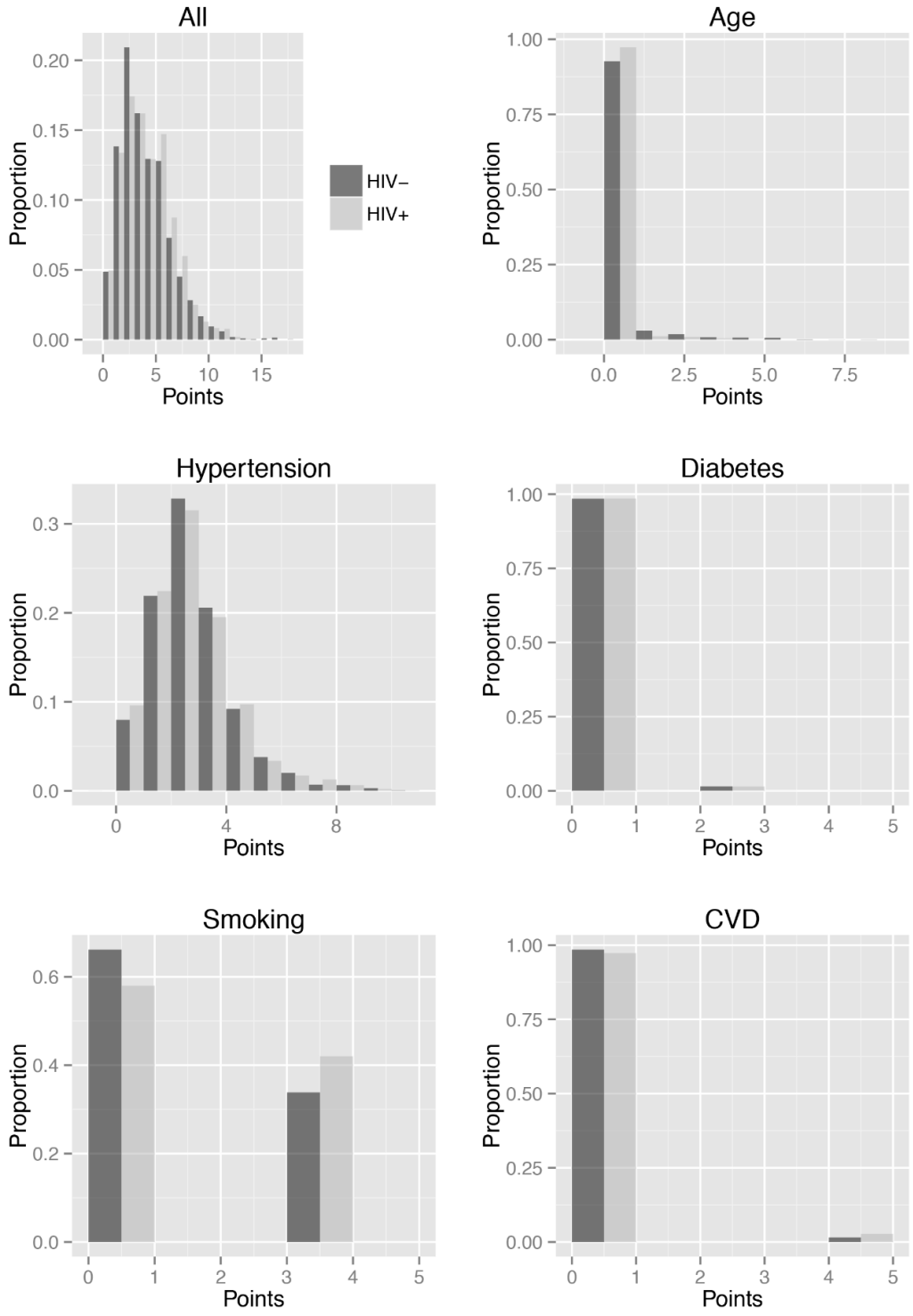
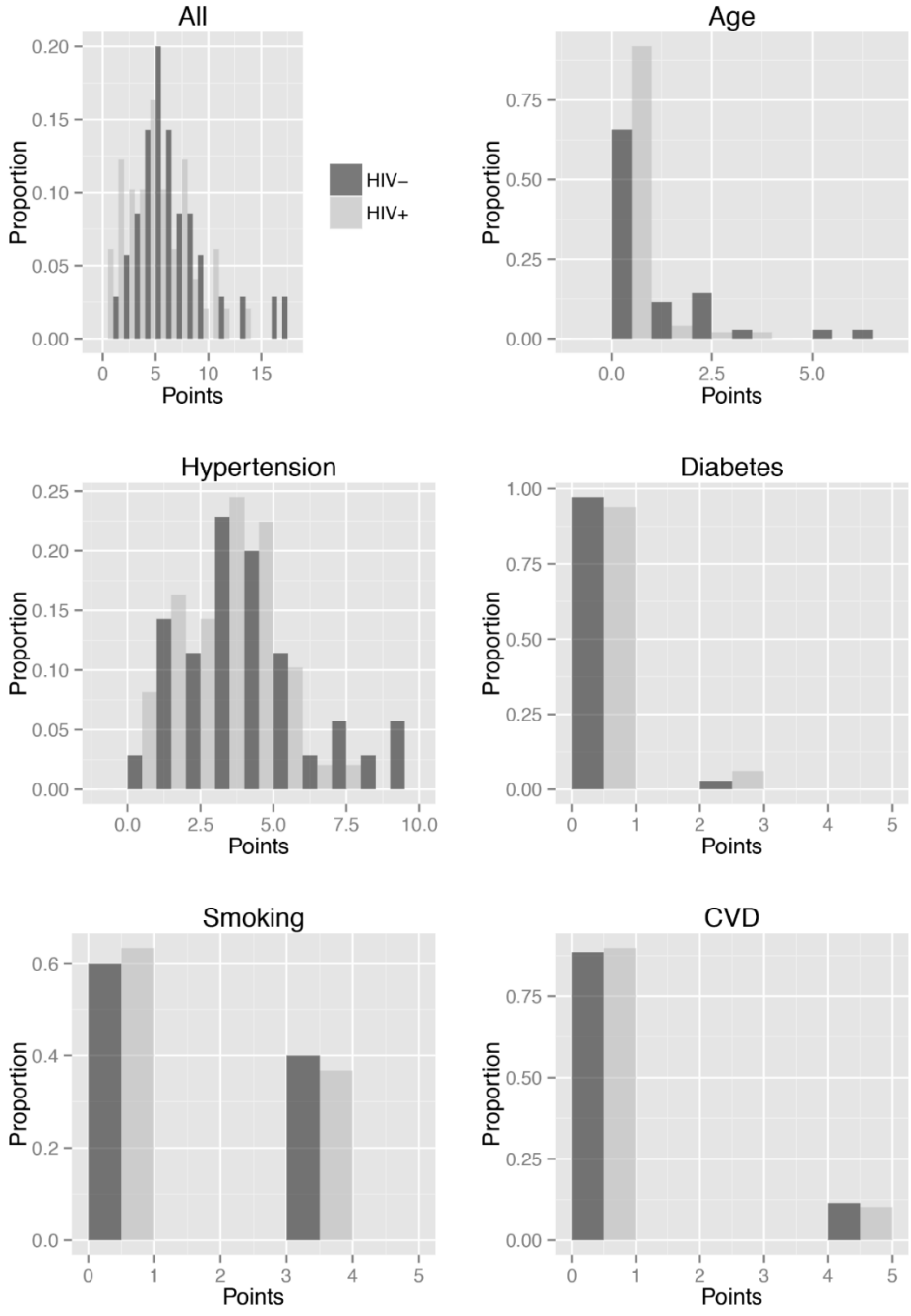


Figure 4. Histograms of the Framingham Risk Score for Stroke point composition of the MACS participants who experienced a stroke.



Curriculum Vita

Farrah Mateen was born September 17, 1981 in Prince Albert, Saskatchewan Canada. She completed her M.D. at the University of Saskatchewan College of Medicine in Saskatoon, SK in 2005, her residency in Adult Neurology at the Mayo Clinic in Rochester, MN in 2009 (American Board of Psychiatry and Neurology), her fellowship in Medical Ethics at Harvard Medical School in Boston, MA in 2008, and her fellowship in Neurological Infections and Neuroimmunology at the Johns Hopkins Hospital in Baltimore, MD in 2012. She is now on faculty as Assistant in Neurology at the Massachusetts General Hospital and Assistant Professor at the Harvard Medical School in Boston, MA.

Farrah has worked, volunteered, and consulted for several international organizations including the Global Polio Eradication Initiative, World Health Organization, United Nations High Commissioner for Refugees, Caritas Lebanon, and others. She served as Chair of the American Academy of Neurology's Ethics Section and currently serves as Co-Chair of the American Neurological Association's International Outreach Team. She is the principal investigator of the Bhutan Epilepsy Project, funded by Grand Challenges Canada and the Thrasher Research Foundation. She has also received support from the Canadian Institute of Health Research, American Brain Foundation, and others to work towards her career goal of bringing focus to neurological disorders in vulnerable populations and raise the profile of neurological disorders on the international health agenda through data-driven approaches.