'Infectious Burden'—New Insights into Stroke Prevention

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Abstract

Chronic and acute infections have been implicated as risk factors that increase the risk for stroke, myocardial infarction (MI), and other vascular events. The lack of consistency among studies attempting to link exposure to infectious pathogens and stroke risk provides empiric evidence that a single pathogen is likely not responsible for stroke. Reconsidering exposure as an 'infectious burden' (IB) aligns with our understanding that the totality of pro-inflammatory agents can contribute to atherosclerosis and vascular risk. We define IB as the cumulative life-course exposure to infectious agents that elicit strong inflammatory responses and review the varied approaches to operationalising this measure. There is promising research investigating the role of acute and chronic IB suggesting that there may be a causal role of pathogens in atherosclerotic progression and plaque destabilization to negatively affect vascular risk; however, the evidence is preliminary.

Keywords

Stroke, atherosclerosis, cerebral thrombosis, cerebral infarction, infection, infectious burden, *Chlamydia pneumoniae*, *Helicobacter pylori*, herpesviruses, risk factors, epidemiology

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Atherosclerosis is a complex inflammatory disease process of the arterial vessels.[1] Chronic and acute infections have been implicated as risk factors that increase the risk for stroke, myocardial infarction (MI), and other vascular events. However, no single pathogen is likely to be responsible for elevated vascular risk.[2] More likely, the combined effect of long-term exposure to multiple pathogens, or 'infectious burden' (IB), is more relevant to the study of vascular disease and stroke. The role of infections in vascular disease may also extend to acute precipitants of vascular events. This article reviews the postulated mechanisms of infection-induced vascular damage, host determinants of infection-mediated vascular effects, and clinical evidence linking individual and composite measures of IB and stroke risk, and also provides a review of recent research evaluating intervention strategies.

Pathogens as Risk Factors for Atherosclerosis

Chlamydia pneumoniae

Chlamydia pneumoniae is a common respiratory pathogen believed to be responsible for approximately 10% of non-hospital-acquired pneumonias.[3] *C. pneumoniae* is one of the best-studied organisms believed to be associated with cardiovascular disease. Studies that have evaluated human samples of atherosclerotic lesions using electron microscopy, molecular DNA methods, and immunostaining techniques have identified *C. pneumoniae* in coronary, carotid, aortic, and popliteal plaque.[4] The majority of these studies have identified pathogen DNA or antigen only; however, a few studies have isolated viable *C. pneumoniae* organisms.[5,6] *C. pneumoniae* has been identified in both early- and late-stage fibrous plaque and, in particular, in carotid and cerebral arterial vessels.[7–9]

Herpesvirus Family

Herpesviruses were initially identified as potential causes of atherosclerosis in animal models, and they have been the target of investigations into the association of infectious agents and atherosclerosis since then.[10] The most thoroughly investigated of the eight herpesviruses known to commonly infect humans are herpes simplex virus 1 (HSV1), HSV2, and cytomegalovirus (CMV). HSV1 infection has been associated with accelerated atheroma formation in apolipoprotein $E^{-/-}$ ($apoE^{-/-}$) mice, with a reduction in progression when treated with antiviral therapies.[11] HSV1 DNA has been found in some but not all samples of carotid atherosclerotic lesions.[12,13] The recurrent outbreaks associated with HSV infection and high population prevalence of exposure have made it an interesting target for epidemiologic study.[11] For CMV, evidence for involvement in vascular disease has been garnered from polymerase chain reaction (PCR) detection of CMV DNA in atherosclerotic lesions. For example, Hendrix et al. found CMV DNA in 90% of advanced atherosclerotic lesions compared with only 50% of control patients with no or minimal atherosclerosis.[14] The complete CMV genome has been identified in asymptomatic individuals, indicating persistent latent stage within the host cells.[14,15] Additional

evidence of CMV causing a vasculopathy is derived from the evidence of association of CMV and advanced progression of atherosclerosis and vasculopathy in heart-transplant recipients.[16]

Helicobacter pylori

Helicobacter pylori is a bacterium responsible for inflammation of the stomach lining, ulceration, and stomach cancer. *H. pylori* has been found at higher rates in carotid endarterectomy plaque samples compared with normal control samples derived from autopsy samples in some[17] but not all studies. Furthermore, specific strains of *H. pylori*, such as those expressing cytotoxin gene A (CagA-positive), are believed to specifically contribute to the atherosclerotic process.[18]

Mechanisms of Pathogen-mediated Effects on Atherosclerosis

Direct infection of cells lining arterial walls can lead to changes in transcription profiles and regulatory pathways that can result in endothelial dysfunction, smooth-muscle proliferation, or increased cytokine secretion.[19] HSV1 infection of human arterial smooth-muscle tissue causes decreased translation of cholesterol ester hydrolase messenger RNA (mRNA)[20] and infection has been documented to interfere with the ability of cells to transport and metabolize cholesterol.[21,22] CMV has been shown to induce vascular injury[23] and double the rate of smooth-muscle-cell proliferation[24] in rat models undergoing aortic allografts. In *in vitro* studies of human arterial smooth-muscle tissue, CMV has been shown to replicate for prolonged periods compared with shorter observed survival in human lung fibroblasts.[25]

Pathogens may also exert non-specific effects on vessel walls, far removed from any localized infections. For example, research indicates that exposure early in life may be sufficient to pre-condition coronary arteries to atherosclerosis. A Swedish study of 175 autopsied children reported increased intima thickening in children with viral (32%) and bacterial (21%) infections compared with groups with no evidence of infection (16%).[26] A case–control study from the same group found increased intima-media thickness (IMT) in cases (0.48±0.02mm) compared with controls (0.41±0.02mm), while no difference between groups was observed during the acute phase of infection.[27] One implication of this research is that atherosclerosis may be a delayed sequela of an acute infection. Other studies of non-specific effects of chronic infection have focused on elevated levels of serum cytokines that may affect atheromatous plaque progression by influencing macrophage activity. *In vitro* studies have shown *C. pneumoniae* to induce cytokine secretion in infected mononuclear cells that can directly affect atheroma progression[28] and induce macrophage conversion to foam cells, a critical aspect of atherosclerotic plaque progression.[29]

Pathogen infection can also increase the risk for pro-thrombotic events by altering platelet activation and dynamics with leukocytes. The P-selectin adhesion molecule is the most critical determinant of platelet activation. *In vitro* analysis of human platelets incubated with *C. pneumoniae* found an increased expression of P-selectin using flow cytometry and the initiation of widespread aggregation as measured by lumiaggregometry.[30] Platelet activation and platelet–leukocyte aggregates were shown to be significantly elevated in recent stroke cases compared with controls of healthy employees.[31]

Evidence for an association between cumulative measures of infectious serologies (CMV, HSV1 and 2, hepatitis A virus [HAV], *C. pneumoniae*, and *H. pylori*) and endothelial dysfunction was observed in a cross-sectional study of 218 patients undergoing coronary angiography.[32] In this study, endothelial reactivity was assessed by measuring constriction in response to intracoronary administration of acetylcholine, and those individuals with more than four positive serologies had more vasoconstriction than those with fewer than four positive serologies.[32] Another study consisting of stroke cases with self-reported infection with an exclusion provision for suspected post-infective stroke found infection-exposed cases to have significantly higher P-selectin mean fluorescence units ± standard deviation (SD) (1.78±0.28 versus 1.49±0.36) and proportion of platelet–leukocyte aggregates (7.28±2.76 versus 4.96±2.58%) levels than those cases without a history of infection.[32]

Immune responses that target 'self' epitopes may be elicited by infection and have been implicated in atherogenesis. Heat-shock protein 60 (HSP60) is a highly conserved protein that is overexpressed in several cell types of atherosclerotic lesions.[33,34] The 'molecular mimicry' hypothesis suggests that exposure to bacterial or virally encoded HSPs homologous to the human variants can elicit an immune response that will target both pathogen and self HSPs. Clinical studies have shown that increased antibody titers to homologous *C. pneumoniae* HSP65 are associated with elevated coronary calcium.[35]

An alternative pathway of infection-mediated atherosclerotic effects is through the toll-like receptor (TLR) proteins. Infection increases expression of TLR, activating nuclear factor kappa beta (NF-κβ) and mitogen-activated protein kinase (MAPK) pathways that in turn set off a pro-inflammatory cascade.[36] TLR proteins are overexpressed in atheromatous plaque and may continue to be overexpressed post-infection even in the absence of the initiating pathogen.[19]

Host and Environmental Determinants of Risk

An important consideration in evaluating IB and stroke is to account for potential confounding and interaction by host characteristics. Socioeconomic status (SES) may be associated with nutritional status, access to care, and many other factors that determine the ability to evoke a successful immune response against a pathogen and clear an infection. Health-seeking behavior may similarly confound the association. Socioeconomic disparities influence the seroprevalence of infection,[37] and exposure to low education and chronic pyschosocial stress may increase antibody titer responses. Developing evidence shows SES may influence immune responses through influence on cortisol levels.[38] However, studies have shown that pathogen burden may be an independent predictor of cortisol levels after adjusting for demographics and socioeconomic position (SEP) measures,[38] although residual influence of SES may continue to shape observed associations.

Underlying host genetic factors can also influence immune susceptibility and inflammatory responses to infection. Risk for CAD in those infected with *C. pneumoniae* has been shown to be elevated in patients with particular interleukin-1 (IL-1) polymorphisms, but IL-1

serum levels were not specified.[39] Polymorphisms in the *TLR4* gene have been demonstrated to increase risk for MI in the presence of infection as well.[40]

Chronic Infection—Single Pathogen Associations with Stroke

Several case–control and cross-sectional studies have found a positive association between *C. pneumoniae* antibody titers and stroke risk.[41,42] For example, in data from the Northern Manhattan Study (NOMAS), 246 stroke cases were matched by age, sex, and race/ethnicity to 474 population-based controls.[43] Elevated *C. pneumoniae* immunoglobulin A (IgA) titers were significantly associated with risk for ischemic stroke after adjusting for other stroke risk factors (adjusted odds ratio [OR] 1.5, 95% confidence interval [CI] 1–2.2). IgG titers were not significantly associated with stroke risk (adjusted OR 1.2, 95% CI 0.8–1.8).[43] There was also a statistically significant interaction between sex and *C. pneumoniae* antibody status, with an increased risk associated with IgA titers among women (p=0.04). The fact that IgA titers were more consistently associated with risk for stroke than IgG titers in this and other studies may reflect the possibility that IgA antibodies, which remain for only three to five days in the circulation, are a marker of persistent, chronic infection, while IgG antibodies, which remain elevated for several years after infection, are a marker of remote, completed infection.[44] Evidence from studies of IgA in other chlamydial diseases support this hypothesis, although there is no consensus on this matter.[45] Prospective studies have also not confirmed an association of *C. pneumoniae* antibody titers and stroke risk.

Clinical studies of herpesviruses have found evidence that herpesvirus infection may also be associated with risk for stroke. For example, in one case–control study 59 subjects with acute ischemic stroke at early age onset (mean age 50.4 years) and 52 hospital controls were assessed.[46] Only positivity to CMV IgG (adjusted OR 2.94, 95% CI 1.27–6.81) and HSV1 IgA (adjusted OR 4.65, 95% CI 1.34–16.16) demonstrated associations with ischemic stroke.

Similarly, some evidence supports an association between *H. pylori* and ischemic stroke. In a case–control study in which 80 ischemic stroke patients were age- and sex-matched to 320 blood donors, *H. pylori* serologies were significantly associated with acute ischemic stroke. *H. pylori* has also been implicated in ischemic heart disease.[47] Interestingly, a large-scale cohort study of 10,938 normotensive patients in Sweden found stomach ulcers to be significantly associated with a first stroke (adjusted relative risk [RR] 2.21, 95% CI 1.03–4.71), after adjusting for risk factors. Although *H. pylori* was not investigated in this study, it is considered to be an important cause of stomach ulcers.

Chronic Infection—Associations of Multiple Pathogens with Stroke: 'Infectious Burden' Construct

The lack of consistency among studies attempting to link exposure to infectious pathogens and stroke risk provides empiric evidence that a single pathogen is likely not responsible for stroke. Pathogens elicit a complex and multifactorial response from the host immune system that occurs in the presence of other vascular risk factors. In fact, the role of chronic infection in atherosclerosis may be complementary in terms of existing risk factors that elicit atherosclerotic effects. Restructuring the measurement construct of 'infectious exposure' as 'pathogen burden' or IB aligns with our understanding that the totality of pro-inflammatory agents can contribute to atherosclerosis. For example, the role of low-density lipoprotein (LDL) in ischemic stroke would be difficult to elucidate by measuring only recent exposure to cheeseburgers. Instead, the cumulative life-course exposure to dietary fat intake is of consequence to atheromatous plaque progression. Analogously, those individuals with the greatest IB, defined as cumulative life-course exposure to infectious agents eliciting strong inflammatory responses, are most likely to have advanced atherosclerosis and be at the greatest risk for vascular events.

Several studies have incorporated IB measures in their study design and have found significant associations with vascular disease. Zhu et al. considered total pathogen burden as an independent predictor of vascular disease.[2] This study primarily selected from a population of individuals with complaints of chest pain or suspected MI, and the outcome was defined as coronary artery disease (CAD) with angiographic-confirmed atherosclerosis. The infectious pathogen measure (IgG antibodies to *C. pneumoniae*, CMV, HAV, HSV1, and HSV2) was categorically partitioned as nought to two, three to four, or five positive serologies. Within this study, 68% of individuals had CAD and 75% of subjects had exposure to at least three pathogens. Positivity for five infectious agents compared with those with positivity for fewer than two was associated with an increased risk for disease (adjusted OR 6.08; p<0.0001). IB was also associated with C-reactive protein (CRP), a serum biomarker of inflammation. This study implicates IB as a risk factor for CAD, and posits that the association may be working through inflammatory mechanisms. CRP levels have previously been shown to be associated with vascular risk.[48]

Another study of 504 randomly selected participants of the AtheroGene cohort measured IB (IgG and IgA antibodies for HSV1, HSV2, CMV, Epstein-Barr virus (EBV), *Haemophilus influenzae*, *C. pneumoniae*, *Mycoplasma pneumoniae*, and *H. pylori*) and carotid atherosclerosis progression.[49] IB was partitioned into categories of nought to three, four to five, or six to eight positive serologies, and progression was confirmed by Doppler ultra-sonography over 2.5 years of follow-up. Those with six to eight serologies were significantly more likely to have progression of carotid IMT than those with nought to three positive serologies (adjusted OR 3.8, 95% CI 1.6–8.8). These results were adjusted for risk factors and a dose-response was observed. A second cohort study by the same group among 572 subjects found advanced atherosclerosis, as measured by angiography, Doppler, and ankle–arm indices, to be significantly higher in those individuals with six to eight serologies.[50] Only 6% of this population was observed to have six to nine positive serologies.

Vascular outcome end-points have also been found to be associated with IB measures. A second study by Zhu et al. in a cohort of 890 subjects with angiographically confirmed CAD who were followed for three years found an association between MI and IB.[51] At least six positive infectious serologies compared with nought to one positive serologies was significantly associated with MI (adjusted RR 3.18, 95%

CI 1.32–7.66) after adjusting for traditional risk factors. Although the reference group sample size was small, the test for trend was significant (p=0.0006) and showed increasing risk for each additional positive serology. Stroke was not evaluated in this study. Among 1,018 patients,[52] Rupprecht et al. assessed IgG or IgA antibodies to CMV, EBV, HSV1, HSV2, *H. influenzae*, *C. pneumoniae*, *M. pneumoniae*, and *H. pylori*, and separated participants into nought to three, four to five, or six to eight positive serologies. Those with six to eight positive serologies comprised 22% of the study population and were at increased risk for death from cardiovascular disease (CVD) (adjusted RR 5.1, 95% CI 1.4–18.3). The association was mainly driven by herpesviridae (p<0.0001) in this population. Smieja et al. analyzed data from a clinical trial among 3,168 patients with 4.5 years of follow-up. They assessed IgG and IgA antibodies for *H. pylori*, *C. pneumoniae*, CMV, and HAV and evaluated a combination of CV outcomes (MI, stroke, or CV death).[53] Those subjects with an IB score of four (17% of the study group) had a modest increased risk for vascular events (adjusted RR 1.41, 95% CI 1.02–1.96) compared with those with a score of nought to one. The authors note that antibody titers may be consistent with several underlying phenomena, including re-infection, persistence, or non-specific immune stimulation. Stroke alone was not found to be associated with this IB score.

A few studies of the association of IB and stroke alone have been performed. In a case–control study of 91 acute strokes or transient ischemic attacks (TIAs) and 87 hospital controls, the authors measured IB with three pathogens (*C. pneumoniae*, *M. pneumoniae*, and *Legionella pneumophila*). Those with three positive serologies were at higher risk for stroke compared with those with one positive serology. The analysis was adjusted for traditional risk factors. Grau et al., in a case–control study of 370 ischemic or hemorrhagic strokes and TIA matched to 370 population controls, observed that more than two non-specific flu-like infections per year was associated with increased stroke risk (adjusted OR 3.68, 95% CI 1.52–8.27) compared with those with fewer than two events per year.[54]

However, not all studies have found an association between IB measures and cardiovascular risk.[55] In an analysis of 1,056 subjects selected from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, there was no association between IB and IMT or coronary artery calcification.[56]

However, there are several limitations to the existing studies. The assessment of IB across these studies fails to distinguish the effects of different pathogens and assumes an equal contribution to disease. Also, the threshold for determination of a positive serology differs among studies and in some cases was established *post hoc*. Furthermore, the prevalence of risk factors and infectious pathogens varies across study samples.

To address some of these concerns of heterogeneity of the IB measurement construct and other potential limitations, NOMAS constructed a novel measure of IB that allows for variable impacts of different pathogens by applying weights for the magnitude of association with stroke, specific to the population under study.[57] The NOMAS cohort is an urban, population-based prospective study in a tri-ethnic population. The IB index comprised weights derived from univariate Cox model parameter estimates of associations between individual infectious serologies (antibodies to *C. pneumoniae*, *H. pylori*, CMV, HSV1, and HSV2) and stroke, using baseline serum samples from 1,625 participants. The mean IB index was significantly (p<0.00001) higher among non-Hispanic blacks (1.05±0.31) and Hispanics (1.07±0.27) compared with Caucasians (0.75±0.41). The IB index was associated with all strokes (adjusted hazard ratio per SD of the IB index 1.39, 95% CI 1.02–1.90). Essentially, the results remained unchanged after excluding those with a history of CAD and after further adjusting for CRP and leukocyte count (adjusted hazard ratio per SD 1.50, 95% CI 1.05–2.13). In a second cross-sectional analysis within NOMAS, this same derived IB index was found to be associated with carotid plaque thickness (0.09mm increase in carotid plaque per SD, 95% CI 0.03–0.15mm) after adjusting for risk factors.[58] These findings support the methodologic approach of deriving IB measures based on the observed weights of individual pathogen associations for a given population. Although promising, these results need to be validated in other study populations.

Acute Infection as a Precipitant to Ischemic Stroke

Acute infections may play a specific role in triggering acute vascular events. Thrombotic complications arise when the site of an atherosclerotic plaque becomes destabilized and exposes the underlying pro-coagulant surface.[59] Although macrophage activity has been shown to play a role in fibrous plaque destabilization,[60] the precipitants of ischemic events remain largely unknown. Empiric evidence across multiple studies and populations suggests acute infections may play a role. The magnitude of association between preceding infections and vascular events appears to increase with increasing proximity in timing. In a case–control study, an increasing association of stroke and respiratory infection was found as the period between infection and stroke decreased: adjusted OR 1.09 at 29–91 days before stroke, OR 1.76 at eight to 28 days before stroke, and OR 1.92 at one to seven days before stroke.[61] Ischemic coronary and cerebrovascular events have been associated with recent respiratory infection and influenza-like illness,[62–64] urinary tract infections,[65] community-acquired pneumonia,[66] and gastroenteritis.[67] Many studies have focused on the role of influenza in precipitating vascular events.[68,69]

Prevention Strategies and Interventions

The accumulated evidence implicating IB in atherogenesis and stroke supports the *a priori* hypothesis that intervening with antimicrobials or vaccination strategies may reduce the risk for vascular events. Observational studies provided early evidence that vaccination strategies may be effective in preventing vascular events in high-risk populations. Influenza vaccination has been associated with a decreased risk for stroke, for example, even after adjusting for risk factors, education, and health-seeking behavior. Vaccine effects were significant in individuals with characteristics consistent with high-risk groups such as age >65 years and previous history of vascular disease. In a large observational study among 286,383 Oxford Health Plan members, influenza vaccination status, as measured by insurance databases, was associated with a 16% reduction (p<0.018) in incident cerebrovascular disease hospitalization during the

1998–1999 time-frame.[70] Based on these and other data, recommendations from professional organizations advise influenza vaccination for secondary prevention of CVD.[71]

On the other hand numerous randomized trials of antibiotic therapy for vascular risk reduction primarily focused on treatment of *C. pneumoniae* have provided mixed results.[72–84] A meta-analysis of 12 of these trials found no significant association between antibiotic treatment and risk for vascular disease or death (OR 0.84, 95% CI 0.67–1.05), although the direction of the effect was protective.[85] However, these studies were not primarily focused on patients with cerebrovascular disease and it remains possible that effects in stroke prevention may differ from those in heart disease. Moreover, despite overall null findings, antibiotic regimens may be effective in subpopulations of patients. Furthermore, most antibiotics in these trials were not expected to be active against latent or hidden forms of infection. Some studies suggest that some antibiotic treatments may be ineffective in removing viable pathogens from circulating monocytes, for example.[86]

Conclusion

Promising research investigating the role of acute and chronic infectious exposure suggests that there may be a causal role of pathogens in atherosclerotic progression and plaque destabilization to negatively affect vascular risk; however, the evidence is preliminary. Further studies are required to identify molecular targets for pharmaceutic intervention along pathways by which IB may elicit atherosclerotic effects, to evaluate the role of IB on small-vessel disease and stroke, to ensure that socioeconomic measures are adequately considered, to assess probable interactions with host genetic and risk-factor profiles, and to further define a standard measure for composite IB. Infectious agents provide tempting targets for interventions, but the role of pathogens in atherogenesis (if it exists) is likely complex and occurs over the life-course. Short-term antibiotic regimes are indicated to clinically treat acute phases of infection, but there is insufficient evidence to support any antibiotic benefits of long-term treatment or treatment earlier in the life-course. Vaccination strategies and development for likely viral contributors of vascular burden may be another viable option. ■

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