Tumor Necrosis Factor Receptor Levels Are Associated With Carotid Atherosclerosis

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Background and Purpose—Recent evidence suggests that atherosclerosis is an inflammatory condition. Serum levels of inflammatory markers may serve as measures of the severity of atherosclerosis and risk of stroke. We sought to determine whether tumor necrosis factor-α (TNF-α) and TNF receptor levels are associated with carotid plaque thickness.

Methods—The Northern Manhattan Stroke Study is a community-based study of stroke risk factors. For this cross-sectional analysis, inflammatory marker levels, including TNF-α and TNF receptors 1 and 2, were measured by immunoassay in stroke-free community subjects undergoing carotid duplex Doppler ultrasound. Maximal carotid plaque thickness (MCPT) was measured for each subject. Analyses were stratified by age <70 and ≥ 70 years. Simple and multiple linear regression analyses were used to calculate the association between marker levels and MCPT. Multiple logistic regression was used to calculate odds ratios and 95% CIs for the association of inflammatory markers with MCPT ≥ 1.5 mm ($>$75th percentile), after adjustment for demographic and potential medical confounding factors.

Results—The mean age of the 279 subjects was 67.6 ± 8.5 years; 49% were men; 63% were Hispanic, 17% black, and 17% white. Mean values for TNF-α and its receptors were as follows: TNF-α, 1.88 ± 3.97 ng/mL; TNF receptor 1, 2.21 ± 0.99 ng/mL; and TNF receptor 2, 4.85 ± 2.23 ng/mL. Mean MCPT was elevated in those in the highest quartiles compared with lowest quartiles of TNF receptor 1 and 2 (1.24 versus 0.79 mm and 1.23 versus 0.80 mm, respectively). Among those aged <70 years, TNF receptor 1 and 2 were associated with an increase in MCPT (mean difference$=0.36$ mm, $P=0.01$ for TNF receptor 1 and mean difference$=0.10$ mm, $P=0.04$ for TNF receptor 2). After adjustment for sex, race-ethnicity, hypertension, diabetes mellitus, LDL cholesterol, smoking, and body mass index, associations remained (mean difference$=0.36$ mm, $P=0.001$ for TNF receptor 1 and mean difference$=0.09$ mm, $P=0.051$ for TNF receptor 2). There was no association for TNF receptors in those aged ≥ 70 years old and no association for TNF-α in either age group. Among those aged <70 years, each unit increase in TNF receptor level increased the odds of the participant's having MCPT ≥ 1.5 mm (adjusted odds ratio$=4.7$; 95% CI, 1.7 to 15.4 for TNF receptor 1; odds ratio$=1.9$; 95% CI, 1.3 to 2.9 for TNF receptor 2).

Conclusions—Relative elevation in TNF receptor levels, but not TNF-α, is associated with carotid atherosclerosis among individuals aged <70 years in this multiethnic, urban population. Chronic subclinical infection or inflammation may account for this association, and modification of these inflammatory pathways may provide a novel approach to stroke prevention. **(*Stroke*. 2002;33:31-38.)**

Key Words: atherosclerosis ■ cerebrovascular disorders ■ epidemiology ■ risk factors

Atherosclerosis is increasingly recognized to be an inflammatory disease.[1] Elevated levels of oxidized LDL cholesterol, as well as other potential contributors to endothelial injury, initiate an inflammatory cascade that leads to activation of monocytes and lymphocytes in the arterial wall, contributing to smooth muscle cell proliferation and thickening of the arterial wall. Inflammatory processes also appear to be involved in the precipitation of acute clinical events through the process of plaque rupture.[2–4]

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Convergent data from several epidemiological studies have provided evidence that serum markers of inflammation are associated with conventional risk factors for atherosclerotic

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diseases and with incident cardiovascular and cerebrovascular events.[5–12] Elevated levels of these markers also appear to predict recurrent events after a first acute cardiac ischemic event[13–17] or stroke.[18,19] C-reactive protein (CRP) has been most consistently shown to be associated with these outcome events, but soluble intercellular adhesion molecule-1, interleukin-6 (IL-6), E-selectin, and other molecules have been associated as well. Tumor necrosis factor-α (TNF-α) has been associated with an elevated risk of recurrent myocardial infarction and cardiovascular death after a first myocardial infarction.[20] Few data are available regarding TNF receptor levels and their association with atherosclerosis or stroke. TNF-α levels were correlated with ankle-brachial index[21] and other measures of atherosclerosis in some studies.[22] Other investigators have suggested that soluble TNF receptor levels may be a better marker of atherosclerotic burden than TNF-α itself.[23]

Recent data provide evidence that carotid duplex Doppler ultrasound is a useful way to study atherosclerotic risk factors, since asymptomatic carotid wall thickening and plaque formation may be precursors to clinical vascular events. Several investigators, including our own laboratory[24] (J-S. Jeng, MD, 1997, unpublished data), have shown that maximal carotid plaque thickness is associated with vascular risk factors, such as diabetes mellitus, hypertension, hypercholesterolemia, and smoking. We sought to determine whether TNF and TNF receptor levels are associated with maximal carotid plaque thickness in a cross-sectional analysis of a stroke-free, elderly, multiethnic urban population.

Subjects and Methods

The Northern Manhattan Stroke Study (NOMASS) includes an ongoing population-based cohort study designed to determine stroke incidence, risk factors, and prognosis in a multiethnic, urban population. Northern Manhattan consists of the area in New York City north of 145th St and south of 218th St, bordered on the west by the Hudson River and on the east by the Harlem River. In 1990, nearly 260 000 people lived in the community, with 40% aged >39 years and a race-ethnic mixture consisting of 20% black, 63% Hispanic, and 15% white residents.[25]

Selection of NOMASS Cohort

The methods of subject recruitment and enrollment have been described in previous publications.[25,26] Briefly, stroke-free community subjects were identified by random-digit dialing with dual-frame sampling to identify both published and unpublished numbers. When a household was contacted, the research objectives were explained, and a resident aged ≥39 years was interviewed briefly to obtain age, sex, race-ethnicity, and risk factor information. Approximately 84 612 telephone numbers were dialed and 22 868 households were contacted by Audits and Surveys, Inc, New York, NY, using trained bilingual interviewers. Of these, approximately 2143 households refused the initial screen to provide any information about eligibility (telephone response rate, 91%), and approximately 5314 were identified as households in which at least 1 household member satisfied eligibility requirements. Seventy-five percent of selected subjects agreed to come in for an in-person interview, for an overall response rate among those eligible subjects called of 68%.

Community participants were enrolled if they (1) had never been diagnosed with stroke, (2) were aged >40 years, and (3) resided in northern Manhattan for ≥3 months in a household with a telephone. In-person evaluations were performed at the medical center; those subjects who were not able to come to the hospital (6%) did not undergo Doppler imaging and were not included in this analysis. The study was approved by the Institutional Review Board at Columbia-Presbyterian Medical Center. All participants gave consent directly or through a surrogate when appropriate.

Index Evaluation of Subjects

Data were collected through interviews by trained research assistants, physical and neurological examinations by the study physicians, in-person measurements, and fasting blood specimens for lipid and glucose measurements, as described elsewhere.[26] When possible, data were obtained directly from subjects with the standardized data collection instruments. When the subject was unable to provide answers, a proxy knowledgeable about the subject's history was interviewed. Direct subject data were obtained from 99% of stroke-free subjects.

Assessments were conducted in English or Spanish depending on the primary language of the participant. Race-ethnicity was based on self-identification through a series of interview questions modeled after the US census and conforming to the standard definitions outlined by Directive 15.[27] All participants responding affirmatively to being of Spanish origin or identifying themselves as Hispanic were classified as such. All participants classifying themselves as white without any Hispanic origin or black without any Hispanic origin were classified as white non-Hispanic or black non-Hispanic, respectively.

Standardized questions were adapted from the Behavioral Risk Factor Surveillance System[28] by the Centers for Disease Control and Prevention regarding the following conditions: 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. Standard techniques were used to measure blood pressure, height, weight, and fasting glucose as described in prior publications.[24] Fasting lipid panels (including total cholesterol, LDL, HDL, and triglycerides) were measured with a Hitachi 705 automated spectrometer (Boehringer Mannheim). Hypertension was defined as in prior publications,[29] and diabetes mellitus was defined by a fasting blood glucose level ≥127 mg/dL, the subject's self-report of such a history, or insulin or oral hypoglycemic use. The definitions are noted in the footnotes of the tables.

Assessment of TNF-α and TNF Receptor Levels

For the measurement of serum inflammatory markers, blood was drawn into a 10-mL EDTA tube with minimally traumatic venipuncture by an experienced research phlebotomist trained in the protocol. The tube was then immediately spun at 3000*g* at 4°C for 20 minutes. Plasma was then divided equally into six 1.5-mL Eppendorf tubes. The samples were then frozen and stored at −70°C. Inflammatory marker levels were then measured in batched samples with the use of enzyme-linked immunosorbent assay utilizing monoclonal antibodies to CRP, IL-6, TNF-α, and TNF receptors 1 and 2 (Biosource International). Assays were performed blinded as to carotid plaque status of subjects. Only subjects enrolled in NOMASS since July 1999 had blood sampled in this way and were included in this analysis.

Assessment of Maximal Carotid Plaque Thickness

The method for assessment of maximal carotid plaque thickness (MCPT) has been described in a previous publication.[24] Briefly, MCPT was assessed by trained ultrasonographers experienced in the use of duplex ultrasonography for research purposes and blinded to the participant's risk factors. Ultrasonography was performed on a Siemens Quantum 2000 duplex ultrasound system with a 7.5-MHz frequency linear array transducer. With the subject lying in a supine position, the extracranial carotid arteries were imaged in transverse and longitudinal planes (anterior, lateral, and posterior views). Both internal carotid arteries and bifurcations were examined for the presence of atherosclerotic plaque, defined as an area of focal hyperechoic wall thickening distinct from wall thickening. If no atherosclerosis was identified, MCPT was recorded as zero. If plaque

TABLE 1. Characteristics of Participants

	n	Prevalence, %, or Mean±SD
Completed high school	125	45.0
Hypertension*	158	57.0
Diabetes mellitus†	62	22.6
Cardiac disease	70	25.3
Current smoker	37	13.5
Ever smoked	162	58.9
Total cholesterol, mg/dL‡	274	196.0±38.8
HDL cholesterol, mg/dL‡	274	48.3±39.7
LDL cholesterol, mg/dL‡	273	124.2±35.4

*H systolic blood pressure recording ≥160 mm Hg, diastolic blood pressure recording ≥95 mm Hg, or patient's self-report of a history of hypertension or antihypertensive use.

†Fasting blood glucose level >126 mg/dL, patient's self-report of such a history, or insulin or hypoglycemic use.

‡Not all subjects had their total, HDL, or LDL cholesterol measured.

was imaged, the view showing the thickest plaque was frozen, and the intimal-medial wall thickness (including the plaque) was measured in a single frame with an electronic cursor and was recorded as the MCPT for that artery. For this analysis, the greater of the right and left MCPT was used.

Statistical Analyses

Means were calculated for continuous variables and proportions for categorical variables, and a Bonferroni multiple comparison procedure was used to test for differences between means. Regression analysis was first performed with the use of each of the inflammatory marker levels as a continuous independent variable and MCPT as the dependent variable. Subjects were then divided into 4 quartiles defined by TNF receptor level. Multivariate linear regression using a nonautomated procedure, incorporating demographic and clinical variables, was then used to build models for the association of TNF-α and TNF receptor level and MCPT. The dependent variable for these analyses, MCPT, was expressed as a continuous variable, despite a skewed distribution, because of the large sample size and stable variance. Conventional atherosclerotic risk factors were chosen for the final model on the basis of association with MCPT in univariate analysis or on the basis of findings of a significant association in previous analyses from our laboratory. Analyses were then conducted to determine the effect of TNF receptor level, considered as a continuous variable, on MCPT as a dichotomous variable with the use of multivariate logistic regression, taking as the cutoff an MCPT ≥1.5 mm (ie, >75th percentile in this population). Subjects with missing values for a particular multivariate analysis were excluded from that analysis. Statistical significance was determined at the $\alpha=0.05$ level with the use of 2-sided tests. Statistical analyses were conducted with SAS computer software (SAS Institute).

Results

The mean age of the 279 participants was 67.6±8.5 years. Forty-nine percent (n=137) were men; 17% (n=48) of the participants were white non-Hispanic, 17% (n=47) black non-Hispanic, 63% (n=175) Hispanic, and 3% (n=8) other race-ethnicity. The distribution of sociodemographic factors, comorbid vascular diseases, and conventional atherosclerotic risk factors is shown in Table 1.

For technical reasons, not all subjects had all tests performed. The distributions of values for the inflammatory markers are given in Table 2, as well as the number of subjects for whom each test was performed. Assessment of TNF-α levels was performed in all 279 subjects, and assessment of TNF receptor 1 and 2 levels was performed in 237 and 238 subjects, respectively. There were some differences in levels of marker by age and race-ethnicity, but none of these differences were statistically significant (Table 2).

The group of 245 participants with MCPT measured did not differ significantly from those without MCPT measured, except that current smoking was more common among those without MCPT measured than among those with it (25% versus 12%; $P=0.04$). The mean MCPT among the 245 participants who had this measured was 0.82±0.88 mm (median, 0.90 mm; interquartile range, 0 to 1.4 mm). There were differences in MCPT among the 3 major race-ethnic groups, as has been shown in prior data from our population[24] (Table 2). MCPT among Hispanics was significantly less than that among non-Hispanics, but there was no significant difference between white and black non-Hispanics. Not surprisingly, mean MCPT was also higher among those aged ≥70 years old than among younger subjects. There were no systematic differences by sex.

In a simple linear regression model, there was no significant association of TNF-α with MCPT in the overall popu-

TABLE 2. Distribution of Inflammatory Markers and MCPT, Overall and Stratified by Age and by Race-Ethnicity

			Stratified by Age and Race-Ethnicity				
Inflammatory Marker	n	Overall	Aged <70 y (n=169)	Aged ≥70 y (n=110)	Whites (n=48)	Blacks (n=47)	Hispanics (n=175)
TNF-α, pg/mL	279	1.88±3.97	1.89±3.67	1.88±4.41	2.71±4.25	1.04±1.63	1.84±4.28
TNF receptor 1, ng/mL	237	2.21±0.99	1.96±0.55	2.64±1.36	2.53±0.93	2.28±0.95	2.12±1.03
TNF receptor 2, ng/mL	238	4.85±2.23	4.33±1.26	5.72±3.08	5.36±3.40	5.11±2.38	4.67±1.76
CRP, mg/dL	279	2.12±3.55	2.49±3.88	1.56±2.90	1.88±2.75	2.64±4.62	2.11±3.50
IL-1β, pg/mL	279	0.34±0.51	0.39±0.56	0.28±0.42	0.23±0.43	0.35±0.59	0.38±0.51
IL-6, pg/mL	279	1.21±1.36	1.13±1.15	1.34±1.63	1.15±1.08	1.36±1.51	1.20±1.41
IL-2, U/mL	236	0.12±0.21	0.11±0.20	0.14±0.22	0.11±0.17	0.11±0.17	0.13±0.23
IL-2 receptor, pg/mL	235	544.50±636.83	530.11±716.68	568.55±477.39	673.66±632.98	568.80±560.73	507.11±665.72
MCPT, mm	245	0.82±0.88	0.61±0.79	1.18±0.92	1.18±0.95	0.93±0.94	0.70±0.82

Values are mean±SD.

TABLE 3. Predicted Mean Differences in MCPT by Level of TNF-α and TNF Receptor 1 and 2, Overall and Stratified by Age

	TNF-α			TNF Receptor 1			TNF Receptor 2		
	n*	Mean Difference per Unit Change in TNF-α, mm	*P*	n*	Mean Difference per Unit Change in TNF Receptor 1, mm	*P*	n*	Mean Difference per Unit Change in TNF Receptor 2, mm	*P*
Overall									
Unadjusted	245	−0.018	0.197	217	0.233	<0.0001	218	0.070	0.005
Adjusted for demographic factors†	239	−0.023	0.074	212	0.116	0.034	213	0.016	0.505
Adjusted for demographic and medical risk factors‡	232	−0.023	0.054	209	0.101	0.071	210	0.012	0.611
Aged <70 y									
Unadjusted	153	−0.012	0.476	138	0.359	0.011	139	0.098	0.041
Adjusted for demographic factors†	147	−0.020	0.256	133	0.344	0.002	134	0.073	0.135
Adjusted for demographic and medical risk factors‡	144	−0.016	0.339	131	0.358	0.001	132	0.091	0.051
Aged ≥70 y									
Unadjusted	91	−0.026	0.215	79	0.082	0.269	79	0.007	0.840
Adjusted for demographic factors†	92	−0.022	0.287	79	0.099	0.194	79	0.010	0.781
Adjusted for demographic and medical risk factors‡	88	−0.025	0.215	78	0.101	0.190	78	0.000	0.992

*The number of subjects for each model differs slightly because not all data were available on all subjects, and those with missing values were excluded from the analyses.

†Adjusted for age (overall model only), race-ethnicity (white non-Hispanic, black non-Hispanic, or Hispanic), and sex.

‡Adjusted for age (overall model only), race-ethnicity (white non-Hispanic, black non-Hispanic, or Hispanic), sex, hypertension, diabetes mellitus, LDL, current cigarette smoking, and body mass index.

lation, but there was a significant association for both TNF receptor 1 and 2 (Table 3). After adjustment for age, sex, and race-ethnicity, an association remained for TNF receptor 1 but not for TNF receptor 2 in the overall population (mean difference for TNF receptor 1=0.116 mm, $P=0.034$; Table 3). After inclusion of other risk factors in the overall model, however, the effect of TNF receptor 1 was also attenuated (mean difference for TNF receptor 1=0.101 mm, $P=0.071$; Table 3). After stratification by age <70 and ≥70 years, there was a significant association for both receptor levels (Table 3). Among those aged <70 years, TNF receptor 1 and 2 were associated with an increase in MCPT (mean difference=0.359 mm per unit change, $P=0.011$ for TNF receptor 1 and mean difference=0.10 mm per unit change, $P=0.041$ for TNF receptor 2). After adjustment for sex, race-ethnicity, hypertension, diabetes mellitus, LDL, smoking, and body mass index, a significant association remained for TNF receptor 1 and a borderline significant association for TNF receptor 2 (mean difference=0.358 mm per unit change, $P=0.001$ for TNF receptor 1 and mean difference=0.09 mm per unit change, $P=0.051$ for TNF receptor 2). There was no association with MCPT for TNF receptors in those aged >70 years old. Levels of TNF-α itself were not associated with MCPT in either age group.

In the overall population, compared with the lowest quartile of TNF receptor 1, mean MCPT was elevated in those in the highest quartile of TNF receptor 1 (1.24 versus 0.79 mm). Similarly, for TNF receptor 2, compared with those in the

TABLE 4. Odds Ratios of MCPT ≥1.5 mm (75th percentile) by Increase in TNF Receptor 1 and 2 Among Those Aged <70 Years (n=117)

	TNF Receptor 1			TNF Receptor 2		
	n*	Odds Ratio†	95% CI	n*	Odds Ratio†	95% CI
Unadjusted	138	3.72	1.63–9.30	139	1.68	1.19–2.43
Adjusted for sex and race-ethnicity	133	3.51	1.48–8.96	134	1.63	1.14–2.38
Adjusted for sex, race-ethnicity, and conventional risk factors‡	131	4.73	1.69–15.41	132	1.90	1.27–2.89

*The number of subjects in each row differs slightly because not all data were available on all subjects, and those with missing values were excluded from the analyses.

†Odds ratios represent increase in risk for each unit increase in TNF receptor level.

‡Conventional risk factors are hypertension, diabetes mellitus, LDL, current smoking, and body mass index.

lowest quartile, those in the highest quartile had an elevated MCPT (1.23 versus 0.80). There was no increase in MCPT in either the second or third quartile of either TNF receptor levels. There was no association of TNF, CRP, or IL-6 level with MCPT.

We also performed analyses with MCPT as a dichotomous outcome, using the 75th percentile of MCPT to dichotomize MCPT. In a logistic regression model, among those aged <70 years, each unit increase in TNF receptor level increased the odds of the participant's having MCPT ≥1.5 mm (adjusted odds ratio=4.7; 95% CI, 1.7 to 15.4 for TNF receptor 1; odds ratio=1.9; 95% CI, 1.3 to 2.9 for TNF receptor 2; Table 4).

Discussion

This cross-sectional study provides evidence for an association between particular serum markers of inflammation and the presence of subclinical carotid plaque. We also suggest that the association may be limited to relatively younger individuals (ie, aged <70 years). We found an association of TNF receptor levels and MCPT in an urban, mostly Hispanic population, in whom the burden of stroke and other vascular diseases is high. The association was not present for TNF-α levels, CRP, or IL-6 in this population.

TNF-α is a potent inflammatory cytokine. The main source of TNF-α is activated mononuclear leukocytes, although it is also secreted by a wide variety of other immune and nonimmune cell types, including fibroblasts, smooth muscle cells, astrocytes, and neurons. TNF receptor 1 (also known as p55) and TNF receptor 2 (also known as p75) are both soluble receptors shed by the many cell types on which they reside. Elevation of TNF-α and TNF receptor levels occurs in a variety of infectious, inflammatory, autoimmune, and neoplastic diseases. Elevated levels of TNF receptor may be a reflection of the inflammatory mechanisms operative in the atherosclerotic plaque. Macrophages and T-lymphocytes are prominent in human atheromas, even at the earliest stages of the disease process,[30] suggesting that immune processes may play an initiating or early role in the development of the lesion in human beings, as well. Our data provide evidence for at least a partial role for activated leukocytes in the chronic process of atherosclerosis.

Several studies of atherosclerotic risk factors using high-resolution carotid duplex Doppler ultrasound have included measurements of inflammatory markers, but TNF receptor levels have been examined in few.[22,23] Elneihoum at al[22] found among a sample of middle-aged asymptomatic subjects with early atherosclerosis that TNF receptor 1 levels correlated with age and systolic blood pressure. They did not study TNF receptor 2. In another study, TNF receptor 2 and TNF-α but not TNF receptor 1 were elevated in patients with either symptomatic coronary artery disease or peripheral arterial disease. We[31] and other investigators[32,33] have found white blood cell count, a crude measure of inflammation or infection, to be associated with carotid atheroma.[34–37]

We found an association with carotid plaque of TNF receptors but not TNF-α itself. This may reflect the fact that TNF-α and TNF receptors have independent significance in terms of carotid plaque formation or progression. Other investigators have similarly shown a dissociation between the effects of TNF-α and its receptors in atherosclerotic disease.[22,23] Alternatively, TNF receptors may be a more stable marker of inflammatory burden than TNF-α. We also did not find associations for other commonly studied inflammatory measures, including CRP and IL-6. Others have similarly failed to find associations of CRP and other inflammatory markers with carotid wall thickness.[38] It is possible that CRP and IL-6, which have been associated with clinical outcome events, are less likely to be associated with subclinical disease, as is seen in our asymptomatic population. It remains uncertain which inflammatory markers are most meaningful in the prediction of vascular risk or prognosis after a first event. In at least 1 study,[20] TNF-α appeared to be a more powerful marker of risk after a first myocardial infarction than did CRP or serum amyloid A, another nonspecific inflammatory measure. TNF receptors were not evaluated in that study.

In the population examined in our study, the association of TNF receptors with atherosclerosis differed by age. Other investigators have similarly found that the association of inflammation and infection with atherosclerosis may differ by age. Among studies in which leukocyte count predicted cardiovascular events, such as the Framingham, Multiple Risk Factor Intervention Trial (MRFIT), and Caerphilly and Speedwell studies, participants were generally relatively young, ie, aged <60 years.[32,33,36,37] In studies examining both middle-aged and older participants,[35] the effect of leukocytes was stronger in those aged <65 years.

The magnitude of the effect of elevated TNF receptors, particularly TNF receptor 1, on MCPT among those aged <70 years in our population may have clinical import. Every unit increase in TNF receptor 1 had an adjusted increase in plaque thickness of 0.36 mm. In the Cardiovascular Health Study, although measurements were made with a different technique, for every increase in intima-media thickness of the internal carotid artery of 0.55 mm there was an increase in risk of stroke or myocardial infarction of 30%.[39]

It has been speculated that the association between elevated levels of inflammatory markers and atherosclerosis reflects chronic subclinical infection, although this hypothesis awaits confirmation. Several observational epidemiological studies,[40–44] including in our own population,[45,46] have suggested an association between chronic infections such as *Chlamydia pneumoniae* and periodontitis and stroke risk or carotid atherosclerosis. Nonetheless, the elevations in TNF receptor levels seen here could also be related to the presence of other noninfectious stimulants of inflammation, including oxidized LDL or smoking. We adjusted for cholesterol levels and smoking history in our analyses, but there could be residual confounding.

Further prospective studies of the relationship between TNF receptors and other inflammatory and infectious markers are needed. While many investigators have examined the relationship between inflammation, infection, and atherosclerotic heart disease, these may not reflect the relationship between these processes and stroke. In northern Manhattan, large-artery atherosclerosis accounts for a minority (only 10% to 20%) of ischemic stroke[47]; embolic and small-vessel causes of stroke are probably more common. Further studies

will need to take into account the several etiologic subtypes of stroke.

Our study has several limitations. Because of its cross-sectional design, we were unable to derive a temporal or causal relationship between elevated TNF receptors and increase in plaque thickness. Increased carotid plaque could itself be the cause of the elevated TNF receptor levels. We also did not have data on clinical infection and therefore are unable to make statements about potential underlying infectious causes of the elevated TNF receptor levels. Prospective study designs that use measures such as progression of atherosclerosis over time are ongoing in our population to address this issue. Our study also assesses a measure of subclinical atherosclerosis, MCPT, rather than clinical end points such as myocardial infarction or stroke, which may be considered more relevant to clinical practice. Several recent studies, however, have provided evidence that measures of subclinical atherosclerosis are predictive of clinical ischemic events.[39,48] These measures thus have the potential to allow stratification of patients for intervention to prevent outcome events.

It is also possible that there is residual confounding by other conditions, including heart failure. Levels of TNF-α are elevated in congestive heart failure[49,50] and could be elevated in patients in our study for that reason. However, this is a generally healthy population identified by random-digit dialing. The prevalence of heart failure in our sample was low, at only 5.8%. Our study population may also not be representative of all US populations. Our method of subject recruitment would exclude those otherwise eligible subjects in our community who do not have a telephone, and not all those contacted agreed to participate. Not all of those enrolled, moreover, had duplex Doppler testing performed. Additional studies in independent populations would be necessary to extend our findings to other groups.

Our measurement of MCPT, a single measurement of the maximum thickness of the internal carotid artery plaque, also differs from that used in other studies of carotid wall thickness.[32,39,48] Using this method, however, we have found associations with atherosclerotic risk factors, including age, smoking, hyperglycemia, hypertension, LDL cholesterol, apolipoprotein A-I and apolipoprotein B, and leukocyte count,[24,31] (J-S. Jeng, MD, 1997) consistent with findings in studies using methods that measure intima-media thickness. One clinical advantage of our method is its ease of use.

In summary, our study supports an association between elevated TNF receptor levels and carotid atherosclerosis in relatively young persons. Several lines of evidence have demonstrated that these and other markers of inflammation and infection may be associated with vascular disease. Strategies aimed at modifying these inflammatory parameters may offer a novel approach to stroke prevention in patients at high risk.

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Editorial Comment

Association Between Tumor Necrosis Receptor Levels and Carotid Atherosclerosis: Is the Association Limited to Younger Individuals?

Together with the complement system, activated mononuclear and polymorphonuclear leukocytes and the proinflammatory mediators that are secreted by these cells are the major elements thought to be involved in the production of local inflammation in an atherosclerotic plaque.[1] Tumor necrosis factor-α (TNF) may arise from various inflammatory cells including circulating mononuclear leukocytes, and macrophages within an atheroma.[2] It is a proinflammatory cytokine that may be important in atherogenesis: it has been associated with apoptotic death of smooth muscle cells[3] and endothelial cells[4] and with adhesion of endothelial cells for T cells.[5] TNF exerts its actions on binding to high-affinity receptors that are found on the surface of cells.[6] These receptors have two isoforms: p55 (also known as TNF receptor 1) and p75 (also known as TNF receptor 2). Apart from those that exist on the surface of cells, these receptors may be found at soluble levels in the plasma. Plasma TNF levels and soluble plasma TNF receptor 2 (p75 isoform) levels have been found to be

higher in people with confirmed atherosclerosis of the peripheral and coronary vessels than among subjects free of symptomatic vascular disease.[7]

Vascular atherosclerosis, as measured by carotid artery intima-media thickness, has been found to be associated with an increased risk of stroke and myocardial infarction in prospective studies.[8,9] These associations remained after controlling for traditional risk factors. Other investigators have found that elevated plasma TNF is associated with an increased risk of recurrent coronary events after myocardial infarction.[10] The question remains as to whether circulating levels of TNF or soluble TNF receptor (sTNFr) levels are associated with carotid artery atherosclerosis and might consequently be used to predict the risk of cerebrovascular or cardiovascular events.

In this article, Elkind et al have utilized a sample of 279 stroke-free controls to assess whether TNF and sTNFr levels were associated with maximal carotid plaque thickness. When adjusted for demographic and traditional risk factors, only sTNFr 1 (p55 isoform) levels remained associated with mean differences in maximal carotid plaque thickness among subjects under (but not over) 70 years of age. Further analysis of the under-70 age group using maximal carotid plaque thickness as a dichotomous variable revealed that each unit increase in both isoforms of sTNFr was associated with an increased odds of participants having a maximal carotid plaque thickness of at least 1.5 mm. These results support an association between sTNFr and carotid atherosclerosis in younger individuals.

The lack of association between maximal carotid plaque thickness and either TNF or the two isoforms of sTNFr in the older age group may not be surprising. It might be partly explained by the smaller number of observations in this age group. Furthermore, it might be influenced by the level of generalized atherosclerosis that may be present in older subjects. If these older subjects have significant disease in any of their other vascular beds, the levels of plasma TNF and sTNFr may be elevated. Elevated levels of TNF and the p75 isoform of sTNFr have been demonstrated to be higher in people with peripheral vascular disease and ischemic heart disease.[7] Consequently, TNF and sTNFr levels that may arise from these other vascular beds may mask any association between these factors and plaque thickness in the carotid artery in older participants. Alternatively, it is also possible that the association between sTNFr and carotid atherosclerosis may be limited to younger individuals.

The findings from this study are consistent with those of previous investigations. The results provide evidence for an association between sTNFr and carotid atherosclerosis in younger individuals. As discussed by the authors, it is still unclear whether the carotid plaque might be a cause, rather than a consequence, of the elevated TNF receptor levels. There remains a possibility that TNF and sTNFr levels might still be potential candidates as markers of subclinical disease and that strategies aimed at modifying these factors might provide a novel approach to prevention of stroke and other vascular disease. The present investigation is part of an ongoing study of stroke in northern Manhattan. Further studies in this or other populations may help to address some of these issues.

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