Infectious Burden and Carotid Plaque Thickness
The Northern Manhattan Study

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Background and Purpose—The overall burden of prior infections may contribute to atherosclerosis and stroke risk. We hypothesized that serological evidence of common infections would be associated with carotid plaque thickness in a multietnic cohort.

Methods—Antibody titers to 5 common infectious microorganisms (ie, Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, and herpesvirus 1 and 2) were measured among stroke-free community participants and a weighted index of infectious burden was calculated based on Cox models previously derived for the association of each infection with stroke risk. High-resolution carotid duplex Doppler studies were used to assess maximum carotid plaque thickness. Weighted least squares regression was used to measure the association between infectious burden and maximum carotid plaque thickness after adjusting for other risk factors.

Results—Serological results for all 5 infectious organisms were available in 861 participants with maximum carotid plaque thickness measurements available (mean age, 67.2±9.6 years). Each individual infection was associated with stroke risk after adjusting for other risk factors. The infectious burden index (n=861) had a mean of 1.00±0.35 SD and a median of 1.08. Plaque was present in 52% of participants (mean, 0.90±1.04 mm). Infectious burden was associated with maximum carotid plaque thickness (adjusted increase in maximum carotid plaque thickness 0.09 mm; 95% CI, 0.03 to 0.15 mm per SD increase of infectious burden).

Conclusion—A quantitative weighted index of infectious burden, derived from the magnitude of association of individual infections with stroke, was associated with carotid plaque thickness in this multietnic cohort. These results lend support to the notion that past or chronic exposure to common infections, perhaps by exacerbating inflammation, contributes to atherosclerosis. Future studies are needed to confirm this hypothesis and to define optimal measures of infectious burden as a vascular risk factor. (Stroke. 2010;41:e117-e122.)

Key Words: atherosclerosis ■ carotid artery ■ carotid stenosis ■ epidemiology ■ infectious disease ■ inflammation ■ neursonology ■ risk factors

Atherosclerosis, a major cause of stroke and myocardial infarction, is an inflammatory process. Recent research has sought to identify inflammatory risk factors that may predict risk of downstream clinical complications associated with atheromatous plaque formation. Atherosclerotic lesions are initiated by endothelial damage, leading to an upregulation of adhesion molecules that recruit both monocytes and lymphocytes into the subendothelial space, where monocytes become macrophages. These macrophages secrete proinflammatory cytokines and ingest oxidized low-density lipoprotein (LDL). Inflammatory cascades involving activated macrophages, T-cells, and mast cells may also weaken plaque fibrous caps and promote plaque rupture, thrombosis, and ischemia. The underlying causes of vascular endothelial injury include traditional risk factors such as oxidized LDL, toxins in cigarette smoke, and shear stress caused by mechanical forces associated with hypertension, but may also include infections. Several studies have linked chronic exposure to infectious agents such as Chlamydia pneumoniae, Helicobacter pylori, and herpes viruses to inflammation, coronary artery disease, and stroke. Among the postulated mechanisms by which infections may contribute to atherosclerosis are direct injury to the plaque, remote signaling of inflammatory mediators, cross-reactivity of pathogen antigens with self-epitopes, and viral dysregulation of cell activity. Single pathogens, however, are unlikely to have a causal relationship with atherosclerosis. It is much more likely that if infections are
related to vascular disease, it is through the combined activity of several infections (ie, an aggregate infectious burden).15–18

Previous data indicated that carotid plaque thickness, imaged through duplex Doppler ultrasound, is associated with traditional vascular risk factors and is a powerful predictor of vascular events.19 We hypothesized that a weighted measure of infectious burden incorporating several common infections and associated with stroke risk would be associated with carotid plaque thickness in a cross-sectional analysis of a healthy, elderly, triethnic urban population.

Methods

Participant Recruitment

The Northern Manhattan Study (NOMAS) is a population-based prospective cohort study of stroke risk factors, incidence, and outcomes in an urban community. Details of participant recruitment and evaluation were published previously.20,21 Briefly, the NOMAS population was recruited through random digit dialing between 1993 and 2001; eligible participants satisfied the following inclusion criteria: (1) no prior stroke; (2) age ≥39 years; and (3) resident of Northern Manhattan for ≥3 months in a household with a telephone. A total of 3298 participants provided informed consent and was enrolled. The NOMAS race–ethnic distribution consists of 63% Hispanic, 20% non-Hispanic black, and 15% non-Hispanic white participants. Carotid ultrasound images in NOMAS were obtained for a total of 1625 participants of the NOMAS cohort who had sufficient stored blood specimens available. This cross-sectional analysis is based on the convenience sample of 861 participants who had both carotid ultrasound and infectious serology samples. This study was approved by the Columbia University Medical Center Institutional Review Board.

Data Collection

Baseline risk factor information was collected through standardized interviews by trained bilingual research assistants and physical examinations and neurological assessments by study physicians. Standardized questions adapted from the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System23 assessed vascular risk factors: hypertension, diabetes, hypercholesterolemia, peripheral vascular disease, transient ischemic attack, cigarette smoking, and cardiac conditions such as myocardial infarction, coronary artery disease, angina, congestive heart failure, atrial fibrillation, other arrhythmias, and valvular heart disease.24 Standardized methods were used for measuring blood pressure, height, weight, fasting lipid panel, leukocyte count, and blood glucose. Race–ethnicity was self-identified by patients using standard census questions. Hypertension was defined by history, medication use, or systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Diabetes mellitus was defined by history, medication use, or fasting blood glucose ≥126 mg/dL. Fasting levels of total cholesterol, LDL, high-density lipoprotein, and triglycerides were measured using a Hitachi 705 automated spectrometer (Boehringer, Mannheim, Germany).

Baseline blood samples were collected in serum tubes, centrifuged, and stored in 1.2-mL cryule vials at −70°C. High-sensitivity C-reactive protein (hsCRP) was assessed using nephelometry (Dade-Behring BNII, Deerfield, Ill) as previously described.20

Serological Testing

Infectious disease serology was evaluated for each of 5 pathogens in baseline blood samples using enzyme-linked immunosorbent assay. Antibody titers were measured for C pneumoniae (both IgG and IgA; Sayvon Diagnostics), H pylori IgG, cytomegalovirus (CMV) IgG (Wampole Laboratories, Princeton, NJ), and herpes simplex virus (HSV) 1 and HSV2 IgG (Focus Diagnostics). Both IgG and IgA titers were measured for C pneumoniae, but based on previous results in our population,20,21 and others,7 IgA titers were used for further analyses. All enzyme-linked immunosorbent assay kits are commercially available and positive serological results were identified according to manufacturer-recommended thresholds. Serological testing protocols were published previously.8,25 All serological testing was conducted by the Center for Laboratory Medicine at Columbia University and technologists were blinded to plaque measurements and clinical outcomes.

Measurement of Maximum Carotid Plaque Thickness

Maximal internal carotid plaque thickness (MCPT) was measured as described previously.26 Trained sonographers, blinded to participant risk factors, performed carotid imaging using a high-resolution B-mode ultrasound system, GE LogIQ 700, with a multifrequency 9/13 MHz linear-array probe and standardized scanning protocols. Both common and internal carotid arteries (including bifurcations) were examined for the presence of atherosclerotic plaque, defined as an area of a focal protrusion 50% greater than the thickness of the neighboring wall. Maximal wall thickness, including plaque thickness, was recorded with use of an electronic cursor from offline digitized images. Absence of atherosclerosis was recorded as MCPT=0. The maximum MCPT value of the 2 sides was used for analysis.

Statistical Analyses

Our development of a weighted infectious burden (IB) index based on the relationship of individual serological test results to risk of stroke was described previously.27 In brief, multivariable Cox proportional hazards models were used to estimate the regression coefficients and 95% CIs for the association of each serological result (positive versus negative) with the risk of stroke in models adjusted for all of the other serologies. Parameter estimates, or β coefficients, from this model 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 result and stroke as an outcome. Individual parameter estimates for those serological results for which an individual was positive were added together to form the IB index. Parameter estimates for those serological results for which the individual was negative are not counted toward the index. Thus, the IB index for an individual was calculated using the following formula given the presence (X=1) or absence (X=0) of the specific pathogen serology with weighted β coefficients from the stroke outcomes model: IB index = 0.26 (X CMV IgG)+0.086 (X H. pylori IgA)+0.69 (X HSV1 IgG)+0.22 (X HSV2 IgG)+0.18 (X CMV IgG). For example, the IB for a participant with individual positive serological results for CMV and HSV2 would equal the sum of the parameter estimates for only CMV and HSV2 (0.69+0.18=0.87). For the present analysis designed to determine whether the IB index was also associated with carotid atherosclerosis, this index was then used as the independent variable in unadjusted and adjusted weighted least squares regression models to calculate the association of the IB index with MCPT. Adjusted models included demographic blood pressure, coronary artery disease, waist size, alcohol consumption, physical activity, smoking, blood glucose, high-density lipoprotein, and LDL. Further models adjusting for hsCRP and leukocyte count were also assessed. In secondary analysis, the index was used as the independent variable in a logistic regression model to calculate the association between IB index and irregular plaque.

Results

Infectious serologies for all 5 pathogens were available in 861 participants (mean age, 67.2±9.6 SD years) with MCPT measurements in the NOMAS cohort. Participants evaluated for MCPT were on average 2.8 years younger (P<0.0001) and slightly more likely to be Hispanic (P=0.018) as com-
Comparing those without MCPT measurements. No other significant differences between the groups with and without MCPT were observed. Detailed descriptive statistics for the participants are presented in Table 1.

The seroprevalence was highest for HSV1 (86.9%) and lowest for H pylori (54.5%). The IB index for this sample had a mean value of 1.00 (SD 0.35) and median 1.08 (interquartile range, 0.86 to 1.26), which was very similar to the distribution of the IB index in the overall cohort (mean, 1.00±0.33 SD; median, 1.08). Carotid plaque was present in 52% of participants and the mean MCPT was 0.90 mm±1.04 SD.

Serological results for individual infectious microorganisms varied in their direction and magnitude of association with plaque thickness. For example, seropositivity for CMV was significantly associated with a mean increase of 0.22 mm (95% CI, 0.06 to 0.38) in MCPT in an adjusted model. In contrast, positive serological results for other individual organisms were not independently associated with MCPT (Table 2).

In a univariate linear regression analysis, IB index was positively, but not significantly, associated with MCPT (increase in MCPT per SD of IB index 0.05 mm; 95% CI, −0.01 to 0.11; P=0.07). After adjusting for demographics (age, sex, race–ethnicity, and education), infectious burden was significantly associated with MCPT (adjusted increase in MCPT per SD of IB index 0.09 mm; 95% CI, 0.02 to 0.14; P=0.01). IB remained associated with MCPT (adjusted increase in MCPT per SD of IB index 0.09 mm; 95% CI, 0.02 to 0.15; P=0.01) after adjusting for diabetes, systolic and diastolic blood pressure, history of coronary artery disease, smoking status, high-density lipoprotein, and LDL (Table 3). There were no interactions detected between IB and other covariates included in the model.

Additionally, the association between IB index and MCPT remained unchanged in magnitude after adjusting for hsCRP and leukocyte counts, both markers of systemic inflammation that have been associated with a risk of vascular events in our patient population.

Table 1. Participant Characteristics: Demographics and Risk Factors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No.</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>861</td>
<td>67.2±9.6</td>
</tr>
</tbody>
</table>
| Men, no. (%)                     | 861 | 333 (38.7%)
| Race–ethnicity, no. (%)          | 861 |           |
| Hispanic                         | 491 | (57.0%)   |
| Non-Hispanic black               | 182 | (21.1%)   |
| Non-Hispanic white               | 164 | (19.1%)   |
| High school diploma, n (%)       | 861 | 399 (46.3%)
| **Traditional risk factors**     |     |           |
| IB index, mean ± SD              | 861 | 1.0±0.3   |
| Diabetes mellitus, n (%)         | 859 | 175 (20.4%)
| Current smoker, n (%)            | 861 | 129 (15.0%)
| Coronary artery disease, n (%)   | 861 | 174 (20.2%)
| Systolic blood pressure, mm Hg, mean ± SD | 859 | 142.8±20.9 |
| Diastolic blood pressure, mm Hg, mean ± SD | 859 | 82.8±11.2 |
| High-density lipoprotein, mean ± SD | 859 | 46.3±13.9 |
| LDL, mean ± SD                   | 851 | 129.8±36.3|
| **Inflammatory risk factors**    |     |           |
| hsCRP, mg/L, mean ± SD           | 860 | 5.2±8.2   |
| Leukocyte count, mean ± SD       | 850 | 6.2±1.9   |
| **Infectious serology positivity, no. (%)** |     |           |
| Chlamydia pneumoniae IgA         | 861 | 556 (64.6%)
| Helicobacter pylori IgG          | 861 | 469 (54.5%)
| CMV IgG                          | 861 | 723 (84.0%)
| HSV1 IgG                         | 861 | 748 (86.9%)
| HSV2 IgG                         | 861 | 488 (56.7%)
| **Plaque measures**              |     |           |
| MCPT, mm, mean ± SD              | 861 | 0.89±1.04 |

Table 2. Association of Individual Infectious Serologies and MCPT

<table>
<thead>
<tr>
<th>Serology</th>
<th>Change in MCPT, mm (95% CI) for Positive Versus Negative Status on Individual Serologies</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Chlamydia pneumoniae IgA</td>
<td>0.14 (0.01–0.26)</td>
</tr>
<tr>
<td>Helicobacter pylori IgG</td>
<td>−0.06 (−0.18–0.07)</td>
</tr>
<tr>
<td>Cytomegalovirus IgG</td>
<td>0.15 (−0.01–0.31)</td>
</tr>
<tr>
<td>Herpes simplex virus 1 IgG</td>
<td>0.03 (−0.14–0.2)</td>
</tr>
<tr>
<td>Herpes simplex virus 2 IgG</td>
<td>−0.13 (−0.25–−0.01)</td>
</tr>
</tbody>
</table>

Demographics: age, sex, race–ethnicity, and high school education.
Risk factors: systolic blood pressure, high-density lipoprotein, LDL, blood glucose, moderate alcohol use, cigarette smoking status, waist circumference, moderate-to-heavy activity level, and coronary artery disease.
population. In secondary analyses, we evaluated the association between IB and irregular plaque, as previously defined. Five percent (n=48) of participants had irregular plaque. Infectious burden was associated with irregular plaque both in unadjusted (OR, 1.79 per SD IB index; 95% CI, 1.17 to 2.74) and fully adjusted models (OR per SD IB index, 1.76; 95% CI, 1.10 to 2.81).

Discussion
In this cross-sectional analysis, we found an association between a weighted measure of infectious burden, previously shown to be associated with stroke risk in our population, and carotid plaque thickness, a known risk factor cerebrovascular events. This analysis provides indirect evidence that if infectious burden is associated with stroke risk, then atherosclerosis, as measured by carotid plaque thickness, may be a mechanism for this increased risk. Therefore, this study enhances the validity of infectious burden as an independent risk predictor of stroke through its effects on atherosclerosis. Infectious burden may be a modifiable marker of stroke risk and measurement of carotid plaque thickness may offer a way to assess the effects of anti-infective strategies. In particular, our secondary findings suggest that infectious burden may be associated measures of plaque instability such as irregular plaque.

The infectious diseases that comprise the NOMAS IB index are common chronic infections that were reported to contribute to atherosclerosis and to have an association with stroke risk. C pneumoniae is an obligate intracellular prokaryote and represents the most studied infectious risk factor for vascular outcomes associated with atherosclerosis. Epidemiological, pathological, and animal studies support an association of C pneumoniae with atheroma development, but not all studies have found this association. Previous studies found C pneumoniae IgA to be more strongly associated with stroke risk than IgG.

H pylori, a well-characterized infectious agent responsible for chronic gastric inflammation, has been linked to stroke caused by small artery occlusion in case–control studies. Additionally, many viral pathogens in the Herpesviridae family, characterized by latent or persistent infection, were implicated in increased stroke risk. For example, CMV was associated with increased stroke risk in some studies but not all studies. Other members of this family such HSV1 and HSV2 have an unclear association with stroke risk, demonstrating inconsistent relationships with stroke risk.

The mechanism for the association of IB with atherosclerosis is uncertain. It is possible that infectious organisms directly invade the vascular wall. For example, a small (n=85) case–control study used polymerase chain reaction to evaluate the presence of persistent infection in atherosclerotic lesions of samples excised from patients undergoing coronary bypass grafting, endarterectomy, or surgery of the abdominal aorta; C pneumoniae DNA was found in 26% of cases and H pylori in 37% of cases, whereas no pathogen DNA was found in controls. Animal models also implicated infection in enhancing the risk for neointimal response to endothelial and smooth muscle injury without direct endothelial localization. Other potential mechanisms include enhanced macrophage lipid uptake; increased gene expression and cell surface localization of adhesion molecules and inflammatory cytokines; increased smooth cell proliferation; localized hypercoagulability; macrophage activation and plaque destabilization; and molecular mimicry, whereby pathogens contain proteins homologous to vascular self-epitopes.

The combined use of multiple serological tests for infectious diseases, to obtain a composite indicator of IB, was used previously in secondary prospective analyses of data from clinical trials such as the Heart Outcomes Prevention Evaluation (HOPE) trial, and in observational studies, including the Framingham Heart Study. In these studies, there was limited evidence of an association with stroke as a primary outcome. However, in prior analyses in the NOMAS cohort, the weighted IB was associated with stroke risk.

Previous studies also sought to elucidate the relationship between positive infectious disease serological results and plaque progression. In one study, serological testing for 8 infectious agents (HSV1/2, CMV, Epstein-Barr virus, Haemophilus influenzae, C pneumoniae, Mycobacterium pneumoniae, and H pylori) were used to compile an independent IB measure with a score ranging from 1 to 8 to predict plaque progression as assessed by multiple diagnostic measures, including angiography and Doppler. This simple score was associated with progression of atherosclerosis after adjusting for age, sex, and cardiovascular risk factors. In another study, 504 patients were evaluated for both carotid artery intima-media thickness and infectious disease serology at baseline and follow-up time points. Infectious burden predicted intima-media thickness progression of ≥0.1 mm/year with an OR=2.89 (95% CI, of 1.14 to 7.28) in the group with 6 to 8 positive serological results as compared with the reference group with 0 to 3 positive serological results. Limitations of these studies include the apparent post hoc nature of the thresholds for measuring infectious burden, the nonquantitative approach that assumes the effect of all infections is equal, and the absence of adjustment for socioeconomic status.

The major strength of our study is the use of a weighted measure to characterize the aggregate effect of infectious disease burden on atherogenesis. Our approach does not assume equal effects for each pathogen. Additional strengths include the use of a large, multiethnic, and urban population-based study with a predominant Hispanic population capturing a traditionally underrepresented group. In addition, we assessed the potential confounding by additional markers of inflammation, including hsCRP and leukocyte counts.

Weaknesses of our approach include performing a cross-sectional analysis and the use of serological measures of infection and carotid plaque thickness at a single time point. Therefore, we cannot assess the temporal relationship between increased MCPT and IB. Future studies in our cohort and others may evaluate the ability of weighted infectious burden measures as they relate to repeated measures of plaque progression. In addition, the NOMAS cohort lacks systematically collected clinical information regarding pre-existing inflammatory conditions, infection status, and immune-related interventions that may confound the relationship of IB and atherosclerosis. Nonetheless, most members of
our population are healthy without significant autoimmune or infectious diseases. Finally, the NOMAS subpopulation under study in this analysis is significantly younger and has a greater proportion of Hispanics than the overall NOMAS cohort, potentially limiting the generalizability of our findings.

Recent clinical trials found no evidence that antibiotic treatment of *C pneumoniae* and other infections reduce vascular risk.43,44 These studies, however, remain subject to weaknesses due to restrictions to cardiac patients, inclusion of participants without serological evidence of infection, and use of antibiotic treatment late after disease onset. Meanwhile, evidence that infection can be a stimulus for atherothrombosis continues to accumulate. These observations, along with the results of this current study, lend support to the notion that past or chronic exposure to common infections, perhaps by exacerbating inflammation, may be an important etiologic factor of atherosclerosis. Future studies are needed to confirm these findings and to define optimal measures of infectious burden as a vascular risk factor. Ultimately, clinical trials using enhanced measurement tools such as the IB index that better capture total infectious burden will be required to test whether preventive or anti-infective strategies can modify the risk of vascular disease associated with infection.

**Disclosures**

None.

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