Infectious Burden and Risk of Stroke

The Northern Manhattan Study

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Objective: To determine the association between a composite measure of serological test results for common infections (Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, and herpes simplex virus 1 and 2) and stroke risk in a prospective cohort study.

Design: Prospective cohort followed up longitudinally for median 8 years.

Setting: Northern Manhattan Study.

Patients: Randomly selected stroke-free participants from a multietnic urban community.

Main Outcome Measure: Incident stroke and other vascular events.

Results: All 5 infectious serological results were available from baseline samples in 1625 participants (mean [SD] age, 68.4 [10.1] years; 64.9% women). Cox proportional hazards models were used to estimate associations of each positive serological test result with stroke. Individual parameter estimates were then combined into a weighted index of infectious burden and used to calculate hazard ratios and confidence intervals for association with risk of stroke and other outcomes, adjusted for risk factors. Each individual infection was positively, though not significantly, associated with stroke risk after adjusting for other risk factors. The infectious burden index was associated with an increased risk of all strokes (adjusted hazard ratio per standard deviation, 1.39; 95% confidence interval, 1.02-1.90) after adjusting for demographics and risk factors. Results were similar after excluding those with coronary disease (adjusted hazard ratio, 1.50; 95% confidence interval, 1.05-2.13) and adjusting for inflammatory biomarkers.

Conclusions: A quantitative weighted index of infectious burden was associated with risk of first stroke in this cohort. Future studies are needed to confirm these findings and to further define optimal measures of infectious burden as a stroke risk factor.

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Stroke is the third leading cause of death in the United States and the leading cause of serious disability.1 Classic modifiable risk factors include hypertension, cardiac disease, dyslipidemia, and smoking, but many strokes occur in patients without any of these risk factors.2 There is therefore interest in identifying additional modifiable stroke risk factors. Seroepidemiological studies in patients with coronary artery disease and stroke have provided evidence of an association of risk with serological evidence of prior infections with various pathogens such as Chlamydia pneumoniae, Helicobacter pylori, and herpesviruses.2-4 In addition, several of these organisms have been identified in atherosclerotic tissue specimens, and some have been found capable of inducing atherosclerosis in animal models.5-9 Mechanistically, these pathogens are thought to promote inflammation, thus contributing to atherosclerosis.10,11 Prospective studies and meta-analyses, however, have suggested that the association of individual serological test results with risk of vascular events is modest.2 A more likely scenario is that a composite measure of multiple serological results, or infectious burden (IB), is associated with risk.3,1-6 According to this hypothesis, the greater the number of infectious exposures during one’s lifetime, the higher the chance of promoting atherosclerosis via inflammation, thus increasing the risk of cardiovascular disease. Some investigators have provided support for this concept by correlating the number of positive serological test results with the presence of atherosclerosis and carotid plaque progres-
sion or risk of vascular events, including stroke. In these studies, however, all positive serological test results are given equal weight in predictive models. It is plausible, however, that different infections would each carry different weights of association with risk of vascular disease.

We hypothesized that a weighted measure of IB would be associated with risk of incident stroke in a prospective cohort study in a multiethnic urban adult population. We further hypothesized that this same weighted measure would also be associated with other vascular event outcomes.

METHODS

PARTICIPANT SELECTION

The Northern Manhattan Study is a community-based prospective cohort study designed to investigate stroke incidence, risk factors, and predictors of severity and outcome, as described previously. Briefly, this is a stroke-free, multiethnic, urban population with a race/ethnic distribution of 63% Hispanic, 20% non-Hispanic black, and 17% non-Hispanic white participants who were recruited by random-digit dialing. A total of 3298 participants were enrolled between 1993 and 2001 if they (1) had no prior diagnosis of stroke, (2) were older than 39 years, and (3) resided in northern Manhattan, New York, for at least 3 months in a household with a telephone. All participants gave informed consent and the study was approved by Columbia University Medical Center institutional review board.

Baseline data were obtained via interviews with bilingual research assistants, physical and neurological examination by study physicians, inpatient assessments, and fasting blood specimen analysis. Blood pressure, height, weight, fasting lipid panels, leukocyte count, and glucose level were measured by standard techniques. High-sensitivity C-reactive protein (hsCRP) level was measured using a BN11 nephelometer (DadeBehring, Deerfield, Illinois). Hypertension (history, taking medications, or systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) and diabetes mellitus (history, taking medications, or fasting blood glucose level ≥126 mg/dL [to convert to millimoles per liter, multiply by 0.0555]) were defined as described previously. Blood samples collected at enrollment were centrifuged and frozen at −70°C in 1-mL aliquots until the time of analysis. Because not all participants had sufficient blood samples available for the multiple serological analyses required for this study, a subsample of 1625 participants was used for the present analyses.

SEROLOGICAL ANALYSES

Serological test results against 5 common pathogens that have each been linked to atherosclerotic disease in prior studies were assessed. Enzyme-linked immunoassay was used to measure antibody titers against *C pneumoniae* (Sawyon Diagnostics, Ashdod, Israel), *H pylori*, cytomegalovirus (CMV) (Wampole Laboratories, Princeton, New Jersey), and herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) (Focus Diagnostics, Cypress, California). Both IgG and IgA titers were measured for *C pneumoniae*, but based on previous results in our population and others, IgA titers were used for further analyses. IgG titers were assessed for the other pathogens. Positive serological test results were based on recommendations from the commercial laboratories providing the assays. All serological testing was conducted by personnel blinded to clinical outcomes.

FOLLOW-UP ASSESSMENTS

Annual follow-up conducted by telephone included assessment of vital status (dead or alive), interval hospitalizations, and presence of symptoms and events consistent with stroke or myocardial infarction (MI) as previously described. Strokes were independently classified by 2 neurologists according to a modified Stroke Data Bank scheme, with subtype assessments determined by consensus. Disagreements were adjudicated by a third neurologist evaluator. A study cardiologist reviewed MIs for validation. First presentation of any type of stroke, ischemic or hemorrhagic, was defined according to the criteria established by the World Health Organization. Myocardial infarction was defined by criteria specified by the Cardiac Arrhythmia Pilot Study and the Lipid Research Clinics Coronary Primary Prevention Trial. All deaths were verified by a study physician and classified as vascular (stroke, MI, heart failure, pulmonary embolus, cardiac arrhythmia, and other vascular causes) or nonvascular.

STATISTICAL ANALYSES

The primary end point was all strokes; secondary end points were ischemic stroke, MI, vascular deaths, nonvascular deaths, all deaths, and a combined end point of stroke, MI, and vascular death. First, Cox proportional hazards models were used to estimate the regression coefficients and 95% confidence intervals (CIs) for the association of each individual serological test result with risk of stroke in unadjusted models and models adjusted for demographics, blood pressure, coronary artery disease, waist size, alcohol consumption, physical activity, smoking, and levels of blood glucose, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. We included variables in multivariate models that were significant in prior analyses of vascular outcomes in the Northern Manhattan Study and that are traditionally accepted risk factors for stroke. We used continuous measures for blood pressure and blood glucose level rather than crude dichotomous measures such as hypertension and diabetes in our main models based on evidence of continuous relationships between these physiologic measures and risk of vascular disease. Additional models were run using hypertension and diabetes mellitus as categorical variables.

Parameter estimates, or β coefficients, from a model containing only the individual serological test results were then used to derive a weighted index designated as the IB. Each parameter estimate represents the strength of the association between the individual positive serological test result and stroke as an outcome. Individual parameter estimates for positive serological test results were added together to form the IB index. Parameter estimates for negative serological test results would not be counted toward the total index score. For example, the IB for a participant with individual positive serological results for CMV and HSV-2 would equal the sum of the parameter estimates for only CMV and HSV-2. This index was then used as the independent variable in unadjusted and adjusted models to calculate the hazard ratios (HRs) and CIs for association with risk of stroke and other outcomes. Further models were calculated among those without history of MI. The final models among those without MI satisfied proportionality assumptions. Associations were expressed per change in standard deviation of IB.

RESULTS

Characteristics among the 1625 participants with serological data are shown in Table 1 and were similar to those in the overall Northern Manhattan Study cohort.
with the exception that the participants in this study had enrolled later compared with those who did not have all 5 infectious serological results at the time of enrollment. Mean (SD) age of participants was 68.4 (10.1) years, and the median follow-up was 7.6 years (interquartile range, 6.4-9.0 years). In follow-up, there were 67 strokes, of which 56 were ischemic; 98 MIs; 150 vascular deaths; 215 nonvascular deaths; and 390 deaths of all causes. Frequencies of positive serological results, using prespecified thresholds, for C pneumoniae, H pylori, CMV, HSV-1, and HSV-2 are given in Table 1. Positive titers were common for these organisms, ranging from 55% for C pneumoniae to 86% for HSV-2.

Each infectious serological result was positively associated with an increased risk for all strokes, with HRs adjusted for age, sex, race/ethnicity, high school education, systolic blood pressure, low-density lipoprotein cholesterol level, high-density lipoprotein cholesterol level, blood glucose level, moderate to heavy activity level, waist size, and coronary artery disease ranging between 1.13 for H pylori to 2.19 for CMV, although none of these associations reached statistical significance (Table 2).

To determine whether composite seropositivity was associated with risk of stroke, individual unadjusted parameter estimates were added, as earlier, to generate the mean IB index for those with positive results for each serological test is shown in the Figure. The mean IB index was higher in non-Hispanic black (1.05 [0.31]) and Hispanic (1.07 [0.27]) individuals compared with non-Hispanic white individuals (0.75 [0.41]; P < .001 for both comparisons). It was slightly higher in women (1.02 [0.31]) than men (0.97 [0.36]; P = .002). There was no difference by age.

Infectious burden was associated with risk of stroke (unadjusted HR per standard deviation, 1.39; 95% CI, 1.04-1.87), and the effect was essentially unchanged after adjusting for demographics (adjusted HR, 1.42; 95% CI, 1.04-1.94) (Table 3). After adjusting for age, sex, race/ethnicity, high school education, systolic blood pressure, low-density lipoprotein cholesterol level, high-density lipoprotein cholesterol level, blood glucose level, moderate to heavy activity level, waist size, and coronary artery disease, the association remained (adjusted HR, 1.39; 95% CI, 1.02-1.90). Further adjusting for leukocyte count and hsCRP level had no effect on this estimate. The magnitude of the correlation of hsCRP level had no effect on this estimate. The magnitude of the correlation of hsCRP level with IB index was only modest (r = 0.09; P < .001).

Additional models were run using history of hypertension and diabetes mellitus, as previously defined, in place of blood pressure and blood glucose measurements, and the results were essentially unchanged (ad-
Table 3. Risk of Stroke Associated With IB Index

<table>
<thead>
<tr>
<th>HR (95% CI) per SD IB</th>
<th>Among Full Cohort (n=1625)</th>
<th>Among Those Without History of MI (n=1525)</th>
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<tbody>
<tr>
<td>Unadjusted</td>
<td>1.39 (1.04-1.87)</td>
<td>1.51 (1.08-2.11)</td>
</tr>
<tr>
<td>Adjusted for demographics</td>
<td>1.42 (1.04-1.94)</td>
<td>1.54 (1.08-2.20)</td>
</tr>
<tr>
<td>Adjusted for demographics and risk factors</td>
<td>1.39 (1.02-1.90)</td>
<td>1.50 (1.05-2.13)</td>
</tr>
<tr>
<td>Adjusted for demographics, risk factors, and log hsCRP level</td>
<td>1.39 (1.02-1.90)</td>
<td>1.52 (1.06-2.17)</td>
</tr>
<tr>
<td>Adjusted for demographics, risk factors, and log leukocyte count</td>
<td>1.40 (1.03-1.91)</td>
<td>1.51 (1.06-2.16)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; IB, infectious burden; MI, myocardial infarction; SD, standard deviation.

Table 4. Risk of Secondary Outcomes Associated With IB

<table>
<thead>
<tr>
<th>Secondary Outcome</th>
<th>Unadjusted HR (95% CI) per SD IB</th>
<th>Adjusted HR (95% CI) per SD IB</th>
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<tr>
<td></td>
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<tr>
<td>Ischemic stroke</td>
<td>1.33 (0.97-1.82)</td>
<td>1.31 (0.94-1.83)</td>
</tr>
<tr>
<td>All MI</td>
<td>0.99 (0.82-1.20)</td>
<td>1.07 (0.85-1.33)</td>
</tr>
<tr>
<td>Vascular deaths</td>
<td>0.99 (0.85-1.16)</td>
<td>0.96 (0.81-1.15)</td>
</tr>
<tr>
<td>Nonvascular deaths</td>
<td>1.21 (1.04-1.40)</td>
<td>1.23 (1.04-1.45)</td>
</tr>
<tr>
<td>All deaths</td>
<td>1.12 (1.01-1.24)</td>
<td>1.12 (0.99-1.26)</td>
</tr>
<tr>
<td>Ischemic stroke, all MI, and vascular death</td>
<td>1.05 (0.92-1.19)</td>
<td>1.05 (0.90-1.21)</td>
</tr>
<tr>
<td>All stroke, all MI, and vascular death</td>
<td>1.06 (0.93-1.20)</td>
<td>1.05 (0.91-1.22)</td>
</tr>
<tr>
<td>All stroke, all MI, and all deaths</td>
<td>1.13 (1.03-1.25)</td>
<td>1.15 (1.03-1.29)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; IB, infectious burden; MI, myocardial infarction; SD, standard deviation.

In this prospective cohort study, a weighted index of exposure to 5 common infections previously implicated in atherosclerotic disease was associated with risk of first stroke. Although individually each infection was positively associated with increased stroke risk, none were individually statistically significant. Our measure of IB considered the association of infections with stroke risk as a weighted measure rather than simply summing up the number of positive serological results, as had been done in prior studies. We therefore did not have an a priori assumption about the strength of association between each of the individual infections and stroke risk. These results need to be validated in independent populations, however, before they can be generalized. These results provide evidence that there is probably no single infectious agent responsible for atherosclerosis or stroke, but that a more likely mechanism for any possible association of infection with stroke is through a more general proinflammatory mechanism.

The rationale for investigating these particular 5 pathogens is multifaceted. First, each of these common pathogens may persist after an acute infection and thus contribute to perpetuating a state of chronic, low-level infection. Second, prior studies demonstrated an association between each of these pathogens and cardiovascular diseases. The best studied of these infections in relation to atherosclerosis, C pneumoniae, is an atypical respiratory pathogen that has been investigated as a stroke risk factor in several case-control and prospective studies, with mixed results. In prior case-control studies from the northern Manhattan population, C pneumoniae IgA titers were associated with increased stroke risk, although IgG titers were not. Studies in other populations have similarly found IgA to be a better marker of stroke risk than IgG titers. These results have not been confirmed in other studies, however, and prospective studies have demonstrated at best a very modest association of C pneumoniae titers with stroke risk. Our present prospective study confirms that the effect of C pneumoniae IgA titers is likely to be modest. Further studies in large populations using well-standardized assays with generally accepted titer cutoffs may be needed to more definitively elucidate the relationship between C pneumoniae and stroke.

Recent studies have implicated H pylori, which is well recognized as a cause of chronic gastric inflammation, ulceration, and cancer, as another pathogen whose persistence might be associated with increased stroke risk. Several case-control studies have demonstrated an increased risk of stroke in seropositive subjects. A meta-analysis of case-control studies on H pylori serostatus revealed an increased risk for stroke (odds ratio, 1.41, 95% CI, 1.11-1.78), and especially for large-vessel stroke. The stroke risk appeared to be especially apparent in subjects seropositive for strains with the virulence factor cytotoxin-
associated gene product A (CagA) toxin.\textsuperscript{28} Other studies have not found this association, however.\textsuperscript{8,17,20,30} We did not limit our analyses to large-vessel stroke, however, nor did we measure \textit{H pylori} CagA toxin serostatus.

Cytomegalovirus is a common viral pathogen, and it can reactivate and cause particularly severe complications in immunocompromised hosts, though even immunocompetent hosts can develop CMV disease. Cytomegalovirus has been implicated as a cause of transplant vasculopathy.\textsuperscript{31,32} There is also some evidence that CMV is associated with stroke risk in otherwise healthy individuals. In 2 case-control studies, CMV seropositivity was associated with increased stroke risk,\textsuperscript{33,34} though this was not confirmed in several prospective studies.\textsuperscript{7,17,35,36} It has been shown that in immunocompromised hosts such as those with diabetes,\textsuperscript{37} or in post–renal transplant patients,\textsuperscript{31} the risk of atherosclerotic events including strokes is increased with CMV seropositivity. Cytomegalovirus was most strongly associated with stroke risk among the 5 pathogens we studied, though it did not reach statistical significance. Many of our participants (21%) had diabetes, and this may have influenced our findings.

Herpes simplex virus 1 and HSV-2 are also viral pathogens with chronic persistence in a latent state. Early work on Marek disease virus, a type of chicken herpesvirus, demonstrated that the virus was associated with atherosclerosis in a chicken model.\textsuperscript{38} Few studies have investigated the association of HSV with stroke risk, and their findings are conflicting. In 1 case-control study, HSV-1 was associated with increased stroke risk,\textsuperscript{39} but in another nested case-control study, neither HSV-1 nor HSV-2 increased stroke risk.\textsuperscript{7} As with CMV, immunocompetent status, even when associated with diabetes, might be a confounding factor.

Our study extends the findings of previous prospective studies aimed at investigating the association of IB and other vascular events. In a secondary analysis of the Heart Outcomes Prevention Evaluation trial, combined serostatus for 4 pathogens vs zero to 1 pathogen was associated with risk of MI, stroke, or cardiovascular death with a magnitude similar to what we found (HR, 1.41; 95% CI, 1.02-1.96).\textsuperscript{15} In that study, however, pathogen burden was not associated with stroke as an independent outcome. In the Framingham study, pathogen burden based on serostatus for \textit{C pneumoniae}, \textit{H pylori}, and CMV was not associated with pooled primary end points of MI, atheroembolic stroke, and coronary heart disease death.\textsuperscript{17} A small case-control study did show a modest association between pathogen burden based on serological results for \textit{Legionella pneumophila}, \textit{Mycoplasma pneumoniae}, and \textit{C pneumoniae} and risk of stroke and transient ischemic attack.\textsuperscript{39} However, other small case-control studies investigating the pathogen burden using similar serological results did not find an increased risk for stroke, though they may have been underpowered.\textsuperscript{8,34}

There are several differences between our study and these other studies. Most importantly, our weighted measure of IB avoided a priori assumptions about the strength of association between each of the individual infections and stroke risk. Our study also included HSV-1 and HSV-2 serostatus in the calculation of the IB index, which may have enhanced the ability to detect its influence on first stroke. Small sample size, selection bias, and case-control study design may account for some of the other differences. The Heart Outcomes Prevention Evaluation trial, moreover, was a secondary prevention trial rather than a population-based study.

Our study also explored the association between IB and secondary vascular outcomes. Nonvascular deaths and the combined end point of all stroke, MI, and death were associated with the IB index, though MI and vascular death were not. Studies of periodontal infection and vascular disease have similarly found that there may be a stronger association for stroke than for MI.\textsuperscript{30,41}

Our study could have potential clinical implications. For example, treatment and eradication of these chronic pathogens might mitigate future risk of stroke. Antibiotic therapy directed against \textit{C pneumoniae} has been tested in randomized controlled trials without evidence of benefit against heart disease.\textsuperscript{82,83} Whether the same holds true for stroke has not yet been established. More studies will be required to further explore IB as a potential modifiable risk factor for stroke.

Our study has several strengths. First, this was a large, multiethnic, population-based prospective study with routine assessment of risk factors and minimal loss to follow-up. We had a large proportion of Hispanic individuals, a traditionally understudied group, in our population. Incidence of first stroke was assessed after adjusting for traditional risk factors, as well as for hsCRP level and white blood cell count, variables previously shown to be associated with incident stroke.\textsuperscript{35,44} The limitations of our study include that participant information regarding the use of specific cholesterol-lowering agents such as statins, preexisting inflammatory diseases, anti-inflammatory medication use, immunosuppression status, and infection status at the time of stroke or other outcomes was unavailable. Availability of these data could have better accounted for known and other unknown confounding factors. Further studies will be needed to confirm the independent predictive effect of IB as a stroke risk factor.

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Author Contributions: Dr Elkind had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Elkind, Rundek, Sacco, and Paik. Acquisition of data: Elkind, Boden-Albala, Liu, Spitalnik, and Rundek. Analysis and interpretation of data: Elkind, Ramakrishnan, Moon, Boden-Albala, Rundek, Sacco, and Paik. Drafting of the manuscript: Elkind and Ramakrishnan. Critical revision of the manuscript for important intellectual content: Elkind, Moon, Boden-Albala, Liu, Spitalnik, Rundek, Sacco, and Paik. Statistical analysis: Moon and Paik. Obtained funding: Elkind and Sacco. Administrative, technical, and material support: Elkind, Ramakrishnan, Boden-Albala, Liu, Spitalnik, Rundek, and Sacco. Study supervision: Elkind, Spitalnik, Rundek, and Sacco.
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