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Infectious burden: a new risk factor and treatment target for atherosclerosis

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Introduction

Atherosclerosis is a chronic inflammatory process, and several common bacterial and viral infections have been hypothesized to contribute to the inflammation of the vascular wall that leads to atherosclerosis. More recently, investigators have found preliminary evidence that the aggregate burden of these chronic infections, rather than any single organism, may contribute to atherosclerosis and risk of clinical vascular events, including ischemic stroke. This aggregate burden of infections, which has been variably labeled “infectious burden” or “pathogen burden,” may be associated with stroke through mechanisms independent of atherosclerosis, as well, including platelet aggregation and endothelial dysfunction. Host factors, moreover, may interact with infectious burden to modify the risk of disease associated with these infections. Currently there is no commonly accepted group of organisms or method of assessing infectious burden, and not all studies confirm an association of infection and stroke risk. Nonetheless, if infectious burden does play a role in atherosclerosis or stroke, it is plausible that preventive anti-infective treatment, such as vaccination, or antibiotics, would reduce the risk of incident or recurrent stroke. While influenza vaccination has been recommended to prevent recurrence among those with coronary disease, similar recommendations for stroke patients have not yet been made. Large scale randomized clinical trials of macrolide antibiotics for coronary patients, moreover, have been negative. Further studies are needed, however, to determine whether an association between infectious burden and stroke exists, and whether infectious burden may be a target for intervention.

Chronic infection as a risk factor for atherosclerosis and stroke

Several individual organisms have been associated with atherosclerosis and stroke. *Chlamydia pneumoniae* (*C. pneumoniae*), an obligate intracellular organism,[1] is probably the best studied of these. Other chlamydial species cause chronic inflammatory diseases with similarities to atherosclerosis. For example, *C. trachomatis* causes trachoma, a chronic infection of either the conjunctiva, with long-term complications of ocular scarring and blindness, or the fallopian tubes, with consequent scarring, infertility, and increased risk of ectopic pregnancy.[2] Estimates of serologic evidence of past infection with *C. pneumoniae* are at least 50% in series throughout the world.[3]

Studies utilizing electron microscopy, immunocytochemistry, and polymerase chain reaction (PCR) have demonstrated that *C. pneumoniae* can be found in diseased blood vessels, including cerebral and carotid arteries,[4,5] suggesting their role as mediators of the endothelial damage leading to atherogenesis.[6,7,8] Viable organisms have been cultured from coronary and carotid

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artery plaques.[9,10] Overall, *C. pneumoniae* is found much more commonly in atherosclerotic tissue than in non-atherosclerotic tissue (52% of atheromatous tissue specimens versus only 5% of non-atheromatous specimens).[11] In vitro studies12 have also shown that *C. pneumoniae* can infect and reproduce in human smooth muscle cells, endothelial cells, and macrophages, the three cell types involved in the pathogenesis of atherosclerosis.

Data from seroepidemiologic studies throughout the world provide conflicting evidence of an association between *C. pneumoniae* and coronary heart disease.[13] The studies differed in methodology, assays used, and populations studied. More recently, several studies have examined the role of *C. pneumoniae* in stroke. Both case-control[14,15,16,17] and prospective studies[18] have found evidence for an association between serological evidence of *C. pneumoniae* infection and stroke risk. Other studies have not confirmed these findings, however.[19,20,21]

Viruses have also been associated with atherosclerosis. The avian virus, Marek's disease herpesvirus, causes atherosclerosis in both normocholesterolemic and hypercholesterolemic chickens, while in the absence of this pathogen even hypercholesterolemic chickens do not develop atherosclerosis.22 Herpes simplex virus (HSV) has been found in early aortic atherosclerotic lesions from human beings.23 Cytomegalovirus (CMV) is a contributor to post-transplant vasculopathy in heart transplant recipients.24 Serologic evidence of CMV infection is also more common in patients with coronary artery disease (CAD) than normal controls. 25 There is also evidence that elevated CMV titers are associated with early carotid atherosclerotic changes, indicated by a thickened intima-media thickness, and later atherosclerotic changes, indicated by carotid stenosis.26 Prospective studies have shown that those with the highest CMV titers have twice the risk of cardiac disease as those with the lowest. 27 Elevated titers against CMV, hepatitis A virus, and HSV2, have been associated with an increased risk of future MI.28 Restenosis after coronary angioplasty also occurs more frequently in patients positive for CMV.[29] CMV has also been detected by PCR in atherosclerotic plaques of those with coronary disease more frequently than in those without atherosclerosis.[30] Other prospective studies have not confirmed that elevated CMV titers predict increased risk of clinical atherosclerotic events.[31]

Infectious burden

The variable results from these studies would seem to suggest that it is unlikely that a single “atherosclerosis bug” or “stroke germ” will be discovered. Atherosclerosis is a complex disease, with many well-recognized precipitants, including oxidized low-density lipoprotein, cigarette smoking, diabetes, hypertension, and others. No single organism is therefore likely to account for atherosclerosis. Instead, if infection plays a role at all, it is probably in a more cumulative and continuous fashion. The concept of “infectious burden” or “pathogen burden” has been used to explain the role that infections in aggregate may play in the development of atherosclerosis or clinical cardiovascular events. According to this model, infections contribute to the overall inflammatory milieu of atherosclerotic plaque, together with other risk factors. Those individuals with the greatest exposure to many different infections throughout life are most likely to develop atherosclerosis and ultimately stroke. 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 also more likely to show vascular changes related to infection.

Assessment of infectious burden

There is at present no standard definition of infectious burden. There is no consensus as to which organisms or clinical infections should be considered, or whether optimal measures should be based on serological evidence of past exposure, or on a history of past exposures

derived from questionnaires, or from independent sources of information, such as chart review or administrative databases. Most serological testing is designed for diagnostic testing in clinical settings, and not for the assessment of burden of remote infections. The situation is complicated by the fact that many of the common infections that are thought to play a role in atherosclerosis, such as *C. pneumoniae*, CMV, and HSV, are often asymptomatic or present with minor, brief, nonspecific illnesses. It is thus difficult to validate these measures of past infection, since there is no clear gold standard.

Alternatives to the use of serological results include the use of questionnaires designed to capture the frequency of common minor illnesses, such as bronchitis and urinary infections. Similarly, administrative records of frequent visits to primary physicians have been used as a measure of infectious burden, but these records may indicate a patient personality or illness behavior that is itself more likely to be associated with risk of vascular disease than an objective measure of infection.

Epidemiologic data

Despite these limitations, preliminary studies have begun to provide evidence of an association between different measures of infectious burden and subclinical measures of atherosclerosis and vascular disease outcomes. For example, the AtheroGene Investigators measured serologies against a number of organisms (*C. pneumoniae*, *Helicobacter pylori*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, CMV, HSV 1 and 2, and Epstein Barr Virus) in 572 hospitalized patients.[32] Infectious burden was quantified by number of positive serologies (primarily IgG) and categorized as 0–3, 4–5, and 6–8. Presence and degree of atherosclerosis was assessed by coronary angiography, carotid ultrasound and ankle-arm indices. After adjusting for other risk factors, there was a dose-response relationship found for an association with advanced atherosclerosis: compared with 0–3 positive serologies, the odds ratio (OR) of advanced atherosclerosis was 1.8 (95% confidence interval (CI) 1.2–2.6) for 4–5 positive serologies and 2.5 (95% CI 1.2–5.1) for 6–8 serologies. In a further study by this group, the number of positive titers to these pathogens correlated with carotid plaque progression over time.[33]

Prevalent CAD was also associated with the number of positive serologies against common infections (*C. pneumoniae*, CMV, hepatitis A virus, and HSV 1 and 2) in a study among 233 participants, even after adjustment for traditional CAD risk factors.[34] The prevalence of CAD was 48% in individuals with antibodies to ≤ 2 pathogens, 69% in those with antibodies to 3 or 4 pathogens, and 85% in those with antibodies to 5 pathogens. Pathogen burden was also directly correlated with C-reactive protein (CRP) levels. Because CRP levels may reflect vascular inflammation and have been associated with coronary disease risk,[35] these data provide evidence that if pathogen burden is associated with coronary disease, it may be through inflammation.

In a follow-up study by the same group, antibodies against these same 5 organisms, as well as *H. pylori*, were measured in 890 patients with significant coronary disease assessed angiographically who were followed for 3 years.[36] The number of positive antibodies was associated with risk of death and MI with a dose-response trend even after multivariate adjustment (p=0.0005). Those participants with 5 or 6 positive serological results were more than 6 times as likely to have an MI or die than those with 0–1 seropositive results. Similar results were found for MI alone. Stroke was not assessed.

The relationship between infectious burden and clinical outcome events has also been assessed in secondary analyses of clinical trials. For example, antibodies against *C. pneumoniae*, *H. pylori*, CMV and hepatitis A virus were measured in stored enrolment sera from 3168 Canadian subjects in the Heart Outcomes Prevention Evaluation (HOPE) trial.[37] Over 4.5 years of follow-

up, the total pathogen score was associated with occurrence of cardiovascular events (adjusted hazard ratio (HR) for 4 pathogens vs. 0–1=1.41, 95% CI 1.02–1.96). Pathogen burden was not associated with stroke as an independent outcome, however.

A small case-control study did show a modest association between pathogen burden based on the total number of positive serological results for *Legionella pneumophilla*, *Mycoplasma pneumoniae*, and *C. pneumoniae* and risk of stroke and TIA.[38] Among 91 stroke or TIA patients > 65 years of age and 86 hospitalized controls, the odds ratio for IgG seropositivity after adjusting for other risk factors was increased in those with 1 (OR 3.89, 95% CI 1.13–13.33), 2 (OR 2.00, 95% CI 0.64–6.21), or 3 (OR 6.67, 95% CI 1.22–37.04) of these infections.

In a case-control analysis among 370 consecutive patients with ischemic or hemorrhagic stroke or TIA and 370 age- and sex-matched randomly selected population-based controls, investigators assessed symptoms of chronic bronchitis and frequency of flu-like illnesses.[39] They found that cough with phlegm during ≥3 months per year (grade 2 chronic bronchitis) was associated with stroke or TIA independent from smoking history, other risk factors, and school education (OR 2.63, 95% CI 1.17 – 5.94). Frequent flu-like infections (>2/year) were also associated with stroke and TIA (OR 3.54; 95% CI 1.52–8.27).

In a preliminary nested case control analysis from the Kaiser Pediatric Stroke Study, performed using the Kaiser Permanente database, 97 children with ischemic stroke were age-matched 1:3 to stroke-free children. Children with stroke had a higher infectious burden as assessed by the number of infection-related medical encounters in preceding time windows. Children with stroke had a median of 3 visits (interquartile range 1–6) for infections, compared to stroke-free children who had a median of 0 visits (interquartile range 0–2; $p<0.0001$) (H. Fullerton, unpublished data).

Other studies of pathogen burden have been negative, however. In a study from Taiwan among 568 patients with coronary disease, the number of positive serologies against 8 common organisms was not associated with the degree of atherosclerosis on angiography, C-reactive protein levels, or the risk of major adverse coronary events.[40] Similarly, among 1056 individuals selected from the 5030 Multi-Ethnic Study of Atherosclerosis (MESA) cohort participants, the total number of positive serologies of antibodies against *C. pneumoniae*, *H. pylori*, CMV, HSV, and hepatitis A virus was not associated with subclinical measures of atherosclerosis such as intimal-medial thickness and coronary calcification.[41]

Limitations of these studies include a post-hoc determination of appropriate thresholds for elevated infectious burden, and the use of a simple scoring system that attributed equal weight to each individual infection. It is possible, for example, that different infections will carry different weights in predicting risk of events.

To address the possibility that different infections may be associated with different magnitudes of association of risk of vascular disease, investigators in the Northern Manhattan Study (NOMAS) created a quantitative index of infectious burden that was based on the individual association of each of 5 common pathogens with stroke risk in a prospective cohort study among a multi-ethnic, urban population.[42] Serologies against *C. pneumoniae*, *H. pylori*, CMV, HSV 1, and HSV 2 were measured using baseline blood samples from 1625 randomly selected stroke-free participants followed for a median of 8 years for incident stroke and other vascular events. Cox proportional hazards models were used to estimate associations of each positive serology with stroke. Individual parameter estimates were then combined into a weighted index of infectious burden and used to calculate hazard ratios for the 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. To determine whether composite seropositivity was associated with risk of stroke, individual unadjusted

parameter estimates were added to generate a weighted infectious burden index (mean 1.00 ± SD 0.33; median 1.08). The mean IB index was higher in non-Hispanic blacks (1.05 ± 0.31) and Hispanics (1.07 ± 0.27) compared to non-Hispanic whites (0.75 ± 0.41; $p<0.0001$ for both comparisons). It was slightly higher in women (1.02 ± 0.31) than men (0.97 ± 0.36; $p=0.0016$). There was no difference by age.

The infectious burden index was associated with an increased risk of all strokes (adjusted HR per standard deviation 1.39, 95% CI 1.02 – 1.90) after adjusting for demographics and risk factors. Results were similar after excluding those with coronary disease (adjusted HR 1.50, 95% CI 1.05 – 2.13) and adjusting for inflammatory biomarkers such as high sensitivity CRP and leukocyte count. Non-vascular deaths (adjusted HR per SD 1.23, 95% CI 1.04–1.45) and the combined endpoint of all stroke, MI and deaths (adjusted HR per SD 1.15, 95% CI 1.03–1.29) were also associated with this infectious burden index. These analyses provide preliminary evidence that more sophisticated measures of infectious burden, i.e., measures that do not utilize a simple scoring system based on each infection contributing one point, may have a role in assessing the risk of vascular disease associated with these infections. These findings need to be validated in other populations, however.

This same measure of infectious burden was also associated with carotid plaque thickness in the NOMAS cohort. Maximal carotid plaque thickness assessed by carotid duplex Doppler ultrasonography increased 0.09 mm (95% CI 0.03–0.15 mm) per standard deviation increase of the infectious burden index, after adjusting of other risk factors. These results support the notion that past or chronic exposure to common infections contributes to atherosclerosis, perhaps by exacerbating inflammation. Future studies are needed to validate these novel approaches to measuring infectious burden and to define optimal measures of infectious burden as a vascular risk factor.

Host factors

Another intriguing possibility is an interaction of infectious burden with host factors, and particularly genetic susceptibility to inflammatory or atherosclerotic complications of infections. Background genetics may influence the susceptibility to infection, the ability to eradicate an infection, or the response to an infection. Particular HLA types are recognized as increasing the risk for and from infections. HLA type DR 13a or 17, for example, may be associated with a propensity to develop *C. pneumoniae*-mediated atherosclerosis.[43] An autoimmune mechanism initiated by an intracellular pathogen like *C. pneumoniae* could thus contribute to atherosclerosis in susceptible individuals. Polymorphisms of the mannose-binding lectin gene have similarly been identified as increasing the risk of infection.[44] These alleles were also found to correlate with both the presence and the size of carotid atherosclerotic plaque in a study of 164 subjects from a vascular disease prevention clinic.[45] There is also evidence that variants in the toll-like receptor 4, an important component of the innate immune response that controls responses to a diverse array of infections, may be associated with atherosclerosis. The Asp299Gly TLR4 polymorphism, which decreases the inflammatory response to gram-negative pathogens, was associated with decreased carotid atherosclerosis and intima-media thickness, for example, in a study from Italy among 810 individuals.[46] Subsequent clinical outcome studies have not confirmed an association with stroke or MI, however.[47] Evidence of chlamydial infection carried a greater risk of symptomatic coronary disease in carriers of IL-1 gene family mutations out of proportion to the risk conferred by either the mutation or the infection alone.[48] Since specific infectious agents may influence atherosclerosis, these and other susceptibility factors may eventually identify high-risk patients who could be screened for potentially pathogenic organisms.

Mechanisms

There are several potential biological mechanisms by which infections may increase risk of ischemic stroke, including contributions to atherosclerosis, platelet aggregability, endothelial dysfunction, and others. Some of these mechanisms may involve direct infection of the cells of the arterial wall, leading to dysfunction, smooth muscle cell proliferation, or increase in levels of circulating cytokines.[49] Alternatively, infections may affect cells remote from the vascular wall, with secondary consequences to the artery. Among 175 unselected Swedish children who died of various causes, pre-mortem infections of any type were associated with coronary intimal thickening, providing indirect evidence of a non-specific effect of infection on blood vessels.[50] In a case-control study by this same group, there was no acute increase in carotid-artery IMT in children hospitalized for acute infection of any type compared with hospitalized control children free of acute infection, but case children had significantly increased IMT upon reexamination 3 months later.[51] These results provide evidence that there may be a delayed pro-atherogenic effect that was not specific to any particular infection, and support the hypothesis that vascular injury may be a general effect of infection and inflammation in children.

There is also evidence that molecular mimicry may play a role in atherogenesis among patients exposed to infections. For example, heat shock proteins (HSPs) are highly conserved proteins that are found in bacteria and humans. They mediate responses to stress and are presented on cell surfaces. Infections may trigger an immune response to bacterial HSPs, then, that lead to cross-reactivity with human HSPs in vascular walls, initiating an autoimmune process. There is evidence that immunization of rabbits with mycobacterial HSPs can induce atherosclerosis.[52] In humans, levels of antibodies directed against HSPs are associated with carotid artery thickening and coronary calcification.[53,54]

Infections can also increase platelet reactivity and platelet-leukocyte interactions, leading to an increased risk of platelet aggregation, potentially precipitating stroke. These changes are not limited to severe infections or sepsis. Platelet activation assessed by P-selectin expression, and platelet-leukocyte aggregates, were both increased in stroke patients compared to controls.[55] Platelet activation and platelet-leukocyte aggregates were also increased in 21 stroke patients with a history of infection within 1 week prior to stroke compared to 37 stroke patients without recent infection. Severity of stroke was also greater among those with recent infection. Other organisms implicated in causing atherosclerosis and ischemic events have also been associated with platelet aggregation, including periodontal infections.[56] Infections may also contribute to hypercoagulability through mechanisms independent of platelets.

Infections may also transiently impair endothelium-dependent relaxation. In children, acute infections are associated with a reduction in endothelial reactivity.[57] Among 135 children with acute infection, brachial artery flow-mediated dilation was 6.3 ± 2.7%, compared with 8.1 ± 3.1% among 166 children 2 weeks out from infection, and 9.7 ± 2.5% in a control group of 299 well children ($p<0.001$ for both comparisons). The reduced brachial artery reactivity returned to normal among acutely infected children by one year. In a randomized, experimental clinical study among 20 human volunteers, *Escherichia coli* endotoxin (lipopolysaccharide) reduced endothelium-dependent forearm vascular reactivity by 40–50%.[58] Four days of simvastatin reduced neutrophil oxidative burst and plasma TNF- levels and completely abrogated the effect of endotoxin on vascular reactivity, however, indicating that statins may protect against acute infection or inflammation-related endothelial dysfunction.

Alternatively, infectious burden could simply be a marker of the underlying burden of other risk factors that are the direct contributors to atherosclerosis. Individuals with multiple medical problems, or those with behavioral risk factors such as heavy drinking or smoking, may be the

same individuals with a high burden of common infections. Socioeconomic status, in particular, is notoriously difficult to measure and could be an important confounder of any associations between infectious burden and stroke risk.

Cross-sectional studies provide evidence of an association between low socioeconomic status and chronic psychological stress and increased pathogen burden.[59] Neuroendocine mechanisms could also play a role in this association. Using a summary index of positive serologies against three common infections (CMV, *C. pneumoniae*, and HSV 1) among 317 healthy volunteers, there was a flattening of the normal slope of decline in salivary cortisol levels among those with a higher pathogen burden, independently of other demographic and socioeconomic variables.60 A decrease in cortisol decline over the course of the day has been associated with coronary artery disease.[61] Most of the effect of pathogen burden on cortisol levels was driven by CMV positivity. It is possible that early life exposure to these infections affects later life hypothalamic-pituitary axis function, leading to changes in cortisol production. Alternatively, the same socioeconomic factors that lead to increased stress in early life (i.e., poverty, poor nutrition, overcrowding) could also lead to increased risk of these infections. Further studies are needed to determine whether changes in hypothalamic-pituitary axis function related to these pathogens are consistently present, and whether these changes have any direct mechanistic effects on vascular function.

There is also evidence, however, that while socioeconomic status inversely correlates with both pathogen burden and risk of vascular disease, pathogen burden does not appear to mediate the risk of vascular disease associated with socioeconomic status. In a cross-sectional study among 451 men and women aged 51–72 without coronary disease, in which socioeconomic status was defined by employment grade, there was a higher infectious burden in those of lower socioeconomic status.[62] Infectious burden was also associated with several cardiovascular risk factors, but the relationship between socioeconomic status and cardiovascular risk remained after accounting for pathogen burden.

Infectious burden as a treatment target

Observational studies provide evidence that vaccination against common infections, particularly viral influenza, prevents stroke. In a case-control study among 370 consecutive stroke or TIA patients and an equal number of community controls, influenza vaccination during the previous season was associated with a 50% reduction in stroke risk after adjusting for other risk factors (a 19.2% vaccination rate in patients compared to a 31.4% rate in control subjects, $p<0.0001$).[63] Vaccinations against other organisms were not associated with this benefit. It is plausible, but not yet tested, that vaccinating stroke patients will lead to a reduction in risk of subsequent events. In children and some adults, varicella infection appears to represent a period of increased stroke risk, and it is possible that vaccination against chickenpox will reduce this risk.64 Recent guidelines, moreover, recommend vaccination against influenza in patients with cardiovascular disease as a means to prevent cardiovascular events.65 This would appear to represent the first anti-infective treatment to be championed as a vascular-disease preventing strategy.

It is important to recognize, however, that the possibility that influenza vaccination reduces risk of stroke and vascular events does not necessarily imply that influenza causes stroke or atherosclerosis directly. It could be that by preventing the general systemic complications of severe illness, such as inflammation, cytokine release, and dehdyradation, vaccination leads to a reduction in stroke risk. Because cervical arterial dissections (carotid and vertebral artery dissections) may even be provoked by severe coughing or vomiting, prevention of these common accompaniments of influenza could reduce the occurrence of dissection and secondary stroke from that mechanism.

Initial observational studies and 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.[66] Subsequent definitive randomized controlled trials, however, were unable to confirm these findings.[67,68,69] Currently, therefore, there is no indication to use 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. It is possible that the effects for stroke would be different than those for coronary disease.

There is precedent for such a discrepancy between the effect of infections on stroke compared to coronary disease. Studies of the association between periodontal disease and vascular disease, for example, have found greater effects on stroke than coronary disease, even within the same populations and databases. In recent studies that have rigorously adjusted for other confounding variables, among participants in the National Health and Nutrition Examination Survey, there has been an association found for stroke70 but not for heart disease.[71] Among 9962 patients with a mean age of 48 followed from initial evaluation in 1975 through 1992, the prevalence of gingivitis, periodontitis, and edentulousness was 25.3%, 16.8%, and 16.8%, respectively. After adjustment for other risk factors, there was a statistically significant increased risk of ischemic stroke (relative risk 2.1) among those with periodontitis. Whether treatment of periodontitis leads to a reduction in atherosclerosis also remains controversial. There is data from at least one prospective uncontrolled study that suggests, however, that treatment of periodontal infection can lead to a reduction in endothelial dysfunction and intima-media thickness.[72] These findings also need to be confirmed in other studies however.

Conclusions

Preliminary evidence has been accumulating that various measures of the aggregate burden of common chronic infections, including both bacterial and viral (particularly herpesvirus) infections, may be associated with subclinical measures of atherosclerosis and clinical outcome events. Several potential mechanisms for this association have been hypothesized, with limited supporting data at this point, but further studies are needed to determine whether multiple infections contribute meaningfully in a causal pathway to development of atherosclerosis. It is likely that host factors, including both behavioral and genetic factors, will interact with infections to influence vascular disease risk. Further studies are needed, moreover, to ascertain that antibiotics or vaccines can reliably reduce or retard atherosclerosis and prevent stroke and other vascular events.

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