C-Reactive Protein as a Prognostic Marker After Lacunar Stroke

Levels of Inflammatory Markers in the Treatment of Stroke Study

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Background and Purpose—Inflammatory biomarkers predict incident and recurrent cardiac events, but their relationship to stroke prognosis is uncertain. We hypothesized that high-sensitivity C-reactive protein (hsCRP) predicts recurrent ischemic stroke after recent lacunar stroke.

Methods—Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS) was an international, multicenter, prospective ancillary biomarker study nested within Secondary Prevention of Small Subcortical Strokes (SPS3), a phase III trial in patients with recent lacunar stroke. Patients were assigned in factorial design to aspirin versus aspirin plus clopidogrel, and higher versus lower blood pressure targets. Patients had blood samples collected at enrollment and hsCRP measured using nephelometry at a central laboratory. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for recurrence risks before and after adjusting for demographics, comorbidities, and statin use.

Results—Among 1244 patients with lacunar stroke (mean age, 63.3±10.8 years), median hsCRP was 2.16 mg/L. There were 83 recurrent ischemic strokes (including 45 lacunes) and 115 major vascular events (stroke, myocardial infarction, and vascular death). Compared with the bottom quartile, those in the top quartile (hsCRP >4.86 mg/L) were at increased risk of recurrent ischemic stroke (unadjusted HR, 2.54; 95% CI, 1.30–4.96), even after adjusting for demographics and risk factors (adjusted HR, 2.32; 95% CI, 1.15–4.68). hsCRP predicted increased risk of major vascular events (top quartile adjusted HR, 2.04; 95% CI, 1.14–3.67). There was no interaction with randomized antiplatelet treatment.

Conclusions—Among recent lacunar stroke patients, hsCRP levels predict the risk of recurrent strokes and other vascular events. hsCRP did not predict the response to dual antiplatelets.

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Key Words: C-reactive protein ■ inflammation ■ prognosis ■ stroke

Basic and clinical studies provide evidence that inflammation plays a major role in atherosclerosis and cardiovascular disease,[1] but relatively little information is available on serum markers of inflammation as indicators of prognosis after stroke.[2] Clinical studies suggest, moreover, that some antiplatelet agents and statins reduce the levels of inflammatory markers, and that the efficacy of these treatments in preventing cardiac disease may be predicted by these levels.[3–5] There have been few major multicenter studies of inflammatory markers in predicting outcomes after stroke and no studies of the role of these markers in choosing antiplatelet therapies.

Lacunes, or small subcortical strokes, comprise ≈25% of brain infarcts, are especially frequent in Latinos and other US minorities, and are the most common cause of vascular dementia.[6–9] Although infrequently fatal, lacunes are associated with a high risk of recurrence and cognitive impairment. The rate of recurrence among patients with lacunar stroke is ≈8% per year, slightly higher than other stroke subtypes.[10–13]

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About 70% of recurrences in these patients are also lacunes, supporting a distinctive pathomechanism.

The major risk factors for lacunar stroke are age, hypertension, and diabetes mellitus. About 70% to 80% of patients have hypertension, and ≈30% have diabetes mellitus.[14–16] Other potential risk factors include smoking, silent brain infarcts, and white matter hyperintensities on MRI.[17–19] Stroke risk factors were absent in 18% of patients in 1 large autopsy study.[20] In the Northern Manhattan Stroke Study, the prevalence of hypertension, diabetes mellitus, smoking, and hypercholesterolemia did not differ between those with lacunar and nonlacunar stroke.[21]

Inflammatory mechanisms have been associated with lacunes and their prognosis. A polymorphism of the interleukin-6 gene associated with increased inflammation was an independent risk factor for lacunar stroke in 1 study.[22] This same polymorphism was found to be associated with carotid artery intima-media thickness as well, providing evidence that polymorphisms related to inflammation may relate to both large vessel and small vessel disease.[23] Others have reported that patients with lacunar stroke with elevated tumor necrosis factor-α and intercellular adhesion molecule-1 levels were more likely to experience early neurological deterioration and poor outcome at 3 months.[24] In a case–control study, elevated *Chlamydia pneumoniae* antibody titers were associated with increased risk of lacunar stroke, as well as large vessel atherosclerotic stroke.[25] An association between leukocyte count and outcomes after stroke has also been found in those with lacunar stroke, as well as in other stroke subtypes.[26] These data support the hypothesis that inflammatory mechanisms contribute to the risk of lacunar disease and its prognosis.

In response to calls for data regarding the role of inflammation in stroke prognosis,[1,2] we designed a prospective observational study to test the hypothesis that inflammatory biomarkers predict recurrence after lacunar stroke. The study was nested within the ongoing Secondary Prevention of Small Subcortical Strokes (SPS3), the results of which have been published.[27,28] The primary aim of the Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS) study was to determine whether serum levels of high-sensitivity C-reactive protein (hsCRP) predict recurrent stroke and other vascular events among patients with a recent history of small artery ischemic stroke. A secondary aim was to determine whether hsCRP predicts which patients respond best to dual antiplatelet therapy.

Methods

Overall Plan

LIMITS was designed as an ancillary study to the SPS3 trial, and the methods for both have been described previously.[27,29,30] In brief, SPS3 was a multicenter, investigator-initiated, National Institutes of Health/National Institute of Neurological Disorders and Stroke–funded phase III trial that focused on secondary prevention of stroke recurrence in patients with small vessel ischemic stroke, or lacunes. The study had a 2×2 factorial design, with 1 arm being a blinded comparison of monotherapy versus combination therapy of antiplatelet agents, and the other an open-label comparison of 2 targets of blood pressure control. Participants had a symptomatic lacune proven on MRI <6 months before randomization. Patients were assigned to 2 interventions: (1) antiplatelet therapy—aspirin (325 mg/day) monotherapy versus aspirin (325 mg/day) plus clopidogrel (75 mg/day) combination therapy (double blind, placebo control); and (2) 2 levels of systolic blood pressure control—higher (130–149 mm Hg) versus lower (<130 mm Hg) targets. LIMITS involved the collection of plasma and serum samples at baseline and at 1 year (≈18 months) of follow-up during the study, and central storage and analysis of samples for inflammatory marker levels. Blood samples were drawn for LIMITS ≥3 weeks after stroke. Sites were required to have the capability to collect, process, and store blood samples at −80ºC. Of 84 sites participating in SPS3, 45 (54%) recruited patients into LIMITS (see Appendix for the list of investigators).

Inclusion and Exclusion Criteria

The population for this ancillary study consisted of patients enrolled into the SPS3 trial at sites participating in LIMITS. Patients with a clinical diagnosis of MRI-proven lacunar stroke, and absence of cortical or large subcortical stroke, carotid stenosis, or cardioembolic source, were eligible. All patients eligible for SPS3 were eligible for this ancillary study; there were no additional exclusion criteria.[27] LIMITS was approved by the Columbia University Medical Center institutional review board and by institutional review boards at all participating sites. Participants provided informed consent.

Blood Collection Kits, Phlebotomy, and Local Processing

Materials for collection and shipping of blood specimens were provided to participating sites. A 10-cc blood sample in EDTA and a 9.5-mL gel serum separator tube were drawn on the day of randomization, before initiation of therapy. Samples were centrifuged locally and aliquotted. Aliquots were frozen locally until shipping to the central laboratory at Columbia University.

Assays for hsCRP

Plasma samples were analyzed in batches blinded to treatment and outcome. hsCRP assays were performed using the Dade-Behring BN-II nephelometric assay system (Dade-Behring; Deerfield, IL), a US Food and Drug Administration–approved, national standard assay for this marker.[1]

Outcomes

Primary outcomes were recurrent ischemic stroke and the combined outcome of a major cardiovascular event (recurrent ischemic stroke, myocardial infarction [MI], or vascular death). Ischemic strokes were defined as a focal neurological deficit persisting for >24 hours and were ascertained via clinical evaluation and use of CT or MRI. Other vascular events were also defined as in the SPS3 trial.[27,29] Additional analyses were performed for the outcome of ischemic or hemorrhagic stroke. Hemorrhagic strokes were defined as neurological deficits associated with intraparenchymal or subarachnoid hemorrhagic lesions confirmed by CT, MRI, or autopsy.

Statistical Analysis and Sample Size Calculations

Descriptive statistics were calculated and compared for patients in the ancillary LIMITS study and the parent trial, and levels of hsCRP were compared across different patient characteristics. An F-test was used to determine whether there were differences in CRP categories for continuous variables, a χ^2 test for nominal categorical variables, and Cochran–Mantel–Haenszel when categorical variables were ordinal.

For the analyses of primary outcomes, hsCRP values at baseline were considered the independent variable of primary interest. Levels of hsCRP were log-transformed to stabilize the variance. Cox proportional hazard regression was used to estimate the unadjusted hazard ratio for hsCRP with time-to-event for ischemic stroke as the dependent variable. Multivariable Cox proportional hazards regression was then used to estimate the adjusted hazard ratio for hsCRP after adjusting for additional potential risk factors, including age, sex, race–ethnicity, region, and traditional stroke risk factors defined either

dichotomously (hypertension, cardiac disease, diabetes mellitus, or smoking) or continuously (body mass index, high-density lipoprotein, or low-density lipoprotein). Because statin therapy may influence both levels of hsCRP and outcomes, analyses were also adjusted for statin use. Observations were censored at the time of last follow-up visit. Additional analyses used quartiles of hsCRP as independent variables, using those in the lowest quartile for each marker as the reference group. In addition, different ranges of standardized levels of risk have been recommended by consensus for primary prevention of cardiac disease: low, medium, and high (CRP <1, 1–3, and >3 mg/L, respectively).[1] There are no consensus levels to be used after stroke, but the results of small studies suggest that levels are higher after stroke.[31,32] We, therefore, tested hsCRP <15 and >15 mg/L as prespecified thresholds.

A sample size of 1440 (57% of the initial planned total of 2500 patients to be enrolled in SPS3) was chosen based on feasibility, assuming that ≈40 enrolling centers would enroll ≥12 patients annually for 3 years. We computed the detectable hazard ratios based on a required power of 80% and a significance level of 0.05 for comparing the outcome rates in the first and fourth quartiles. Where possible, sample size calculations were based on the same assumptions used in the SPS3 trial (annual 7% rate of recurrent stroke and 10% rate of recurrent ischemic stroke, MI, or death). In addition, a 10% 3-year loss to follow-up was assumed. Because event rates in SPS3 were lower than expected, the sample size of the parent SPS3 trial was increased to 3000.[33] All hypothesis tests performed during analysis of the primary and secondary end points are 2 sided and use an α of 0.05.

Results

Characteristics of the LIMITS Cohort

A total of 1244 patients in SPS3 were enrolled in LIMITS. This represented 41% of the number of participants in the parent SPS3 trial. Participants enrolled in LIMITS were broadly representative of the population enrolled only in SPS3 (Table 1). The mean age of LIMITS participants was 63.3±10.8 years, and 63.4% were men. The race–ethnicity distribution differed in that a larger proportion of patients in LIMITS were Hispanic (40.6% in LIMITS versus 23.1% in SPS3-only patients), reflecting an increased number of participating sites from Spain and South America. Other significant differences between participants in LIMITS and SPS3-alone were, in the former, a slightly lower proportion of patients with diagnosed hyperlipidemia and current smoking, a slightly shorter time from qualifying stroke to randomization, and a slightly lower proportion already using aspirin at baseline (Table 1).

Levels of hsCRP in LIMITS Participants and Association With Patient Characteristics

Among the 1244 patients in LIMITS, median hsCRP was 2.16 mg/L (interquartile range, 0.93–4.86), which differed by age, sex, smoking, and low-density lipoprotein levels (Table 2). Median hsCRP among current smokers was 3.1 mg/L compared with 2.0 mg/L among both former and never smokers. Median time between stroke and LIMITS sample collection was 60 days (interquartile range, 34–105 days; mean, 75±52 days). hsCRP levels were inversely and weakly correlated with proximity to stroke date (r=−0.06; P=0.039).

Association of hsCRP With Outcomes

There were 83 recurrent ischemic strokes (including 45 lacunes), 16 hemorrhagic strokes, and 115 major vascular events (stroke, MI, and vascular death) among LIMITS participants during a median follow-up of 3 years.

Compared with the bottom quartile of hsCRP (<0.93 mg/L), those in the top quartile (≥4.86 mg/L) were at increased risk of recurrent ischemic stroke (unadjusted HR, 2.54; 95% CI, 1.30–4.96; Table 3). There was a trend toward an intermediate level of increased risk for those in the second and third quartiles, but these increased risks were not significantly elevated. The increased risk for those in the top quartile persisted after adjusting for age, sex, race, region, hypertension, smoking, previous history of stroke, diabetes mellitus, body mass index, and lipid levels (model 3 in Table 3) and remained elevated after further adjusting for statin use (adjusted HR, 2.32; 95% CI, 1.15–4.68). The risk of ischemic or hemorrhagic stroke was of similar magnitude (adjusted HR for those in the top quartile, 2.08; 95% CI, 1.11–3.89; Table 4). Approximately 70% of recurrent ischemic strokes were lacunes; there was no evidence of an independent, statistically significant predictive effect on lacunar stroke as an outcome, but the results for lacunar stroke were more generally consistent with the effect on ischemic stroke (adjusted HR, 2.27; 95% CI, 0.90–5.75).

hsCRP was associated with an increased risk of major vascular events as well (unadjusted HR for those in the top quartile compared with lowest, 2.14; 95% CI, 1.22–3.75). The results were similar after adjusting for demographics, risk factors, and baseline statin use (adjusted HR, 2.04; 95% CI, 1.14–3.67; Table 5).

Results were similar using clinically recommended hsCRP thresholds of high risk in primary prevention. Compared with those with hsCRP <1 mg/L, those with hsCRP >3 mg/L had ≈2-fold increase in the risk of ischemic stroke (adjusted HR, 2.16; 95% CI, 1.13–4.11; Table 3) and a 70% increase in the risk of major vascular events (adjusted HR, 1.72; 95% CI, 1.02–2.90; Table 5). Using a prespecified higher threshold (>15 mg/L),[31] there was no evidence of a difference of risk (Tables 3–5). hsCRP assessed as a continuous measure was also not significantly associated with risk.

hsCRP Levels as a Predictor of the Effect of Antiplatelet and Blood Pressure Therapy

There was no interaction of randomized antiplatelet treatment or blood pressure target with hsCRP levels for stroke risk, indicating a lack of evidence for a differential effect of dual versus single antiplatelet therapy depending on the hsCRP level. Furthermore, there was no interaction of hsCRP with statin use at baseline.

Discussion

Our data provide evidence that hsCRP predicts the risk of recurrent ischemic stroke, total stroke, and major vascular events among patients with recent lacunar stroke. Patients with the highest levels of hsCRP measured in the subacute phase after lacunar stroke (median, 60 days) had more than twice the risk of recurrent ischemic stroke and approximately twice the risk of total stroke or a major vascular event. There was some evidence that hsCRP levels >1 mg/L and in the intermediate 2 quartiles were also associated with an approximate doubling of risk, although the increased risk at those levels was not statistically significant, and there was no definite evidence of a linear trend across quartiles. These findings

Table 1. Descriptive Characteristics of Patients Enrolled in Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS; Ancillary Study) and Secondary Prevention of Small Subcortical Strokes (SPS3; Parent Study) Only

Baseline Characteristics	LIMITS (N=1244)	SPS3 Only (N=1776)	*P* Value
Demographics			
Age	63.3±10.8	63.3±10.7	0.85
Men	789 (63.4)	1113 (62.7)	0.67
Race–ethnicity			
White	531 (42.7)	1007 (56.7)	<0.01
Hispanic	505 (40.6)	411 (23.1)	
Black	191 (15.4)	301 (16.9)	
Other/multiple	17 (1.4)	57 (3.2)	
Region			
North America	679 (54.6)	1281 (72.1)	<0.01
Latin America	428 (34.4)	266 (15.0)	
Spain	137 (11.0)	229 (12.9)	
Medical history			
Previous symptomatic lacune	140 (11.3)	168 (9.5)	0.11
Subcortical TIA	60 (4.8)	105 (5.9)	0.20
Hypertension (SPS3 criteria)	1119 (90.0)	1590 (89.5)	0.71
Diabetes mellitus	447 (35.9)	659 (37.1)	0.51
Hyperlipidemia	576 (46.3)	895 (50.4)	0.03
Ischemic heart disease	86 (6.9)	139 (7.8)	0.35
Congestive heart failure	5 (0.4)	9 (0.5)	0.68
Intermittent claudication	41 (3.3)	54 (3.0)	0.69
Current smoking	223 (17.9)	394 (22.2)	<0.01
Qualifying stroke at entry			
Rankin score	1.3±0.8	1.3±0.8	0.92
Days between qualifying stroke and randomization	73.3±49.0	78.6±45.6	<0.01
Types of lacunar syndrome			
Pure motor hemiparesis	421 (33.9)	574 (32.3)	0.17
Pure sensory stroke	118 (9.5)	185 (10.4)	
Sensorimotor	403 (32.4)	533 (30.0)	
Other	301 (24.2)	483 (27.2)	
Use of aspirin at the time of qualifying event	314 (25.5)	524 (29.8)	<0.01
Use of statin at baseline	840 (67.5)	1241 (69.9)	0.17
Use of statin at any time during follow-up	1033 (83.0)	1488 (83.8)	0.59

Numbers represent N (%) or mean ± standard deviation. TIA indicates transient ischemic attack.

provide indirect evidence that there may be a threshold effect at ≈1 mg/L, above which the risk is increased, rather than a continuous relationship between hsCRP and subsequent risk after stroke. This result is also confirmed by our findings of a significantly increased risk for hsCRP levels above the clinically recommended high-risk threshold of 3 mg/L, as well as the absence of an effect for the continuous measure of hsCRP.

CRP is an acute phase reactant, a member of the pentraxin family of proteins, and part of the innate, nonspecific immune response. It is produced in the liver on stimulation by interleukin-6. The high-sensitivity assay for CRP has several features that make it ideal both as an epidemiological tool and for clinical purposes, including stability after freeze-thaw cycles, limited diurnal and seasonal variability, and ability to be measured in the nonfasting state.[1,2] Several studies have demonstrated its utility as a measure of future risk of atherosclerotic coronary artery disease, and these findings have been interpreted as a sign that inflammation may be an important contributor to future risk of cardiovascular disease. The fact that even relatively minimal elevations, within the range of hsCRP traditionally considered to be normal (ie, <10 mg/L), have been interpreted to mean that even minor inflammation, perhaps related to or because of low-grade inflammation within atherosclerosis throughout the arterial tree, contributes to the risk of cardiovascular events.[1] Our findings are consistent with this hypothesis; although because of its observational design and absence of systematic measures of atherosclerosis, our study cannot shed light on the source of hsCRP.

Relatively few studies have examined hsCRP as a risk factor for ischemic stroke, and the majority of these have focused

Table 2. Distribution of High-Sensitivity C-Reactive Protein (hsCRP) Levels by Baseline Characteristics

		hsCRP by Quartile, N (%)					hsCRP (Standard Clinical Thresholds), N (%)			
Characteristics	Overall Median (IQR)	Quartile 1, <0.93 mg/L (n=312)	Quartile 2, 0.93–2.16 mg/L (n=310)	Quartile 3, 2.16–4.86 mg/L (n=312)	Quartile 4, ≥4.86 mg/L (n=310)	*P* Value	<1 mg/L (n=332)	1–3 mg/L (n=417)	>3 mg/L (n=495)	*P* Value
Age, y										
<50	2.2 (0.9–5.2)	32 (25.4)	31 (24.6)	29 (23.0)	34 (27.0)	0.02	33 (26.2)	44 (34.9)	49 (38.9)	0.05
50–65	2.4 (1.0–5.9)	146 (24.5)	131 (22.0)	142 (23.8)	177 (29.7)		153 (25.7)	178 (29.9)	265 (44.5)	
>65–75	2.0 (0.9–4.0)	77 (24.0)	89 (27.7)	95 (29.6)	60 (18.7)		85 (26.5)	119 (37.1)	117 (36.4)	
>75	1.7 (0.8–3.9)	57 (28.4)	59 (29.4)	46 (22.9)	39 (19.4)		61 (30.3)	76 (37.8)	64 (31.8)	
Sex										
Men	2.0 (0.9–4.6)	216 (27.4)	202 (25.6)	189 (24.0)	182 (23.1)	<0.01	230 (29.2)	264 (33.5)	295 (37.4)	<0.01
Women	2.4 (1.1–5.7)	96 (21.1)	108 (23.7)	123 (27.0)	128 (28.1)		102 (22.4)	153 (33.6)	200 (44.0)	
Race										
Hispanic	2.1 (0.9–4.6)	127 (25.1)	128 (25.3)	129 (25.5)	121 (24.0)	0.18	136 (26.9)	173 (34.3)	196 (38.8)	0.08
Non-Hispanic white	2.1 (0.9–4.8)	145 (27.3)	126 (23.7)	134 (25.2)	126 (23.7)		155 (29.2)	172 (32.4)	204 (38.4)	
Black	2.7 (1.1–5.8)	38 (19.9)	48 (25.1)	46 (24.1)	59 (30.9)		38 (19.9)	64 (33.5)	89 (46.6)	
Other/multiple	2.1 (1.3–4.5)	2 (11.8)	8 (47.1)	3 (17.6)	4 (23.5)		3 (17.6)	8 (47.1)	6 (35.3)	
Region										
United States	2.2 (0.9–5.7)	157 (25.0)	148 (23.6)	146 (23.3)	176 (28.1)	0.26	163 (26.0)	197 (31.4)	267 (42.6)	0.26
Canada	1.6 (0.8–4.0)	16 (30.8)	15 (28.8)	12 (23.1)	9 (17.3)		18 (34.6)	17 (32.7)	17 (32.7)	
Latin America	2.1 (0.9–4.6)	107 (25.0)	108 (25.2)	111 (25.9)	102 (23.8)		115 (26.9)	147 (34.3)	166 (38.8)	
Spain	2.1 (1.0–3.8)	32 (23.4)	39 (28.5)	43 (31.4)	23 (16.8)		36 (26.3)	56 (40.9)	45 (32.8)	
Hypertension										
Yes	2.2 (0.9–5.0)	275 (24.6)	280 (25.0)	279 (24.9)	285 (25.5)	0.17	295 (26.4)	374 (33.4)	450 (40.2)	0.33
No	2.1 (0.8–4.1)	37 (29.6)	30 (24.0)	33 (26.4)	25 (20.0)		37 (29.6)	43 (34.4)	45 (36.0)	
Smoking										
Never	2.0 (0.9–4.3)	133 (25.5)	140 (26.9)	139 (26.7)	109 (20.9)	<0.01	141 (27.1)	190 (36.5)	190 (36.5)	<0.01
Former	2.0 (0.9–4.6)	134 (26.8)	127 (25.4)	118 (23.6)	121 (24.2)		144 (28.8)	164 (32.8)	192 (38.4)	
Current	3.1 (1.2–7.0)	45 (20.2)	43 (19.3)	55 (24.7)	80 (35.9)		47 (21.1)	63 (28.3)	113 (50.7)	
Ischemic disease										
Yes	2.1 (1.1–5.7)	17 (19.8)	27 (31.4)	20 (23.3)	22 (25.6)	0.68	19 (22.1)	32 (37.2)	35 (40.7)	0.51
No	2.2 (0.9–4.9)	295 (25.5)	283 (24.4)	292 (25.2)	288 (24.9)		313 (27.0)	385 (33.2)	460 (39.7)	
Diabetes mellitus										
Yes	2.2 (0.9–5.0)	110 (24.6)	112 (25.1)	109 (24.4)	116 (26.0)	0.66	114 (25.5)	151 (33.8)	182 (40.7)	0.49
No	2.1 (0.9–4.8)	202 (25.3)	198 (24.8)	203 (25.5)	194 (24.3)		218 (27.4)	266 (33.4)	313 (39.3)	
Laboratories										
LDL	…	108.3±40.3	112.7±39.2	112.0±39.1	116.3±39.4	0.10	109.0±40.0	111.7±40.0	115.1±38.7	0.10
HDL	…	46.3±19.2	45.1±13.1	45.8±20.9	41.8±14.5	<0.01	46.2±18.7	45.2±15.4	43.3±17.7	0.05
BMI	…	28.1±11.9	28.2±5.0	29.3±6.1	30.3±6.5	<0.01	28.1±11.6	28.3±5.0	30.2±6.6	<0.01

BMI indicates body mass index; HDL; high-density lipoprotein; IQR, interquartile range; and LDL, low-density lipoprotein.

on first stroke.[2,34–38] In the prospective Northern Manhattan Study (NOMAS), hsCRP predicted MI and death, but not first ischemic stroke, after adjusting for potential confounders.[38] In a large individual participant meta-analysis of 54 prospective cohort studies (n=160 309), the risk ratio of ischemic stroke per SD increase in $\log_e$CRP was 1.44 (95% CI, 1.32–1.57) after adjusting for age and sex, but was attenuated to 1.27 (95% CI, 1.15–1.40) when further adjusting for other risk factors.[39] Nonvascular mortality, including cancer, was increased significantly and by a greater magnitude in these analyses (adjusted risk ratio, 1.54; 95% CI, 1.40–1.68), providing some evidence that elevated CRP may more generally be a marker of illness rather than of vascular disease risk alone.

Until the present study, most studies of hsCRP as a risk marker after stroke included small selected samples, often from single centers, and with limited duration of follow-up; most also focused on mortality alone or on combined vascular events, and they incompletely adjusted for other predictors of outcome. In 1 study, hsCRP levels ≥10.1 mg/L measured <72 hours of stroke predicted increased mortality >4 years.[31] In another study, patients in the highest quintile of hsCRP levels assessed ≥3 months after first ischemic stroke or transient

Table 3. Risk of Recurrent Ischemic Stroke by Levels of High-Sensitivity C-Reactive Protein (hsCRP) Before and After Adjusting for Potential Confounders

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
hsCRP continuous (per SD)	1.09 (0.93–1.28)	0.27	1.11 (0.94–1.32)	0.21	1.11 (0.93–1.32)	0.25	1.12 (0.93–1.34)	0.22
hsCRP								
Quartile 1 (referent)	…	0.05	…	0.04	…	0.09	…	0.11
Quartile 2	1.94 (0.96–3.95)		2.03 (0.99–4.13)		2.08 (1.02–4.26)		2.07 (1.01–4.23)	
Quartile 3	1.66 (0.81–3.40)		1.85 (0.90–3.81)		1.91 (0.92–3.96)		1.95 (0.94–4.05)	
Quartile 4	2.54 (1.30–4.96)		2.68 (1.36–5.26)		2.46 (1.22–4.95)		2.32 (1.15–4.68)	
hsCRP (standard clinical thresholds)								
<1 mg/L (referent)	…	0.04	…	0.03	…	0.05	…	0.06
1–3 mg/L	1.75 (0.90–3.38)		1.83 (0.94–3.55)		1.82 (0.94–3.56)		1.82 (0.94–3.55)	
>3 mg/L	2.20 (1.19–4.10)		2.36 (1.26–4.42)		2.22 (1.17–4.24)		2.16 (1.13–4.11)	
hsCRP								
≤15 mg/L (referent)	…	0.55	…	0.62	…	0.61	…	0.65
>15 mg/L	1.29 (0.56–2.95)		1.24 (0.54–2.85)		1.24 (0.54–2.88)		1.22 (0.52–2.83)	

Model 1, unadjusted; model 2, adjusted for demographics (age, sex, race, and region); model 3, adjusted for demographics (age, sex, race, and region) and comorbidities (hypertension, smoking, history of ischemic stroke, diabetes mellitus, body mass index, and low-density and high-density lipoproteins); and model 4, adjusted for demographics (age, sex, race, and region), comorbidities (hypertension, smoking, history of ischemic stroke, diabetes mellitus, body mass index, and low-density and high-density lipoprotein), and statin use at baseline. CI indicates confidence interval; and HR, hazard ratio.

ischemic attack attributable to large vessel stenosis had a significantly increased risk of subsequent stroke or MI.[40] In a population-based follow-up study after first ischemic stroke of all etiologic subtypes in which several outcomes were assessed, hsCRP measured predominantly within the first 72 hours after stroke was associated with increased risk of mortality during the next 5 years after adjusting for confounding factors.[41] There was no increased risk of recurrent stroke alone, nor of the combined outcome of stroke and other vascular events. hsCRP in that study, moreover, was significantly associated with stroke severity, a major predictor of poststroke mortality.

There have been few multicenter studies addressing hsCRP after stroke. In a secondary nested case–control analysis of stored blood specimens from the Perindopril Protection Against Recurrent Stroke Study (PROGRESS), a multicenter secondary stroke prevention trial, those in the highest tertile of CRP had

Table 4. Risk of Recurrent Ischemic Stroke or Cerebral Hemorrhage by Levels of High-Sensitivity C-Reactive Protein (hsCRP) Before and After Adjusting for Potential Confounders

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
hsCRP continuous (per SD)	1.06 (0.90–1.25)	0.51	1.07 (0.91–1.27)	0.40	1.07 (0.90–1.27)	0.44	1.08 (0.90–1.28)	0.41
hsCRP								
Quartile 1 (referent)	…	0.06	…	0.05	…	0.07	…	0.06
Quartile 2	1.94 (1.05–3.59)		1.93 (1.04–3.59)		1.99 (1.07–3.71)		1.99 (1.07–3.71)	
Quartile 3	1.37 (0.72–2.60)		1.46 (0.76–2.79)		1.52 (0.79–2.92)		1.55 (0.80–2.98)	
Quartile 4	2.09 (1.15–3.80)		2.25 (1.23–4.11)		2.17 (1.16–4.04)		2.08 (1.11–3.89)	
hsCRP (standard clinical thresholds)								
<1 mg/L (referent)	…	0.13	…	0.08	…	0.11	…	0.12
1–3 mg/L	1.58 (0.89–2.80)		1.60 (0.90–2.84)		1.61 (0.91–2.87)		1.62 (0.91–2.88)	
>3 mg/L	1.74 (1.01–2.99)		1.87 (1.08–3.23)		1.81 (1.03–3.19)		1.78 (1.01–3.13)	
hsCRP								
≤15 mg/L (referent)	…	0.89	…	0.92	…	0.86	…	0.90
>15 mg/L	1.06 (0.47–2.43)		1.05 (0.46–2.40)		1.08 (0.47–2.48)		1.06 (0.46–2.44)	

Model 1, unadjusted; model 2, adjusted for demographics (age, sex, race, and region); model 3, adjusted for demographics (age, sex, race, and region) and comorbidities (hypertension, smoking, history of ischemic stroke, diabetes mellitus, body mass index, and low-density and high-density lipoproteins); and model 4, adjusted for demographics (age, sex, race, and region), comorbidities (hypertension, smoking, history of ischemic stroke, diabetes mellitus, body mass index, and low-density and high-density lipoprotein), and statin use at baseline. CI indicates confidence interval; and HR, hazard ratio.

Table 5. Risk of Major Vascular Events (Stroke, Myocardial Infarction, Vascular Death) by Levels of High-Sensitivity C-Reactive Protein (hsCRP) Before and After Adjusting for Potential Confounders

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
hsCRP continuous (per SD)	1.07 (0.92–1.24)	0.39	1.08 (0.93–1.25)	0.34	1.08 (0.92–1.26)	0.36	1.08 (0.92–1.27)	0.34
hsCRP								
Quartile 1 (referent)	…	0.05	…	0.04	…	0.07	…	0.09
Quartile 2	1.91 (1.07–3.41)		1.92 (1.07–3.44)		1.95 (1.08–3.50)		1.95 (1.08–3.50)	
Quartile 3	1.53 (0.85–2.77)		1.63 (0.90–2.97)		1.60 (0.87–2.94)		1.63 (0.89–2.99)	
Quartile 4	2.14 (1.22–3.75)		2.24 (1.27–3.94)		2.12 (1.18–3.81)		2.04 (1.14–3.67)	
hsCRP (standard cut-offs)								
<1 mg/L (referent)	…	0.08	…	0.05	…	0.11	…	0.12
1–3 mg/L	1.50 (0.88–2.56)		1.52 (0.89–2.60)		1.52 (0.89–2.60)		1.53 (0.89–2.61)	
>3 mg/L	1.77 (1.07–2.93)		1.87 (1.13–3.10)		1.75 (1.04–2.94)		1.72 (1.02–2.90)	
hsCRP								
≤15 mg/L (referent)	…	0.34	…	0.38	…	0.35	…	0.37
>15 mg/L	1.40 (0.71–2.76)		1.36 (0.68–2.69)		1.40 (0.69–2.72)		1.37 (0.69–2.74)	

Model 1, unadjusted; model 2, adjusted for demographics (age, sex, race, and region); model 3, adjusted for demographics (age, sex, race, and region) and comorbidities (hypertension, smoking, history of ischemic stroke, diabetes mellitus, body mass index, and low-density and high-density lipoproteins); and model 4, adjusted for demographics (age, sex, race, and region), comorbidities (hypertension, smoking, history of ischemic stroke, diabetes mellitus, body mass index, and low-density and high-density lipoprotein), and statin use at baseline. CI indicates confidence interval; and HR, hazard ratio.

a modestly increased risk of recurrent ischemic stroke (odds ratio, 1.39; 95% CI, 1.05–1.85).[42] Results were not, however, fully adjusted for all risk factors, including diabetes mellitus.

The present study differs from these other studies in including only patients with lacunar stroke and in collecting hsCRP measurements at a much longer time interval after stroke (median, 60 days), at a time when they are less likely to be merely reflective of stroke severity. Studies such as NOMAS with access to samples both before and after stroke have shown that hsCRP is elevated acutely after stroke.[43] Taken together, these data may be interpreted to indicate that among patients with smaller infarcts, in whom the levels of hsCRP are not as confounded by stroke severity and in whom hsCRP is measured after the acute phase reaction to stroke has diminished, hsCRP has a prognostic value for recurrent vascular events, including stroke.

The treatment implications of elevations in hsCRP at the time of stroke remain uncertain. In particular, hsCRP levels did not predict a response to dual antiplatelet therapy, although 1 prespecified hypothesis of the study was that dual antiplatelet therapy would be of greater benefit in those who had elevations in hsCRP. Recent results from a large primary prevention trial using rosuvastatin provide indirect evidence of the utility of measuring hsCRP as a determinant of whether to initiate statin therapy in otherwise low-risk patients.[44] However, the relevance of this finding to patients with stroke is unclear. In the present study, the effect of hsCRP on the risk of recurrent stroke and other events persisted after adjusting for statin use and was not influenced by statin use at baseline. Statin use was not randomized, however, and was left to the discretion of the treating physician, although the majority of patients were treated with a statin. Future studies will be needed to determine whether other specific therapies should be used in stroke patients with elevated hsCRP. The present study provides some evidence, however, that hsCRP may be useful as a prognostic marker for potential risk stratification in future trials.

Our study has several strengths. One major strength of LIMITS is its international multicenter design with a central laboratory, thereby minimizing interlaboratory error. We also had a race-ethnically diverse population, including a significant proportion of Hispanics who have been traditionally understudied in stroke trials.[45] In addition, we were able to assess the role of hsCRP in relation to a specific stroke subtype using a well-defined population of MRI-proven lacunar strokes, thereby limiting some confounding because of stroke size, cause, and severity. Third, by nesting LIMITS within a secondary prevention trial, we were able to address interactions of treatment effect and hsCRP level. Future analyses will allow us to determine the effect of both antihypertensive and antiplatelet therapies on hsCRP levels, as well as the association of hsCRP with other outcomes, including cognition, collected in SPS3.

Our study had some limitations as well. The nesting of LIMITS within SPS3 may have led to selection bias because of the inclusion and exclusion criteria inherent in a clinical trial. Because our study was nested, we also had limited data about chronic inflammatory diseases or clinical infections. However, the proportion of patients with clinically apparent conditions is likely to be low. Rates of infection at the time of stroke range from 6% to 25%, depending upon the population studied and methods used to detect infection.[46–49] The likelihood of infection at the time of stroke is related to stroke severity.[50] Also, higher rates of infection were seen in population-based studies than in secondary analyses of clinical trials, probably because patients in clinical trials tend to have fewer comorbidities. Patients with permanently disabling strokes were excluded from SPS3, and most patients were likely to be enrolled at a time after acute infections were likely to have resolved.

In conclusion, LIMITS provides evidence that hsCRP levels measured in the subacute phase after recent lacunar stroke predict the risk of recurrent stroke and other vascular events, although they do not predict the response to dual antiplatelet therapy. Future studies of this cohort may elucidate whether hsCRP predicts cognitive change after lacunar stroke, and whether hsCRP may be used to stratify patients in future clinical trials.

Appendix

The LIMITS Investigators

Clinical Sites in Order of Number of Participants (n) Enrolled

United States, 31 sites (n=639)

University of Kentucky, Lexington, KY—Creed Pettigrew, MD; Anand Vaishnav, MD; Peter Sawaya, MD; Anna Fowler, RN; Nedda Hughes, PA; Johnya Rice, RN; Kathy Vanderpool, RN (51); University of California San Diego, San Diego, CA—Brett Meyer, MD; Christy Jackson, MD; Paul Gamble, MD; Nancy Kelly, RN; Janet Warner, RN; Jo Bell, RN (47); Rochester Stroke Center, Rochester, MN—Irene Meissner, MD; John Graves, MD; Deb Herzig, RN; Jody Covalt, RN (45); St Louis University, St Louis, MO—Salvador Cruz-Flores, MD; H. Douglas Walden, MD; Eve Holzemer, RN (44); University of Arizona, Tucson, AZ—Bruce Coull, MD; Lien Howard, MD; Mina Malekniazi, RN; Melissa VanSkiver; Denise Bruck; Stacey Redman, RN (42); Metrohealth Medical Center, Cleveland, OH—Joseph Hanna, MD; Thomas Zipp, MD; Scott Bailey, RN; Dana Cook, RN; Alice Liskay, RN; Dana Simcox, RN; Joan Kappler, RN (38); Mayo Clinic Scottsdale, Scottsdale, AZ—Bart Demaerschalk, MD; Michael Hogan, MD; Daniel Wochos, MD; Judith Wieser, RN; Barbara Cleary, RN; Lori Wood, RN (37); Henry Ford Hospital, Detroit, MI—Angelo Katramados, MD; Brian Silver, MD; Jerry Yee, MD; Krisy Aiello, RN; Kathleen Wilson, RN; Sharon McCarthy, RN (35); Boston University, Boston, MA—Carlos Kase, MD; Irene Gavras, MD; Helena Lau, RN; Matt Ogrodnik; Nancy Allen, RN (34); Berman Center for Clinical Research, Minneapolis, MN—David Anderson, MD; Richard Grimm, MD; Donna Brauer, RN (27); University of South Alabama, Mobile, AL—Dean Naritoku, MD; Richard Zweifler, MD; Michael Culpepper, MD; Mel Parnell, RN; Robin Yunker, RN; Kelly Boots, RN; Renay Drinkard, RN; Rachel Backlin (25); Columbia University Medical Center, New York, NY—Mitch Elkind, MD; Russell John Crew, MD; Jai Radhakrishan, MD; Tania E. Corporan, MD; Julisa Diaz, MD; Rebeca Aragon, BS (25); University of Texas Health Science Center at San Antonio, San Antonio, TX—Oscar Benavente, MD; Robert Hart, MD; Pablo Pergola, MD; Santiago Palacio, MD; Irma Castro; Arlene Farias; Ana Roldan, MD, MS (21); Cooper Health System Department of Medicine, Camden, NJ—Tom Mirsen, MD; Susan McAllister, MD; Arnaud Bastien, MD; Patricia Niblack, MLT (17); University Hospitals Case Medical Center, Cleveland, OH—Sophia Sundararajan, MD; Mahboob Rahman, MD; Tom Horvath; David Korosec, RN; Chris Murphy, RN (16); Oregon Health and Science University, Portland, OR—Helmi Lutsep, MD; Don Girard, MD; Kali Seisler; Megan Cingel; Megan Ross; Rachel Stone; Darren Larsen, RN; Ann Doherty, RN (15); Medical College of Wisconsin, Milwaukee, WI—Diane Book, MD; Sunu Eapen, MD; Clarence Grimm, MD; Barbara Blaney; Stephanie Rozman; Linda Gaertner, RN; Erin Bradenburg; Laura Loomis, RN; Jolene Monarch-Cotton, RN; Jean Ravavelli-Meyer, RN; Anna Golembieski, RN (15); University of Miami, Miller School, Miami, FL—José Romano, MD; Gustavo Ortiz, MD; Maria del Carmen Lichtenberger, MALS (15); University of Texas Southwestern, Dallas, TX—Mark Johnson, MD; Yinghui Liu, MD; Robert Goldsteen, MD; April Blair, MSW; Gregg Wright; Naomie Gathua, RN (14); Ruan Neurology Clinic and Research Center, Des Moines, IA—Michael Jacoby, MD; David Jones, MD; Jeffrey DeFrancisco, MD; Theresa Hamm, RN (14); University of Rochester Medical Center, Rochester, NY—Scott W. Burgin, MD; Joshua Hollander, MD; Walter Polashenski, MD; Patricia Wallace, RN; Cheryl Weber, RN (12); Helen Hayes Hospital, West Haverstraw, NY—Jason Greenberg, MD; Laura Lennihan, MD; Marjorie King, MD; Laura Tenteromano, RN (9); Research Foundation of SUNY, Buffalo, NY—Lorainne Pereira, MD; Marilou Ching, MD; Robert Sawyer, MD; Kathy Parkes, RN; Cheryl Conover (8); North General Hospital, New York, NY—Jesse Weinberger, MD; Lewis Wright, MD; Dorothy Burch, RN (8); University of Rochester, Rochester, NY—Curtis Benesch, MD; John Bisognano, MD; Ann Leonhardt, RN; Justine Zentner, RN; Molly Hildreth, LVN (6); Marshfield Clinic Department of Neurology, Marshfield, WI—Percy Karanjia, MD; Narayana Murali, MD; Richard Dart, MD; Kathleen Mancl, CCRP (5); Wake Forest University, Winston-Salem, NC—David Lefkowitz, MD; Levy Pavel, MD; Nancy Buchheimer; Sara Vaughn, BA; Emily Smith; Jean Satterfield, RN (4); University of Washington at St Louis, St Louis, MO—Renee Van Stavern, MD; Angela Brown, MD; Jannie Serna, RN; Jill Newgent, RN; Julie Naylor; Laura Carpenter (4); University of Alberta Stroke Research NACTRC, Edmonton, AB—Ashfaq Shuab, MD; Khurshid Khan, MD; Naeen Dean, MD; Frederika, Herbert, RN; Karen Kastelic, RN (3); Sutter Neuroscience Institute, Sacramento, CA—Richard Atkinson, MD; Roger Lieberman, MD; Teresa Carter, RN; Pat Zrelak, RN; Nola Kenney, RN (2); St John's Mercy Medical Center, St Louis, MO—William Logan, MD; David Carpenter, MD; Sally Schroer, RN (1)

Canada, 2 sites (n=52)

Charles LeMoyne Hospital, Greenfield Park, QC—Leo Berger, MD; Sylvain Brunet, MD; Johanne Pontbriand, RN; Martine Mainville; Denise Racicot, RN (29); McGill University Montreal General, Montreal, QC—Robert Côté, MD; Laurence Green, MD; Lisa Wadup, LVN; Anne-Marie Fontaine, RN (23)

Chile, 2 sites (n=90)

Hospital Clinico Universidad Católica de Chile, Santiago—Jorge Tapia, MD; Ivan Esteban Godoy, MD; Marcela Valdes, RN (46); Universidad Católica, Santiago, and Hospital Naval, Viña del Mar—Gonzalo Matamala, MD; Helmut Goecke, MD; Marcela Parra, RN; Jessica Pozo, RN (44)

Ecuador, 1 site (n=171)
Hospital-Clínica-Kennedy, Guayaquil—Oscar del Brutto, MD; Rocio Santibáñez, MD; Joffre Lara, MD; Mauricio Zambrano (171)

Mexico, 2 sites (n=18)
Hospital Civil/México, Guadalajara, Jalisco—José Luis Ruiz Sandoval, MD; Eduardo Salcido Vásquez, MD; Carmen Ruiz (12); Instituto Nacional de Neurología y Neurocirugía, México City—Antonio Arauz, MD; G. Amin Cervantes, MD; Adolfo Leyva, MD; Itzel Camacho, MD (6)

Peru, 1 site (n=151)
Hospital Sabogal, Lima, Perú—Edwin Javier Pretell, MD; José Valdivia, MD; Marissa Pretell (151)

Spain, 6 sites (n=137)
Hospital de Girona Dr Josep Trueta, Girona—Joaquín Serena Leal, MD; Mar Castellanos, MD; Verónica Cruz; Mercè Cepeda, RN (36); Hospital Del Sagrat Cor, Barcelona—Adrià Arboix, MD; Antoni Pelegrí, MD; Lorena Blanco (31); Hospital del Bellvitge, Barcelona—Francisco Rubio Borrego, MD; Francisco Gudiol, RN (28); Hospital Universitario German Trias i Pujol, Barcelona—Meritxell Gomis, MD; Juan Arenillas, MD; Antonio Dávalos, MD; Ana Suñol, RN; Silvia Reverté, RN (17); Hospital del Mar, Barcelona—Jaume Roquer, MD; Ana Oliveras Serrano, MD; Jordi Jiménez Conde, MD; Ana Rodríguez, MD; Gemma Romeral, RN (13); Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela—José Castillo Sánchez, MD; Miguel Blanco González, MD; Manuel Rodríguez, MD; Isabel Jiménez; Jaime Rodríguez, RN (12)

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