Elevated C-Reactive Protein Values and Atherosclerosis in Sudden Coronary Death
Association With Different Pathologies

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Background—Elevations in serum C-reactive protein measured by high-sensitivity assay (hs-CRP) have been associated with unstable coronary syndromes. There have been no autopsy studies correlating hs-CRP to fatal coronary artery disease.

Methods and Results—Postmortem sera from 302 autopsies of men and women without inflammatory conditions other than atherosclerosis were assayed for hs-CRP. There were 73 sudden deaths attributable to atherothrombi, 71 sudden coronary deaths with stable plaque, and 158 control cases (unnatural sudden deaths and noncardiac natural deaths without conditions known to elevate CRP). Atherothrombi were classified as plaque ruptures (n = 55) and plaque erosion (n = 18); plaque burden was estimated in each heart. Total cholesterol, high-density lipoprotein cholesterol, diabetes, smoking history, and body mass index were also determined. Immunohistochemical stains for CRP and numbers of thin cap atheromas per heart were quantitated in coronary deaths with hs-CRP in the highest and lowest quintiles. The median hs-CRP was 3.2 μg/mL in acute rupture, 2.9 μg/mL in plaque erosion, 2.5 μg/mL in stable plaque, and 1.4 μg/mL in controls. Mean log hs-CRP was higher in rupture (P < 0.0001), erosion (P = 0.005), and stable plaque (P = 0.0003) versus controls. By multivariate analysis, atherothrombi (P = 0.02), stable plaque (P = 0.003), and plaque burden (P = 0.03) were associated with log hs-CRP independent of age, sex, smoking, and body mass index. Mean staining intensity for CRP of macrophages and lipid core in plaques was significantly greater in cases with high hs-CRP than those with low CRP (P = 0.0001), as were mean numbers of thin cap atheromas (P < 0.0001).

Conclusions—hs-CRP is significantly elevated in patients dying suddenly with severe coronary artery disease, both with and without acute coronary thrombosis, and correlates with immunohistochemical staining intensity and numbers of thin cap atheroma. (Circulation. 2002;105:2019-2023.)

Key Words: death, sudden ▪ inflammation ▪ protein, C-reactive ▪ coronary disease ▪ thrombosis

C-reactive protein (CRP), a member of the pentraxin family of proteins, is an acute-phase reactant, increasing 1000-fold in response to infection, ischemia, trauma, burns, and inflammatory conditions.1 Ligand-bound or aggregated CRP binds C1q and in so doing activates the classical complement pathway.2 Although primarily synthesized in the liver, there is evidence for local expression of CRP in macrophages of the lung and the brain.3–5 In atherosclerotic plaques, it has been found associated with complement proteins and within foam cells.6,7

A growing number of studies suggest that CRP is an independent risk factor for atherosclerotic vascular disease. Plasma CRP concentrations in the highest quartile are associated, depending on the subject group, with 1.5- to 7-fold increases in relative risk of symptomatic atherosclerosis.1,9 The baseline plasma concentration of C-reactive protein predicts the risk of future myocardial infarction and stroke10 and is associated with a poor prognosis in unstable angina.11–13

Elevations of CRP in acute coronary syndromes highlight the importance of inflammation in atherosclerotic lesions. There have been no morphological studies, however, correlating serum CRP levels with histopathological findings. In sudden coronary death, the mechanism of death is diverse.

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There may be acute coronary thrombosis attributable to acute plaque rupture, which is considered to be caused by inflammatory infiltration of a thin fibrous cap,14 or acute thrombus with plaque erosion, in which the role of inflammation is less clear,15 or there may be no thrombosis. The purpose of this study was to compare the serum levels of CRP in these types of sudden death with controls and to correlate serum CRP findings with immunohistochemical localization of CRP within plaques and numbers of thin cap atheromas (vulnerable plaques).

Methods

Hearts were examined as previously described.16 Initial inclusion for study was any case of sudden unexpected death, natural or unnatural. For purposes of CRP category, these cases were classified on the basis of histological examination of internal viscerum in addition to full autopsy data into 2 groups. The first group (n=302) included cases without known predisposing cause for elevations of CRP other than atherosclerosis and formed the final study. These were healthy control hearts without evidence of significant coronary disease (n=158), stable coronary atherosclerosis (≥1 epicardial artery with ≥75% cross-sectional luminal narrowing in the absence of other causes of death or evidence of other processes known to elevate CRP, n=71), acute coronary thrombosis attributable to plaque rupture without myocardial necrosis (n=53), and coronary plaque erosion without myocardial necrosis (n=20). The second group (n=172), which was excluded for additional study, included cases with conditions known to elevate serum CRP other than atherosclerosis. These were 61 inflammatory conditions (15 bronchopneumonia, 5 infectious endocarditis, 5 sepsis, 4 pyelonephritis, 2 influenza, 1 staphylococcal mediastinitis, 7 hepatitis, 5 myocarditis, 5 pancreatitis, 4 sarcoidosis, 3 chronic prostatitis, 2 autoimmune disease, 2 colitis, and 1 inflammatory pseudotumor), 40 cases of congestive heart failure (left ventricular dilatation) in the presence of ischemia (healed infarct, n=15) or dilated cardiomyopathy (n=25), 33 cases of acute myocardial infarction with coronary disease, and 38 miscellaneous conditions (13 nonhepatitic liver disease, 11 trauma with survival >6 hours, 2 aortic dissection, 4 insulin-induced hypoglycemia, 4 deep venous thrombosis, 2 stroke, and 2 chronic dialysis). In each case, coronary arteries were serially sectioned at 3- to 4-mm intervals, and all areas of ≥50% cross-sectional luminal narrowing were studied histologically. Acute thrombi were classified as plaque rupture or erosion, as previously described.15,17 Hearts were weighed to the nearest gram after removal of intracavitary blood. Plaque burden was assessed in sudden coronary death and control cases and was calculated by adding the maximal percent cross-sectional area luminal narrowing in 4 arterial beds: left main, left anterior descending, left circumflex, and right coronary artery) were randomly selected from 10 hearts with plaque erosion, 10 hearts with plaque rupture, and 10 hearts without coronary thrombi. Selection was based on 5 cases each with hsCRP in the highest and lowest quintiles. Immunohistochemical staining was carried out using standard avidin-biotin techniques and a commercially available antisera for CRP (Sigma Corp) at a dilution of 1:200. Deparaffinized sections were incubated in 1 mmol/L EDTA buffer with steam heat before staining for antigen retrieval. Grading of staining intensity was assessed on macrophages and lipid core. A qualitative score of 0 to 4 was applied to each. Zero indicated no staining; 1+, <10% of macrophages staining or <10% of necrotic core area; 2+, 10% to 40% of macrophages staining or 10% to 40% of necrotic core area; 3+, 40% to 75% macrophages staining or 40% to 75% of necrotic core area; and 4+, >75% of necrotic core area or >75% macrophages staining. A sum of the 2 scores resulted in an overall grading system of 0 to 8.

Results

The characteristics of the cases in group 1 are summarized in Table 1. The percentage of cases with elevated hsCRP increased from controls to atherosclerosis without and with thrombus. By univariate analysis, log hsCRP was signifi-
cantly elevated in all groups versus controls. Although log hsCRP, percent of patients with elevated hsCRP, and median hsCRP were all higher in acute thrombi attributable to erosion or rupture compared with stable plaque, the differences between acute thrombosis and stable plaque were not statistically significant. Group 2 cases with conditions known to elevate hsCRP other than atherosclerosis showed significant elevations of hs-CRP that were greater than controls ($P<0.0001$). The median hsCRP was 9.6 $\mu$g/mL in patients with heart failure, 14.5 $\mu$g/mL in patients with acute myocardial infarction, and 27.3 $\mu$g/mL in inflammatory conditions and miscellaneous conditions known to elevate hsCRP.

Table 2 demonstrates characteristics of the groups, excluding cases of nonatherosclerotic causes for elevated hsCRP (group II). The mean age was significantly higher in cases with stable plaque compared with controls ($P=0.0001$), erosion, or rupture ($P=0.02$). Smoking frequency was elevated in those with thrombus attributable to rupture or erosion, total cholesterol was greatest in those with acute rupture, and body mass index was elevated in all atherosclerotic groups versus controls. In the 158 controls, age ($r^2=0.03$, $P=0.007$) and body mass index ($r^2=0.04$, $P=0.005$) were significantly associated with log hsCRP; log hsCRP was elevated in smokers ($0.26 \pm 0.33$) versus non-smokers ($0.16 \pm 0.30$, $P=0.03$). There were no significant associations between log hsCRP and sex ($0.20 \pm 0.32$, males; $0.18 \pm 0.29$, females; $P=0.8$), glycohemoglobin ($r^2=0.01$, $P=0.15$), total cholesterol ($r^2=0.01$, $P=0.23$), or HDL-cholesterol ($r^2=0.01$, $P=0.08$). There was a positive but statistically insignificant association between plaque burden and hs-CRP ($r^2=0.07$, $P<0.0001$). Table 3 demonstrates an independent association between log hsCRP and stable plaque ($P=0.03$), acute thrombus ($P=0.02$), and plaque burden ($P=0.03$), after adjusting for age, sex, smoking, and body mass index in the linear regression models.

The overall staining score in the high hs-CRP group was 6.2±0.6 and in the low hs-CRP group was 2.9±0.5 ($P=0.0001$). The mean immunohistochemical score for arterial plaques in hearts with erosions was 4.5±0.9; for ruptures, 4.4±0.8; and for stable plaques, 4.8±0.9. There was no difference in CRP staining intensity between the 3 groups ($P>0.5$). The Figure demonstrates immunolocalization of CRP in tissue sections with a necrotic core and within macrophages. The mean numbers of thin cap atheromas was

| TABLE 2. Risk Factor Data: Controls and Coronary Deaths |
|----------------------------------|----------------------------------|----------------------------------|
| Age, Years±SD | Men:Women | % Smokers | TC, $\mu$g/mL±SD | HDL-C, $\mu$g/mL±SD | BMI, ±SD | % Glycohemoglobin |
| Controls | 46±10 | 122:36 | 43 | 202±56 | 51±23 | 26±6 | 6.4±1.6 |
| Stable | 56:14 | 56:15 | 40 | 222±59 | 43±18 | 28±7 | 7.1±2.2 |
| Erosion | 48±10 | 14:6 | 78 | 228±48 | 34±12 | 27±5 | 7.3±2.4 |
| Rupture | 48±10 | 50:3 | 74 | 241±54 | 36±18 | 29±6 | 6.6±1.6 |

| TABLE 3. Relationship Between Log CRP and Stable Plaque, Acute Thrombus, and Plaque Burden: Multivariate, Controls, and Coronary Deaths |
|----------------------------------|----------------------------------|----------------------------------|
| Log CRP | 0.03 | 0.02 | 0.03 |
| Smoking | 0.90 | 0.0003 | 0.02 |
| Body mass index | 0.13 | 0.05 | 0.007 |
| Age | <0.0001 | 0.33 | <0.0001 |
| Male sex | 0.94 | 0.48 | 0.13 |

Immunolocalization of CRP in tissue section. A, Lower magnification of an epicardial coronary artery with near total occlusion demonstrates diffuse CRP staining of lipid core area (arrow). B, Higher magnification of this area shows CRP staining adjacent to cholesterol clefts. C, Localization in the cytoplasm of macrophages at the rim of the lipid core.
Discussion

A relationship between unstable angina and elevated serum CRP levels was shown more than 10 years ago. More recently, it has been established that elevated serum CRP is associated with a poor prognosis in unstable angina patients and that it may predict future risk for myocardial infarction or stroke in apparently healthy men. It has been suggested that increased serum CRP, which is synthesized primarily in the liver in response to interleukin-6 and other cytokines, may not only be a marker of low level inflammation but may directly enhance inflammation in plaques. This amplification may be mediated by its involvement in binding to complement C1q when ligand bound or in an aggregate state and in activated endothelial cells with upregulation of chemokines and adhesion molecules.

The present study demonstrates a modest elevation of serum hsCRP in autopsy samples of sudden coronary death, regardless of the apparent mechanism of death. There were significant elevations of serum hsCRP compared with controls in patients dying with acute coronary thrombi associated with plaque rupture or erosion as well as patients dying with stable plaque without evidence of thrombosis. The present study also demonstrated (as expected) marked elevations of serum hsCRP in patients with acute myocardial necrosis and inflammatory conditions. These data in patients with myocardial infarction and inflammatory conditions were presented to validate the postmortem method of hsCRP measurement as well as highlight the increase in serum hsCRP that results with myocardial necrosis, which accompanies underlying coronary artery disease with thrombosis.

There have been several immunohistochemical studies demonstrating CRP within atherosclerotic plaques, primarily localized to macrophages and extracellularly within the lipid core. The present study is the first to correlate serum levels with histologic staining for CRP in fatal lesions. There was a positive correlation between the intensity of CRP staining with serum levels independent of mechanism of death (rupture, erosion, and stable plaque). In addition, the study demonstrates a strong correlation between hsCRP levels and increased numbers of thin cap atheromas in the coronary tree. These results support the concept that hs-CRP in the serum reflects the numbers of atherosclerotic plaques with superficial foam cells and large necrotic cores and is correlated with local accumulation of CRP within the coronary lesions. The significance of this finding is unclear but suggests that the increased risk of patients with elevated serum hs-CRP in developing future coronary events lies in the increased numbers of plaque substrates (thin cap atheroma) prone to rupture.

The results of the present study suggest that hsCRP is a marker for coronary atherosclerosis, especially those lesions rich in lipid core and macrophages. Elevated serum levels of hsCRP were greatest in hearts harboring acute rupture and erosion. However, we were unable to demonstrate any significant increase or spike in serum hsCRP with lethal acute coronary thrombosis compared with patients dying with stable plaque. It is unclear whether the lack of significance is attributable to relatively small sample size or whether inflammatory mechanisms represent only a portion of the risk for developing fatal thrombosis. We have previously demonstrated that traditional risk factors such as smoking and hypercholesterolemia are independently associated with lethal coronary thrombi. Although hs-CRP is strongly related to the presence of thin cap atheromas, as the present study demonstrates, additional factors that may not be directly related to inflammation clearly play an important role in the subsequent development of occlusive fibrous platelet thrombi.

Clinical studies have suggested that although there is an association between elevations of hsCRP and acute coronary events, the link between plaque burden and hs-CRP is less clear. In the Cardiovascular Health Study and the Family Heart Study, there was little evidence of association between hsCRP and carotid artery wall thickness as assessed by ultrasound. In addition, imaging studies that measure correlates of plaque burden (ie, CT scans for calcification) failed to demonstrate a strong association between serum hsCRP and calcification. The present study demonstrates, however, that there is a significant association between coronary plaque burden and hs-CRP. The discrepancy among these results is unclear but likely related to the different methodologies used, because neither calcification nor carotid artery thickness directly measure coronary plaque composition. In addition, the correlation between plaque burden and hsCRP in the present study, although significant, was weak, with a low regression coefficient.

Most clinical studies have demonstrated an association between serum hsCRP and unstable coronary syndromes, independent of other risk factors. However, Doggen et al demonstrated in a case-control study that the effect of serum hsCRP on the risk of developing first acute myocardial infarction was greatly reduced if covariates of cholesterol, triglycerides, and high-density lipoprotein cholesterol were considered. In the present study, we found that the association of hsCRP with plaque burden and acute coronary thrombosis was lessened when covariates of glycohemoglobin and high-density lipoprotein were included in the analysis. This lessening is expected, because both hsCRP and glycohemoglobin are strongly associated with the metabolic syndrome and both hsCRP and HDL (negatively) are sensitive to inflammatory status. However, the effect of elevated hsCRP was significant to a similar degree to other measured risk factors, when risk for dying with stable plaque was considered. Therefore, despite relatively few cases, we observed a significant association of hsCRP with risk of dying from stable plaque, strengthening the role of hsCRP as a major risk factor for the development of clinical manifestations of coronary artery disease.

Limitations

Inherent limitations of the present study include the case-control nature of the autopsy study, the fact that controls included natural and unnatural deaths, and the fact that generalizations to other populations are impossible. In addition, methods of determining risk factors were limited to a single collection at postmortem, duration of risk factor...
exposure was generally not available, and information regarding risk factor modification (eg, aspirin, hormone replacement therapy, and statin use) was often incomplete.

Conclusion
This is the first autopsy study to corroborate the association between mild elevations of serum hs-CRP and coronary atherosclerosis. Specifically, elevated hs-CRP is associated with sudden death attributable to severe coronary disease, independent of the other risk factors most closely associated with hs-CRP, namely, age, smoking, and body mass index. The rise in hs-CRP was associated with thin cap atheromas and immunohistochemical deposition of CRP within plaques and, although higher in fatal thrombosis, was also elevated in patients dying with stable plaque. These results corroborate the concept that inflammation is an important component to plaque instability reflected by serum hs-CRP.

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References
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