Inflammatory Mechanisms of Stroke

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Abstract—Basic and clinical research provides evidence that inflammatory mechanisms play a central role in the pathogenesis and progression of atherosclerosis, plaque rupture, thrombosis, and stroke. Inflammatory biomarkers such as high-sensitivity C-reactive protein have been identified as predictors of first stroke and prognosis after stroke. The value of high-sensitivity C-reactive protein and other markers may depend on the characteristics of the study population; their utility may be less among populations with high vascular risk. A recent randomized, clinical trial suggests that the use of rosuvastatin therapy in otherwise healthy patients with high-sensitivity C-reactive protein $>2$ mg/dL can reduce the risk of a first stroke by $50\%$. The prognostic role of high-sensitivity C-reactive protein among patients after stroke, however, is less clear, and other biomarkers, including lipoprotein-associated phospholipase A$_2$, may provide complementary information about the risk of stroke recurrence. Infections, moreover, may contribute to inflammation and stroke risk. Although no single infectious organism is likely to be identified as the direct cause of atherosclerosis, summary measures of multiple chronic infectious exposures, or “infectious burden,” have been associated with the risk of stroke and atherosclerosis affecting the carotid arteries. Acute infections have also been found to serve as stroke triggers in epidemiologic studies. Recommendations to vaccinate patients with cardiovascular disease against influenza represent the first specific anti-infective strategy to be used in vascular prophylaxis. Further studies are needed to determine the role of treatment of inflammation and infection in stroke prevention. (Stroke. 2010;41[suppl 1]:S3-S8.)

Key Words: atherosclerosis inflammation infection infectious burden statins stroke cerebral thrombosis risk factors

Basic and clinical research provides evidence that inflammatory mechanisms play a central role in the pathogenesis of atherosclerosis, plaque rupture, thrombosis, and stroke. Inflammatory biomarkers such as high-sensitivity C-reactive protein (hsCRP) have been identified as predictors of stroke and prognosis after stroke. Infections, moreover, may contribute to inflammation and stroke risk. Although no single infectious organism is likely to be identified as the direct cause of atherosclerosis, summary measures of multiple chronic infectious exposures, or “infectious burden” (IB), have been associated with the risk of stroke and atherosclerosis affecting the carotid arteries. Acute infections have also been found to serve as stroke triggers. This article, based on a presentation given at the 2010 Princeton Conference, focuses on recent epidemiologic and clinical studies evaluating the hypotheses that inflammatory biomarkers and infections are associated with the risk of stroke, with an emphasis on the author’s own investigations.

Inflammatory Biomarkers in Primary Prevention

Epidemiologic Studies of hsCRP and Stroke Risk

Acute-phase proteins have been extensively studied as markers of coronary artery disease, and to a lesser extent, stroke. hsCRP, in particular, has many features that recommend it as a molecular marker of the risk of stroke associated with inflammation. A disadvantage to the assay is that it is very nonspecific, so acute increases in the levels of hsCRP may occur in the setting of acute infection or other illness. hsCRP has predicted incident cardiovascular events in several generally healthy populations. In multivariate models, hsCRP may improve the predictive ability of models over those containing lipid values and other risk factors alone ($P<0.001$).

The relation of hsCRP to incident stroke risk probably depends on the study design and population studied. In the Cardiovascular Health Study, among the elderly, hsCRP predicted incident ischemic stroke, although the effect was modest. In a study among elderly European subjects, hsCRP was associated not only with an increased risk of fatal stroke but also with a risk of death from all causes. A large individual-person meta-analysis of 54 prospective cohort studies ($N=160\,309$) was recently performed. The risk ratio of ischemic stroke per 1-SD increase in the log$_{10}$CRP value was 1.44 (95% CI, 1.32 to 1.57) when adjusted for age and sex but was attenuated to 1.27 (95% CI, 1.15 to 1.40) when further adjusted for other risk factors. Of note, however, nonvascular mortality, including cancer, was also increased significantly and by a greater magnitude in these analyses (adjusted risk ratio=1.54; 95% CI, 1.40 to 1.68). These
results suggest that elevated CRP may be a marker of general illness rather than a specific marker of vascular disease risk. Other studies have not confirmed a consistent, independent relation of hsCRP to stroke risk. In the Framingham Study, during >10 years of follow-up, men in the highest quartile of CRP had twice the risk of stroke as those in the lowest quartile, and women had 3 times the risk.11 For men, however, there was no increased risk after adjusting for confounders. Among healthy Japanese-American men in the Honolulu Heart Program, investigators found an almost 4-fold increase in stroke risk among those in the highest compared with the lowest quartile of hsCRP.12 The associations were strongest among those ≤55 years and in those without a history of hypertension or diabetes.

Our recent analysis of data from the Northern Manhattan Study (NOMAS) similarly did not confirm the ability of hsCRP to predict first stroke (Figure 1).13 NOMAS represents a stroke-free, multiethnic, community-based cohort study in participants age ≥40 years. hsCRP measurements were available for 2240 participants (mean±SD age, 68.9±10.1 years; 64.2% women; 18.8% white, 23.5% black, and 55.1% Hispanic). After a median follow-up of 7.9 years, compared with those with an hsCRP <1 mg/L, those with an hsCRP ≥3 mg/L were at increased risk of ischemic stroke in a model adjusted for demographics (hazard ratio [HR]=1.60; 95% CI, 1.06 to 2.41), but the effect was attenuated after adjusting for other risk factors (adjusted HR=1.20; 95% CI, 0.78 to 1.86; Figure 1A). Of note, an hsCRP >3 mg/L was also associated with risk of myocardial infarction (adjusted HR=1.70; 95% CI, 1.04 to 2.77; Figure 1B) and death (adjusted HR=1.55; 95% CI, 1.23 to 1.96) in the cohort. Other studies have similarly failed to confirm an association of hsCRP levels with stroke risk in the elderly.14

The association between hsCRP and ischemic stroke is thus probably attenuated in certain older populations and in those with more risk factors.15 The finding of an association between hsCRP and stroke risk may depend on both the degree to which other risk factors are included in the analyses as well as on age and the absolute risk of stroke in the population. Those studies in which predictive associations are found, for example, tend to include relatively young, healthy cohorts. The relation between hsCRP and measures of subclinical cerebrovascular disease, as assessed by brain magnetic resonance imaging, is also uncertain. In NOMAS, an association between hsCRP and white-matter hyperintensity volume was not found, though other inflammatory biomarkers were associated with white-matter disease.16

Role of Statins in Preventing First Stroke Among Patients With Evidence of Inflammation

The hypothesis that statin treatment among apparently healthy men and women with elevated hsCRP values may be associated with a reduction in risk of vascular events was recently tested in a large randomized, clinical trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin [JUPITER]). Patients were eligible for enrollment if they had no evidence of cardiovascular disease, diabetes, or hyperlipidemia but had an hsCRP ≥2.0 mg/L. They were randomized to rosvustatin or placebo. The study was stopped early because of evidence of benefit from rosvustatin therapy, with a significant reduction in the incidence of major cardiovascular events, including stroke.17 There was a 48% relative reduction in risk of stroke (HR=0.52; 95% CI, 0.34 to 0.79) among those taking rosvustatin.

There were several limitations to the study design, however, which have curbed enthusiasm regarding the use of statins as anti-inflammatory therapy to prevent vascular disease. First, the benefit was modest in absolute terms. Second, it was not tied to baseline levels of hsCRP, although there was some evidence that the greatest benefit was seen in those whose hsCRP was reduced to <2.0 mg/L. Third, it remains uncertain whether the mechanism through which statins appear to work is related to inflammation or to some other effect, with the effect on hsCRP a bystander or epiphenomenon. JUPITER provides only indirect evidence, therefore, that statins are of benefit among those with elevated hsCRP values, as the results may simply reflect a
general benefit of statin therapy among all patients, with the greatest magnitude seen among those at higher risk.

**Inflammatory Biomarkers in Secondary Stroke Prevention**

The utility of hsCRP measured after stroke as a predictor of long-term risk of recurrence is unsettled. In a secondary nested, case-control analysis from a multicenter secondary stroke prevention trial, those in the highest tertile of hsCRP had a modest increase in risk of recurrent ischemic stroke (odds ratio [OR]=1.39; 95% CI, 1.05 to 1.85). Results were not, however, fully adjusted for all risk factors. In a data from NOMAS, elevated levels of hsCRP (top quartile) were associated with a doubling of the risk of death for several years after first ischemic stroke but were not associated with an increase in risk of recurrent stroke. Lipoprotein-associated phospholipase A2 (LpPLA₂) was associated with risk of recurrent stroke and other vascular events. Because hsCRP was associated with stroke severity, however, whereas LpPLA₂ was not, it seems likely that hsCRP serves as a measure of general illness and stroke severity, whereas LpPLA₂ may be more specific to vascular inflammation. Both markers may therefore provide complementary information. The timing of measurement of hsCRP and LpPLA₂ may have important implications for the results of these studies. hsCRP levels increase acutely after stroke and remain elevated for 28 days or more, whereas levels of LpPLA₂ decrease. These changes imply that measurements made soon after stroke and myocardial infarction are not reflective of prestroke levels and may be less reliable for long-term risk stratification.

The decision to measure hsCRP or other markers in stroke patients may be based on Centers for Disease Control and Prevention/American Heart Association guidelines until further data are available. No data yet demonstrate the validity of such an approach, however, and no current guidelines recommend measurement of inflammatory markers in patients with stroke or even provide appropriate levels to determine absolute risk. Because of limitations in the available data, members of the CRP Pooling Project concluded that there was not yet enough data to routinely recommend hsCRP testing for prognostication in stroke patients. Ongoing studies are designed to test the prognostic utility of inflammatory measures after stroke (eg, the Levels of Inflammatory Markers in the Treatment of Stroke study, or LIMITS).

**Chronic Infection as a Risk Factor for Atherosclerosis and Stroke**

Among potential proinflammatory causes of atherosclerosis and stroke, infection remains 1 of the most controversial. Infection could contribute to risk in at least 2 ways. First, infections could serve as a chronic risk factor through effects on the vascular wall accumulating over many years, much like conventional risk factors such as hypertension. Alternatively, acute infections could contribute to short-term stroke risk (that is, a stroke trigger), a possibility considered in the next section.

With regard to infection as a chronic risk factor, individual organisms have been associated with atherosclerosis and stroke risk. Electron microscopy, immunocytochemistry, and polymerase chain reaction have demonstrated *Chlamydia pneumoniae* in diseased blood vessels, including cerebral and carotid arteries. Viable organisms have been cultured from carotid plaques. *C pneumoniae* is found more commonly in atherosclerotic tissue (52%) than in nonatherosclerotic tissue (5%). Data from seroepidemiologic studies provide conflicting evidence of an association between *C pneumoniae* and coronary artery disease. More recently, both case-control and prospective studies have found evidence for an association between serologic evidence of *C pneumoniae* and stroke risk, although other studies have not confirmed these findings.

Viruses have also been associated with atherosclerosis. Herpes simplex virus has been found in human early aortic atherosclerotic lesions. Cytomegalovirus (CMV) is a contributor to vasculopathy in heart transplant recipients, and CMV infection is also more common in coronary artery disease. Elevated CMV titers are associated with early carotid atherosclerotic changes (increased intima-media thickness) and later carotid stenosis. Prospective clinical studies, however, have not confirmed that CMV titers predict increased risk of clinical atherosclerotic events.

**Infectious Burden**

The variable results from these studies indicate that it is unlikely that a single ‘stroke germ’ will be found. Instead, if infection plays a role at all, it is likely to be in a cumulative fashion. The concept of IB has been used to explain the role that infections in aggregate may play in atherosclerosis. According to this hypothesis, infections contribute to the overall inflammatory milieu of atherosclerotic plaque, together with other risk factors, and individuals with the greatest exposure to different infections throughout life are most likely to develop atherosclerosis and stroke (Figure 2). It is likely also the case that those individuals with a more robust inflammatory response to these organisms, perhaps due to polymorphisms in infection-response genes, are more likely to show vascular changes related to infection.

Some but not all studies have begun to provide evidence of an association between different measures of IB and subclinical measures of atherosclerosis and vascular disease. For stroke specifically, in a case-control analysis, cough with phlegm during ≥3 months per year (chronic bronchitis) was associated with stroke or transient ischemic
attack independent of smoking history, other risk factors, and school education (OR = 2.63; 95% CI, 1.17 to 5.94). Frequent flu-like infections (>2/y) were also associated with stroke/transient ischemic attack.

Limitations of these studies include post hoc determinations of appropriate thresholds for IB and use of simple scoring systems that attribute equal weight to each infection. To address the possibility that different infections are associated with different magnitudes of risk, we created in NOMAS a quantitative index of IB based on the individual association of each of 5 common pathogens with stroke risk.53 Serologies against C pneumoniae, Helicobacter pylori, CMV, herpes simplex virus 1, and herpes simplex virus 2 were measured in 1625 participants followed up for a median of 8 years. Each individual infection was positively though not significantly associated with stroke risk after adjusting for other risk factors (the Table). Individual unadjusted parameter estimates were then added to generate a weighted IB index. The mean IB index was higher in non-Hispanic blacks and Hispanics compared with non-Hispanic whites (P < 0.0001 for both comparisons). This IB index was associated with an increased risk of all strokes (adjusted HR per SD = 1.39; 95% CI, 1.02 to 1.90) after adjusting for risk factors. Results were similar after adjusting for inflammatory biomarkers. The combined end point of all stroke, myocardial infarction, and deaths (adjusted HR per SD = 1.15; 95% CI, 1.03 to 1.29) was also associated with this IB index. This same IB index was also associated with carotid plaque thickness in NOMAS, with an increase of 0.09 mm (95% CI, 0.03 to 0.15 mm) per SD increase of IB index, after adjusting for risk factors.54 These analyses provide evidence that more sophisticated measures of IB may have a role in assessing the risk of vascular disease. They further support the notion that past exposure to common infections contributes to atherosclerosis, perhaps by exacerbating inflammation. Future studies are needed to validate these novel approaches to measuring IB, define optimal measures of IB as a vascular risk factor, and elucidate host factors, including genetics, that modify the infection-associated risk of vascular disease.

**Acute Infection as a Stroke Trigger**

Case-control studies have also found recent (that is, within 1 week) infection to be associated with acute stroke.55–59 Case-control studies, however, may be limited by interindividual confounding. To limit such confounding, we recently undertook a case-crossover analysis among participants in the Cardiovascular Health Study by comparing hospitalization for infection during case periods (90, 30, or 14 days before stroke) and control periods (equivalent time periods exactly 1 or 2 years before stroke).60 During a median follow-up of 12.2 years, 669 incident ischemic strokes were observed in participants without a baseline history of stroke. Hospitalization for infection was more likely during case than control time periods; for 90 days before stroke, OR = 3.4 (95% CI 1.8 to 6.5). Risks were higher when we examined shorter intervals: for 30 days, OR = 7.3 (95% CI 1.9 to 40.9) and for 14 days, OR = 8.0 (95% CI 1.7 to 77.3). Survival analyses confirmed these findings.

Similarly, in a prospective analysis among 50 000 patients in the UK General Practice Research Database, both upper respiratory infections and urinary tract infections were associated with an increased risk of stroke.61 The risk of stroke in the 3 days after infection was 3 times as high as during infection-free periods and gradually diminished during the following 3 months. There is also evidence from observational studies that vaccination against common infections, particularly influenza, can prevent stroke. Influenza vaccination during the previous season is associated with slightly >50% reduction in risk of stroke.62 This protective effect was not present for vaccinations against other organisms, however. The mechanism of this benefit from flu vaccination is uncertain but may reflect reduced immune activation of atherosclerotic plaque or coagulation.63,64 Alternatively, vaccination could lead to a reduction in illness-associated dehydration and respiratory impairment. Whether other viruses can be similarly implicated in short-term stroke risk remains uncertain. In children, however, varicella infection appears to represent a period of increased stroke risk.65 Further studies, including in stroke patients, are warranted.

**Infections as a Treatment Target**

Recent guidelines recommend vaccination against influenza in patients with cardiovascular disease as a means to prevent cardiovascular events.66,67 This represents the first antiinfective treatment to be championed as a vascular prevention strategy. There are data from a prospective, uncontrolled study that suggest, however, that treatment of periodontal infection can lead to a reduction in endothelial dysfunction and intima-media thickness.68 Although pilot clinical trials provided some evidence that antibiotics, particularly macrolide antibiotics directed against Chlamydiae, might reduce the risk of recurrent coronary events in patients with atherosclerosis,69 subsequent definitive randomized, controlled trials were unable to confirm these findings.70,71 Currently, therefore, there is no indication for antibiotics in patients with atherosclerotic disease. It should be noted, however, that these studies were largely confined to patients with coronary disease. Similar trials of antibiotics for patients with stroke have not been performed, and it is possible that the effects for stroke would differ.

The identification of a short-term state of elevated stroke risk after acute infection could have direct therapeutic impli-
cations, however, independent of the use of antibiotics. For example, increased doses of antiplatelet agents or statins may be warranted during times of fever or infection, when benefits may outweigh the risks of dose-related side effects. In addition, the period during and soon after hospitalization for infection could constitute a “treatable moment,” during which patients could be evaluated for cardiovascular risk and standard preventive strategies instituted.

Sources of Funding
M.S.V.E. receives funding from the National Institute of Neurological Disorders and Stroke (NINDS R01 NS 48134 and NINDS R01 NS50724) for research discussed in this review.

Disclosures
M.S.V.E. discloses that he receives research funding from diaDexus, Inc and BMS-Sanofi Partnership for research related to stroke biomarkers. He is also on an BMS-Sanofi Partnership Speakers Bureau.

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Stroke. 2010;41:S3-S8
doi: 10.1161/STROKEAHA.110.594945

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