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Plasma Concentration of C-Reactive Protein and Risk of Ischemic Stroke and Transient Ischemic Attack

The Framingham Study

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Background—The role of C-reactive protein (CRP) as a novel plasma marker of atherothrombotic disease is currently under investigation. Previous studies have mostly related CRP to coronary heart disease, were often restricted to a case-control design, and failed to include pertinent risk factors to evaluate the joint and net effect of CRP on the outcome. We related plasma CRP levels to incidence of first ischemic stroke or transient ischemic attack (TIA) in the Framingham Study original cohort.

Methods—There were 591 men and 871 women free of stroke/TIA during their 1980 to 1982 clinic examinations, when their mean age was 69.7 years. CRP levels were measured by using an enzyme immunoassay on previously frozen serum samples. Analyses were based on sex-specific CRP quartiles. Risk ratios (RRs) were derived, and series of trend analyses were performed.

Results—During 12 to 14 years of follow-up, 196 ischemic strokes and TIAs occurred. Independent of age, men in the highest CRP quartile had 2 times the risk of ischemic stroke/TIA (RR=2.0, $P=0.027$), and women had almost 3 times the risk (RR=2.7, $P=0.0003$) compared with those in the lowest quartile. Assessment of the trend in risk across quartiles showed unadjusted risk increase for men (RR=1.347, $P=0.0025$) and women (RR=1.441, $P=0.0001$). After adjustment for smoking, total/HDL cholesterol, systolic blood pressure, and diabetes, the increase in risk across CRP quartiles remained statistically significant for both men ($P=0.0365$) and women ($P=0.0084$).

Conclusions—Independent of other cardiovascular risk factors, elevated plasma CRP levels significantly predict the risk of future ischemic stroke and TIA in the elderly. (*Stroke*. 2001;32:2575-2579.)

Key Words: atherosclerosis ■ C-reactive protein ■ inflammation ■ ischemic stroke ■ risk factors ■ TIA

An increasing body of evidence has linked inflammation with the pathogenesis of atherothrombotic stroke. Infections and inflammation may promote atherosclerosis and thrombosis by elevating serum levels of fibrinogen,¹ leukocytes,² clotting factors,³ and cytokines⁴ and by altering the metabolism and functions of endothelial cells and monocyte macrophages.⁵ Low-grade infections, reflected in elevated levels of various acute-phase proteins,⁶ may be partly responsible for the inflammatory processes observed in atherosclerotic lesions, which in turn may relate to the occurrence of ischemic symptoms.

C-reactive protein (CRP), an acute-phase reactant, is an indicator of underlying systemic inflammation and a novel plasma marker for atherothrombotic disease. The recent use of highly sensitive CRP assays, with international reference standards set by the World Health Organization (WHO),⁷ has

enhanced the usefulness of CRP as a reliable predictor of cardiovascular events. A strong and consistent association between clinical manifestations of atherothrombotic disease and baseline CRP levels has been described in epidemiological studies of patients with acute myocardial ischemia⁸ or myocardial infarction,^{9,10} stable and unstable angina pectoris,¹¹ and myocardial infarction or recurrent ischemia among those hospitalized with angina pectoris.^{12,13} Large prospective studies in apparently healthy subjects confirmed the prognostic relevance of CRP to (1) the risk of cardiovascular disease in men,¹⁴ women,^{15,16} and the elderly^{17,18}; (2) the risk of fatal coronary disease among smokers with multiple risk factors for atherosclerosis¹⁹; (3) the development of peripheral vascular disease²⁰; and (4) the risk of coronary heart disease (CHD) in a large cohort of initially healthy middle-aged men.²¹

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Most researchers have used a case-control design,^{8–13} have focused on selected subject populations,^{14–21} and have largely investigated men,^{8–14,20,21} with few studies of women.^{15–17} Furthermore, limited availability of other pertinent risk factors in some of these studies has not permitted determination of the joint and net effect of CRP levels on the outcome. Last, the research findings linking CRP to atherosclerotic cardiovascular disease have mostly focused on CHD^{8–11,13–17,19,21} or have used cardiovascular “events” (encompassing fatal CHD, nonfatal myocardial infarction or stroke, and coronary revascularization procedures) instead of stroke as the specific outcome of interest,^{15,16} and there have been only a few large-scale prospective epidemiological studies of stroke.¹⁴ To address the issue of baseline CRP levels and risk of subsequent stroke events, we measured the concentration of CRP in members of the Framingham Study original cohort who were free of stroke or transient ischemic attack (TIA) at the time of their 1980 to 1982 clinic examination and related the baseline plasma concentrations of CRP to incident first ischemic stroke or TIA in these subjects during a 12- to 14-year follow-up period.

Subjects and Methods

Subjects and Definition of Clinical Outcome

The Framingham Study was begun in 1948 to explore risk factors for and consequences of cardiovascular disease in a longitudinal community-based population sample. At entry, there were 5209 male and female participants who were aged 28 to 62 years. The subjects have been examined biennially with routine assessment of medical history, physical examination, blood tests, and 12-lead ECGs. The examination procedures were approved by the Institutional Review Board of Boston Medical Center, and all subjects gave informed consent. Study design, response rates, and completeness of follow-up have been reported elsewhere.²²

For the present study, we related CRP level at biennial examinations 16 and 17 (1980 to 1982) to ischemic stroke or TIA incidence during 12 to 14 years of follow-up at examination 23 (1994). Of the initial Framingham Study cohort, 2999 subjects were alive and stroke free on January 1, 1982. At the time of the clinic examinations from 1980 to 1982, nonfasting blood specimens were obtained from 591 men and 871 women of the original cohort who were free of stroke or TIA. This study population represented approximately 60% of living subjects who attended the clinic examination, and frozen specimens were available for almost all of these participants (an overall inclusion rate of 50% of the living subjects). Subjects were followed up over a 12- to 14-year period for the development of incident ischemic stroke, including atherothrombotic brain infarction, cerebral embolism, or TIA. Most subjects with stroke or

suspected stroke had been hospitalized in the only general hospital in Framingham, where they were evaluated by a Framingham Study neurologist within a few days (often within hours) after onset. The criteria for stroke were met by the presence of a neurological deficit of sudden or rapid onset that persisted for ≥ 24 hours, and the events were adjudicated by a panel of 2 neurologists. CT scans and MRI studies of the brain and arteries were available to confirm the diagnosis; since 1982, 91.5% have had at least 1 CT or MRI scan of the brain and arteries, and many have undergone >1 study.²³

Assessment of the Risk Factors

Baseline covariables assessed for the present analysis included plasma CRP, age, sex, systolic blood pressure, cigarette smoking, total and HDL cholesterol levels, and diabetes. Nonfasting blood specimens were obtained from all subjects at biennial examinations 16 and 17, and the serum aliquots were stored at -20°C until mid-1997. Plasma concentrations of CRP were measured by use of a standardized commercial biochemical assay (Hemagen Diagnostics). Total and HDL cholesterol levels were assessed from fresh nonfasting plasma samples by standard Lipid Research Clinics techniques.²⁴ Diabetes was defined as use of insulin preparations or oral hypoglycemic agents or any recorded blood glucose level of ≥ 11.1 mmol/L (≥ 200 mg/dL). Persons reporting cigarette smoking during the past year were considered smokers. Systolic blood pressure was recorded with patients in the sitting position after at least 5 minutes of rest. On the basis of 2 consecutive measurements, elevated systolic blood pressure of ≥ 140 mm Hg and/or diastolic blood pressure of ≥ 90 mm Hg was defined as hypertension.

Laboratory Procedures

Serum concentrations of CRP were measured with an ultrasensitive enzyme immunoassay by using monospecific polyclonal and monoclonal antibodies produced by immunization with highly purified CRP. Briefly, the IgG fraction of polyclonal goat anti-human CRP antiserum was immobilized on the inner surface of microwell plates. One hundred microliters (at 1:100 dilution) of each serum sample was introduced into the test wells. The titer plates were incubated for 30 minutes at room temperature and then washed 4 times with rinsing solution. Aliquots (100 μL) of secondary rabbit anti-human CRP antibody conjugated to horseradish peroxidase were added to each well and incubated for 30 minutes at room temperature. At the end of the incubation, the plates were rinsed 4 times with rinsing solution. Horseradish peroxidase activity was determined by the addition of 100 μL of the substrate 3,3',5,5'-tetramethylbenzidine, followed by incubation for 30 minutes at room temperature. The enzymatic reaction was stopped by transferring 50 μL of 1N H_2SO_4 . The optical density of the product was quantified in an enzyme immunoassay plate reader at a wavelength of 450 nm. A standard curve was generated by using known concentrations of human serum CRP as an internal control in each experiment. The concentration of CRP in the test samples was determined from the standard curve. To verify the accuracy of CRP results interpolated from the standard curve, the WHO standard preparation^{7,25} was serially diluted in the

TABLE 1. Subject Characteristics at Exams 16 and 17

	Men (N=591 [40.4%])	Women (N=871 [59.6%])
Age, y	69.1 \pm 6.7 (60–90)	70.2 \pm 7.1 (59–91)
Total cholesterol, mg/dL*	215.2 \pm 37 (105–379)	239.1 \pm 40.4 (123–395)
HDL cholesterol, mg/dL*	43.7 \pm 13.4 (20–134)	53.5 \pm 14.7 (20–112)
Systolic blood pressure, mm Hg	140.5 \pm 19.7 (90–220)	141.6 \pm 21.8 (86–225)
Smoking, n (% total)	125 (21.15)	207 (23.80)
Diabetes, n (% total)	71 (12.01)	68 (7.8)
CRP, $\mu\text{g}/\text{mL}$	5.4 \pm 7.3 (0–48.3)	6.2 \pm 8.4 (0–50.2)

Data are mean \pm SD (range), unless otherwise specified. Total number of subjects was 1462.

*To convert cholesterol values to mmol/L, multiply by 0.02586.

TABLE 2. Unadjusted Relative Risk of Incident Ischemic Stroke or TIA According to Plasma Concentration of CRP

Quartiles of CRP Concentration*	Men (n=82/591)				Women (n=114/871)			
	Range, $\mu\text{g/mL}$	RR	95% CI	<i>P</i>	Range, $\mu\text{g/mL}$	RR	95% CI	<i>P</i>
Q ₁	0–1.08	Referent	0–1.00	Referent
Q ₂	1.10–3.0	0.9	0.48–1.92	0.906	1.02–3.19	1.2	0.66–2.28	0.507
Q ₃	3.03–6.80	1.9	1.07–3.69	0.037	3.20–7.31	1.8	1.02–3.23	0.033
Q ₄	6.90–48.30	2.0	1.09–3.78	0.028	7.33–50.20	2.9	1.69–5.07	0.0001

*In calculations of RRs, the first quartile of CRP was used as referent, where RR=1.

working range of the kit. The response of the Hemagen CRP150 Kit was linear with a coefficient of determination (r^2) >0.99 and colinear with the standard curve of the assay. On split specimens, the correlation coefficient was 0.86.

Statistical Analyses

Analyses were performed separately for men and women by using sex-specific quartiles (Q₁ to Q₄) of CRP (men: Q₁=0 to 1.08, Q₂=1.10 to 3.0, Q₃=3.03 to 6.80, and Q₄=6.90 to 48.30 $\mu\text{g/mL}$; women: Q₁=0 to 1.00, Q₂=1.02 to 3.19, Q₃=3.20 to 7.31, and Q₄=7.33 to 50.20 $\mu\text{g/mL}$).

Unadjusted, bivariate (adjusted for age), and multivariate (adjusted for age, smoking, total and HDL cholesterol, systolic blood pressure, and diabetes) risk ratio (RR) estimates (with 95% CIs) for plasma CRP quartiles were generated by Cox proportional hazards modeling by using CRP quartiles as the independent variable. These RRs were derived by using the lowest quartile (Q₁) as the referent group.

For each of the unadjusted, bivariate, and multivariate Cox regression analyses, a trend analysis was performed to determine whether the risk of first ischemic stroke/TIA increased as the CRP quartile increased.

Results

Study participant characteristics were recorded at biennial examinations 16 and 17 (Table 1). The cohort (n=1462) was followed for up to 14 years. During this time, 196 (13.4%) first cerebrovascular events (ischemic stroke or TIA) occurred; 82 (13.9%) occurred in men, and 114 (13.1%) occurred in women.

Unadjusted relative risk of first ischemic stroke or TIA increased significantly with each increasing quartile of base-

line plasma CRP concentrations in both sexes (Table 2). Men with plasma CRP levels in the third quartile (≥ 3.03 $\mu\text{g/mL}$) had an unadjusted relative risk of first ischemic stroke/TIA almost 2 times greater than the relative risk for those in Q₁ (RR=1.9, $P=0.037$). A similar association was found for women in the third quartile of CRP (RR=1.8, $P=0.033$). Even greater relative risks were observed for men (RR=2.0, $P=0.028$) and women (RR=2.9, $P=0.0001$) in the highest sex-specific quartiles of CRP.

Adjustment for age in Table 3 did not attenuate the response, and the top 2 CRP quartiles were consistently associated with an increased risk of first ischemic stroke/TIA in men and women.

After multivariate adjustment, the Q₄ plasma CRP levels were related to a 1.6-fold increase in risk of first ischemic stroke/TIA in men, a result that was no longer statistically significant ($P=0.123$). However, for women in the highest CRP quartile, the relative risk of first ischemic stroke or TIA remained significantly increased after multivariate adjustment (RR=2.1, $P=0.008$; Table 3).

In the series of trend analyses, the unadjusted relative risk of first ischemic stroke/TIA for an increase in CRP from one quartile to the next higher quartile was 1.347 in men ($P=0.0025$) and 1.441 in women ($P=0.0001$). Statistical significance of this trend was not altered by the age adjustment (men: RR=1.346, $P=0.0027$; women: RR=1.411, $P=0.0002$). Multivariate adjustment did not attenuate the response in men (RR=1.248, $P=0.0365$) or women (RR=1.288, $P=0.0084$) (Table 4).

TABLE 3. Adjusted Relative Risk of First Ischemic Stroke or TIA According to Plasma Concentration of CRP

Adjustment*	Men			Women		
	RR	95% CI	<i>P</i>	RR	95% CI	<i>P</i>
C-reactive protein Q ₂						
Age	0.9	0.48–1.91	0.904	1.2	0.66–2.29	0.468
Multivariate	0.9	0.46–1.86	0.839	1.2	0.63–2.27	0.508
C-reactive protein Q ₃						
Age	1.9	1.04–3.61	0.04	1.8	1.03–3.26	0.03
Multivariate	1.5	0.80–2.87	0.259	1.6	0.89–2.93	0.087
C-reactive protein Q ₄						
Age	2.0	1.10–3.79	0.027	2.7	1.59–4.79	0.0003
Multivariate	1.6	0.87–3.13	0.123	2.1	1.19–3.83	0.008

*Multivariate adjustment was made for age, smoking, total and HDL cholesterol, systolic blood pressure, and diabetes.

TABLE 4. Trend in Risk of Ischemic Stroke/TIA Across CRP Quartiles in 1462 Framingham Study Participants

Data Category	Unadjusted			Age-Adjusted			Multivariate-Adjusted*		
	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P
Men (n=591)	1.347	1.105–1.642	0.0025	1.346	1.104–1.642	0.0027	1.248	1.012–1.539	0.0365
Women (n=871)	1.441	1.210–1.713	0.0001	1.411	1.188–1.677	0.0002	1.288	1.073–1.546	0.0084

*Multivariate adjustment was made for age, smoking, total and HDL cholesterol, systolic blood pressure, and diabetes.

Discussion

More than 700 000 strokes occur in the United States each year,²⁶ and it is paramount to identify new risk markers and preventive strategies for ischemic cerebrovascular disease.

Ischemic cerebrovascular disease accounts for a substantial proportion of all strokes.²⁷ Although the proximate cause of most brain infarcts is thrombus formation, atherosclerosis is the chief underlying cause.^{28,29} The lesions of atherosclerosis represent a series of specific cellular and molecular responses that include lipoprotein, hematologic, mechanical, and inflammatory components.²⁹ CRP, one of the acute-phase reactants, is an indicator of underlying systemic inflammation³⁰ and a novel plasma marker of atherothrombotic disease.^{31–33} It is likely that CRP has many pathophysiological roles in the inflammatory process, including binding of phosphocholine and recognition of foreign pathogens and phospholipid constituents of damaged cells.³⁰ A recent meta-analysis of several prospective studies of CRP and cardiovascular disease showed that the relative risk for CHD among individuals with CRP values in the top third compared with those in the bottom third was 1.7 (95% CI 1.4 to 2.1).³¹ However, few prospective epidemiological studies of stroke and markers of inflammation have been performed to date. Previous data on plasma CRP levels and ischemic stroke have suggested that this plasma marker is an independent predictor of the risk of future stroke.^{14,15} Men with the highest baseline CRP values had twice the risk of ischemic stroke,¹⁴ and women with the highest CRP levels had a 5-fold increase in risk of any vascular event and a 7-fold increase in risk of the combined outcome of myocardial infarction or stroke.¹⁵

Our prospective data from a large community-based cohort of men and women free of stroke and TIA demonstrate a strong relationship between plasma CRP and incidence of first ischemic stroke or TIA in both sexes. This relationship is linear and consistent with the CRP quartile gradation in both men and women. These data demonstrate a graded increase in the incidence of ischemic stroke and TIA with increased levels of CRP, even when the values were within the normal range. Furthermore, in trend analyses, shown in Table 4, this relationship persisted after adjusting for a number of potential confounders, including smoking, systolic blood pressure, total and HDL cholesterol, and diabetes. Elevated serum levels of CRP are not disease specific but are sensitive markers produced in response to tissue injury, infectious agents, immunologic stimuli, and inflammation. Cytokines such as interleukin-6, interleukin-1, and tumor necrosis factor- α are highly correlated with CRP levels and their function.³⁴

Inflammation is only one of multiple factors that can foster an increased risk of acute ischemic events. CRP levels are known to be greater in smokers,^{35,36} obese individuals (body mass index >130% of the ideal), individuals with abnormal fibrinolytic activity (plasmin-antiplasmin complex), and individuals with subclinical atherosclerosis.^{37,38} Overall, these data support the view that CRP, as a marker of low-level inflammation, predicts an increased risk of atherothrombotic events in otherwise healthy individuals. In addition, inflammation not only appears to be a response to the underlying atherosclerotic disease process but also may be an integral part of it.³⁶ This is consistent with the beneficial effects of anti-inflammatory agents, such as aspirin, in reducing the risk of cardiovascular events in men.¹⁴ The substantial reduction of risk of myocardial infarction in subjects with high baseline CRP levels who were treated with aspirin¹⁴ may suggest a beneficial anti-inflammatory effect of the drug that becomes detectable in low-risk patients. Unfortunately, we do not have adequate aspirin intake data in the present study to address this issue.

Our findings are based on the 1-time measurement of the plasma CRP levels, which may not completely and accurately reflect the status of the study participants over a prolonged follow-up period. However, this source of variability could not account for the relationship observed in the present study, because a random misclassification of such nature would tend to underestimate study findings and bias the results toward the null hypothesis. The nested prospective cohort design allows us to exclude the possibility that acute ischemia affected the levels of plasma CRP in the study participants. The data were obtained in an elderly cohort of men and women, and this may limit the applicability of the results to younger men and women.

We conclude that elevated plasma CRP levels significantly predict greater risk of ischemic stroke or TIA in elderly men and women. If the results of the present study are confirmed in other analyses of large population-based cohorts of men and women, the inclusion of CRP as a risk factor for ischemic stroke or TIA would have important implications. The addition of CRP levels in the highest quartile to the risk factor profile of the elderly may significantly increase the predictability of incident ischemic stroke or TIA. Thus, the use of CRP values may aid in identifying a potentially large number of men and women who are at risk for cerebrovascular events. This, in turn, may lead to the development of new treatment strategies for primary stroke prevention in those individuals identified as being at risk for developing cerebrovascular disease.

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