C-Reactive Protein, Interleukin-6, and Fibrinogen as Predictors of Coronary Heart Disease

The PRIME Study

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Objective—This study was undertaken to examine the association of plasma inflammatory markers such as C-reactive protein (CRP), interleukin-6, and fibrinogen with the incidence of coronary heart disease within the prospective cohort study on myocardial infarction (PRIME study).

Methods and Results—Multiple risk factors were recorded at baseline in 9758 men aged 50 to 59 years who were free of coronary heart disease (CHD) on entry. Nested case-control comparisons were carried out on 317 participants who suffered myocardial infarction (MI)-coronary death (n=163) or angina (n=158) as an initial CHD event during a follow-up for 5 years. After adjustment for traditional risk factors, incident MI-coronary death, but not angina, was significantly associated with CRP, interleukin-6, and fibrinogen, but only interleukin-6 remained significantly associated with MI-coronary death when the 3 inflammatory markers were included in the model. The different interleukin-6 levels in Northern Ireland and France partly explained the difference in risk between these countries. Interleukin-6 appeared as a risk marker of MI-coronary death, and it improved the definition of CHD risk beyond LDL cholesterol.

Conclusions—This association may reflect the underlying inflammatory reaction located in the atherosclerotic plaque or a genetic susceptibility on the part of CHD subjects to answer a proinflammatory stimulus and subsequent increase in hepatic CRP gene expression. (Arterioscler Thromb Vasc Biol. 2003;23:1255-1261.)

Key Words: coronary heart disease ■ C-reactive protein ■ interleukin-6 ■ fibrinogen

Evidence now indicates that inflammation contributes considerably to the initiation and progression of atherosclerosis,1,2 and histopathological and immunochemical observations suggest that active inflammatory processes may trigger plaque rupture and enhance the risk of coronary thrombosis leading to a clinical ischemic event.3 Inflammation is characterized by a local reaction that may be followed by the activation of an acute phase reaction.4 Some systemic inflammatory markers can indicate the severity of inflammation, and their levels have actually been associated with coronary disease. Fibrinogen, which was previously recognized as an independent coronary heart disease (CHD) risk factor,5–7 is now considered an inflammatory marker and not only a coagulation component.8 Another acute phase reactant such as C-reactive protein (CRP) has also been demonstrated to be an independent cardiovascular risk factor in prospective population-based studies.8–14 However, fibrinogen and CRP are products of acute phase reaction,15 and because their concentrations may be subject to posttranscription regulation, they may not reflect all of the relevant effectors of inflammation.16

Interleukin 6 (IL-6) is the major initiator of acute phase response by hepatocytes and a primary determinant of hepatic CRP production.17,18 as suggested by IL-6-deficient animals showing impaired acute phase reaction.19 Experimental studies indicate that vascular endothelial and smooth muscle cells produce IL-620–26 and that IL-6 gene transcripts are expressed in human atherosclerotic lesions.22,27,28 Given the role of IL-6 in CRP regulation and the hypothesis that atherosclerosis fundamentally represents a chronic inflammatory disorder,1,2 the predictive value of IL-6 for cardiovascular ischemic events was evaluated in a prospective cohort study and IL-6 was associated with increased risk of future myocardial infarction (MI) in healthy middle-aged men.29 However, several of the participants in this study were taking the anti-inflammatory drug aspirin at the time of blood sampling.
a drug known to affect IL-6 levels\textsuperscript{30} and so inducing a potential bias.

The Prospective Epidemiological Study of Myocardial Infarction (PRIME) study is a cohort study set up to prospectively investigate the association of different risk factors and CHD simultaneously in France and Northern Ireland.\textsuperscript{31} Although several prospective cohort studies have evaluated the role of plasma inflammatory markers such as CRP, IL-6, and fibrinogen in predicting CHD risk, none has simultaneously analyzed these markers to determine the most predictive ones. Furthermore, their association has been evaluated with MI and coronary death incidence, but none used angina pectoris as an end point. In this work, we have studied the value of CRP, IL-6, and fibrinogen in predicting CHD risk in the PRIME prospective cohort according to the type of first clinical event during follow-up: MI-coronary death on the one hand, and angina on the other hand.

Methods

The PRIME study has been described in great detail.\textsuperscript{32} The PRIME study is a prospective cohort study that was set up to investigate risk factors of ischemic heart disease. From 1991 to 1994, 10,600 men aged 50 to 59 years living in France and Northern Ireland were included. On entry, nurses distributed questionnaires, made physical measurements, recorded ECGs, and analyzed them following the Minnesota code.

In the morning between 8 and 10 AM after a 12-hour fast, blood samples were taken and placed in tubes containing EDTA. Plasma was separated by centrifugation at 4°C within 15 minutes at each clinic. Aliquots of plasma were immediately frozen at −80°C for measurements of CRP, IL-6, and fibrinogen. These samples were sent weekly by air (with the exception of those from the Lille Center) to the Central Laboratory at the Pasteur Institute of Lille, where they were stored in liquid nitrogen until analysis. All samples were treated in exactly the same way (delay, temperature) whatever the center. Other analyses such as lipid measurements were carried out by usual methods as previously described.\textsuperscript{32,33} Additional questionnaires were posted or phoned to participants every year over 5 years (98.5% response). For subjects reporting a possible clinical event, clinical information was sought directly from the hospital or general practitioners’ files. All details of ECG, hospital admissions, enzymes, surgical operations, angioplasty, and treatment were collected and classified according to MONICA criteria.\textsuperscript{34} Death certificates were also used to complete information on the cause of death.

A medical committee was established to provide independent validation and classification of coronary events. CHD categories retained for analysis were nonfatal MI or coronary death and angina at the first event.\textsuperscript{35} The former category included subjects who had had at least 1 nonfatal MI or who died from CHD during follow-up. MI was defined by at least 1 of the following sets of conditions: (1) new diagnostic Q wave or another typical aspect of necrosis at ECG; (2) typical or atypical pain symptoms and new (or increased) ischemia at ECG and a myocardial enzyme level higher than twice the upper limit; or (3) postmortem evidence of recent MI or thrombosis. Definite coronary death was defined as death with a documented coronary event. When a coronary death was suspected with no other documentation or explanation, it was classified as possible coronary death. Sudden death was defined as death occurring within 1 hour after the onset of symptoms without explanation. However, when significant coronary atheroma was present at autopsy, death was considered as definite coronary death. The 3 death categories were grouped together as coronary deaths. Angina pectoris was defined by the presence of chest pain at rest or on exertion and 1 of the following criteria: (1) angiographic stenosis greater than 50%; (2) a positive scintigraphy (if no angiographic data); (3) positive exercise stress test (if no angiographic or scintigraphic data); or (4) ECG changes at rest (if no angiographic, scintigraphic, or exercise stress test data) but without any set of conditions for MI and no evidence of a noncoronary cause in the clinical history. Unstable angina was defined as a crescendo pain (change in frequency or severity of chest pain on exertion or appearance of chest pain at rest following preexisting pain on exertion) or chest pain at rest with either enzyme changes or ischemic ECG changes. In the absence of enzyme or ECG data, the diagnosis was rejected.

The number of subjects lost to follow-up, ie, those who could not be contacted in the fifth year of surveillance or who refused to participate any longer in the study at any time during the follow-up, was 228. As LDL cholesterol used in statistical analysis was calculated according to the Friedewald formula,\textsuperscript{36} subjects with triglycerides up to 400 mg/dL (n = 217) were excluded. Furthermore, only subjects who were without any history of CHD on entry were included in this study. Therefore, the number of subjects free of CHD on entry and not lost to follow-up was 9758, 7359 living in France and 2399 in Northern Ireland.

To evaluate CRP, IL-6, and fibrinogen as markers of coronary risk in the PRIME study, assays were made on baseline plasma samples of the 320 study participants who subsequently developed a coronary ischemic event during follow-up and from 2 controls per case. Matched controls were study participants recruited in the same center and on the same day (±2 days) as the corresponding case and were free of CHD at the date of the ischemic event of the case.

CRP was measured by immunonephelometry (Dade Behring), IL-6 by ELISA (R& D Systems) according to the instructions available from the supplier, and fibrinogen according to the method of Clauss.\textsuperscript{36} CRP, IL-6, and fibrinogen were measured in different central laboratories, at the University Hospital of Nantes, Pasteur Institute of Lille, and Laboratory of Hemostasis of La Timone Hospital in Marseille, France, respectively. Plasma samples were sent from the central plasma bank (Lille) to each laboratory in dry ice. For all 3 parameters, measurements were carried out as batch analyses. Accuracy and precision were assured by a strict internal quality control program using quality control from the supplier (CRP) or a single batch of normal plasma pooled from 50 healthy subjects. The coefficients of variation were 4.4%, 7.8%, and 4.3% for CRP, IL-6, and fibrinogen, respectively. Laboratory personnel was unaware of case or control status.

Statistical Analysis

All statistical analyses were carried out using the statistical SAS package (SAS Institute). Values of continuous variables are expressed as mean±SD, but the median value of triglycerides, CRP, IL-6, and fibrinogen are given because of their rightward skewed distribution. A conditional logistic regression analysis suitable for a nested case-control design was performed to identify discriminating predictive parameters. The same type of analysis was used to determine the relative risks of future CHD event after controlling for the presence of diabetes, hypertension, or smoking and possibly for LDL cholesterol, HDL cholesterol, and triglyceride levels. Relative risks related to IL-6 and LDL cholesterol distribution among controls were assessed using conditional logistic regression analysis after controlling for nonlipid risk markers. HDL cholesterol, and triglycerides. Correlations between continuous variables were calculated using Spearman’s rank correlation coefficients. All tests were considered significant at the 0.05 level.

Results

The characteristics and biological values of 317 cases and 609 controls included in the nested case-control study are presented in Table 1. Compared with their matched controls without CHD events, the subjects with incident CHD during the 5-year follow-up were of similar age. As expected, body-mass index (BMI), cholesterol, LDL cholesterol, and triglycerides were significantly higher in the case group, whereas HDL cholesterol was lower. Furthermore, the prevalence of smoking, hypertension, and diabetes was also higher in cases (Table 1). In these
univariate analyses, CRP, IL-6, and fibrinogen were significantly higher in cases than in controls.

Correlations between inflammatory markers and anthropometric or lipid parameters shown in Table 2 were calculated over the whole group of subjects because they were similar to those obtained separately in cases and controls. CRP and IL-6 were positively correlated with BMI and triglycerides and inversely with HDL cholesterol. No correlation was noted between inflammatory markers, as for instance the variance in the levels of these inflammatory marker levels with age and LDL cholesterol and inversely with HDL cholesterol. Thus, less than 5% of the variance in the levels of these inflammatory marker levels were positively correlated with BMI and triglycerides and those obtained separately in cases and controls. CRP and IL-6 were significantly differentially associated with MI-coronary death and angina events (Table 3). On the contrary, none of these parameters was significantly associated with angina pectoris. However, the comparison of $\beta$ regression coefficients computed in the 2 clinical categories showed that only IL-6 was differentially associated with MI-coronary death and angina events ($Z$ score $=2.21$, $P<0.05$).

Tertiles of CRP, IL-6, and fibrinogen were derived from the distribution of control subjects and used to model the risk of MI-coronary death using stratified conditional logistic regression after adjustment for nonlipid (diabetes, hypertension, smoking) and lipid (LDL-cholesterol, HDL-cholesterol, triglycerides) risk factors in each of the 2 clinical categories. CRP, IL-6, and fibrinogen were significantly associated with the appearance of future MI-coronary death events (Table 3). On the contrary, none of these parameters was significantly associated with angina pectoris. However, the comparison of $\beta$ regression coefficients computed in the 2 clinical categories for each parameter showed that only IL-6 was differentially associated with MI-coronary death and angina events ($Z$ score $=2.21$, $P<0.05$).

The Prime Medical Committee enabled us to divide cases into the 2 categories of MI and coronary death ($n=163$) and angina pectoris ($n=158$) according to the first occurrence of the disease. The sum of the numbers of the 2 clinical categories is higher than the total number of cases, because 4 cases had angina before MI and were included in both analyses, MI-coronary death, and angina. Medians of plasma levels for MI-coronary death and angina, respectively, were 2.00 and 1.92 mg/L for CRP, 1.65 and 1.29 pg/mL for IL-6, and 353 and 329 mg/dL for fibrinogen. These levels were separately compared with control subjects using conditional logistic regression after adjustment for nonlipid (diabetes, hypertension, smoking) and lipid (LDL-cholesterol, HDL-cholesterol, triglycerides) risk factors in each of the 2 clinical categories. CRP, IL-6, and fibrinogen were significantly associated with the appearance of future MI-coronary death events (Table 3). On the contrary, none of these parameters was significantly associated with angina pectoris. However, the comparison of $\beta$ regression coefficients computed in the 2 clinical categories showed that only IL-6 was differentially associated with MI-coronary death and angina events ($Z$ score $=2.21$, $P<0.05$).

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### TABLE 1. Baseline Patient Characteristics of Participants

<table>
<thead>
<tr>
<th>Metric</th>
<th>Cases (n=317)</th>
<th>Controls (n=609)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55.3±2.9</td>
<td>55.2±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3±3.6</td>
<td>26.7±3.5</td>
<td>0.008</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>234±39</td>
<td>223±41</td>
<td>0.0003</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>154±35</td>
<td>144±37</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>44±13</td>
<td>47±13</td>
<td>0.0002</td>
</tr>
<tr>
<td>ApoA1, mg/dL</td>
<td>141±23</td>
<td>147±24</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>137 (99 to 1.96)</td>
<td>125 (02 to 179)</td>
<td>0.01</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>56</td>
<td>41</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>27</td>
<td>15</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>10</td>
<td>6</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Mean±SD is presented for age, BMI, cholesterol, and LDL and HDL cholesterol; median (25th to 75th percentile) for triglycerides, CRP, IL-6, and fibrinogen. The comparison between cases and controls was performed by using conditional logistic regression analysis. NS indicates not significant ($P>0.05$).**

### TABLE 2. Spearman's Rank Correlation Coefficient Between CRP, IL-6 and Fibrinogen With Age, BMI and Lipid Parameters

<table>
<thead>
<tr>
<th>Metric</th>
<th>CRP</th>
<th>IL-6</th>
<th>Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.09†</td>
<td>0.08*</td>
<td>0.11‡</td>
</tr>
<tr>
<td>BMI</td>
<td>0.24‡</td>
<td>0.13‡</td>
<td>0.05</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.04</td>
<td>0.00</td>
<td>0.09‡</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.00</td>
<td>-0.03</td>
<td>0.08*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.22</td>
<td>-0.20</td>
<td>-0.15†</td>
</tr>
<tr>
<td>ApoA1</td>
<td>-0.14</td>
<td>-0.14</td>
<td>-0.14‡</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.13‡</td>
<td>0.17‡</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP</td>
<td>1.00</td>
<td>0.53‡</td>
<td>0.52‡</td>
</tr>
<tr>
<td>IL-6</td>
<td>...</td>
<td>1.00</td>
<td>0.39‡</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.01; ‡P<0.0001; otherwise not significant.

### TABLE 3. Univariate Conditional Logistic Regression of CHD Risk on Inflammatory Parameters

<table>
<thead>
<tr>
<th>Metric</th>
<th>MI-Coronary Death</th>
<th>Angina Pectoris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Logistic Regression Coefficient (SD)</td>
<td>Wald $\chi^2$</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>+0.050 (0.019)</td>
<td>6.63</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>+0.200 (0.059)</td>
<td>11.58</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>+0.00291 (0.00107)</td>
<td>4.51</td>
</tr>
</tbody>
</table>

**Subjects with MI or coronary death on the one side, angina pectoris on the other side were separately compared with controls.**
both models, IL-6 level seemed to be an independent risk factor for MI-coronary death, unlike CRP and fibrinogen (Table 5).

Because LDL cholesterol was a strong lipid risk marker for CHD and IL-6 seemed to be the most discriminating risk marker among the 3 inflammatory parameters that were analyzed in this study, we assessed their bivariate relationship to the risk of MI-coronary death. To do so, we divided the sample into 9 subgroups defined by the tertiles of the distribution level of controls, the limit values being 127 and 159 mg/dL and 0.93 and 1.58 pg/mL for LDL cholesterol and IL-6, respectively. As expected, the risk increased in each IL-6 tertile along with the increase in LDL cholesterol (Figure). Relative risk also increased with IL-6 in each LDL cholesterol tertile with the exception of the subgroup of subjects with the highest levels of IL-6 and LDL cholesterol, even if the calculated relative risk of this subgroup remained clearly higher than that of subjects with low LDL cholesterol and IL-6. The risk was particularly high (6- to 10-fold) in subjects with both high LDL cholesterol and IL-6 compared with subjects with low values for both.

The analysis of MI-coronary death risk according to IL-6 level after adjustment for nonlipid and lipid variables was performed separately on French and Northern Irish subjects. The respective β regression coefficients, 0.251 ± 0.089 (P=0.005) and 0.170 ± 0.081 (P=0.03), were not significantly different (Z score=0.36; P>0.05). The hazard ratio for MI or coronary death between Northern Irish and French men in their fifties was estimated at 1.79. Because the mean value of IL-6 is higher in Northern Irish controls than in French ones (2.06 ± 0.18 [SEM] pg/mL versus 1.58 ± 0.08 pg/mL), we might speculate that an IL-6 increase, if causal, might partly explain the between-country difference in coronary risk. Using β regression coefficient for IL-6 in the multivariate analysis of risk, we estimated that the adjusted hazard ratio attributable to higher IL-6 in Northern Ireland compared with France was 1.10 (95% CI, 1.04 to 1.38), which represents approximately 13% of the marginal excess relative risk between the 2 countries. LDL cholesterol levels were also different in controls in the 2 countries, 149 and 141 mg/dL in Northern Ireland and France, respectively. This difference explains approximately 10% of the marginal excess relative risk between the 2 countries.

### TABLE 4. Relative Risk (RR) of Future MI-Coronary Death Among Apparently Healthy Men Included in the Prime Study According to Tertiles of Baseline Plasma Concentrations of CRP, IL-6, and Fibrinogen

<table>
<thead>
<tr>
<th>Tertile</th>
<th>CRP, mg/L</th>
<th>RR (95% CI)</th>
<th>P for Linear Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;0.75</td>
<td>0.81 (0.47 to 1.40)</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>≥0.75 to &lt;1.97</td>
<td>2.16 (1.26 to 3.72)</td>
<td>0.005</td>
</tr>
<tr>
<td>3</td>
<td>≥1.97</td>
<td>0.009</td>
<td>NS</td>
</tr>
</tbody>
</table>

### TABLE 5. Multivariate Conditional Logistic Regression of MI-Coronary Risk on Inflammatory Parameters

<table>
<thead>
<tr>
<th>Logistic Regression Coefficient (SD)</th>
<th>Wald χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>+0.018(0.021)</td>
<td>0.8</td>
</tr>
<tr>
<td>IL-6</td>
<td>+0.165(0.066)</td>
<td>6.3</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>+0.043(0.130)</td>
<td>0.11</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>+0.011(0.022)</td>
<td>0.25</td>
</tr>
<tr>
<td>IL-6</td>
<td>+0.152(0.063)</td>
<td>5.87</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>+0.090(0.122)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

In model 1, CRP, IL-6, and fibrinogen were included after adjustment for nonlipid parameters (age, diabetes, smoking, high blood pressure). In model 2, the same nonlipid parameters as in model 1 were included plus LDL cholesterol, HDL cholesterol, and triglyceride levels.
Discussion

Prospective data from the PRIME population–based study reported in the present paper shows that in apparently healthy men, baseline plasma concentrations of CRP, IL-6, and fibrinogen are predictive of the risk of a first coronary heart ischemic event. However, these 3 risk markers are strongly correlated with each other, and IL-6 appears as the most discriminating marker. Moreover, IL-6 is associated with MI-coronary death but not with angina end points. Finally, IL-6 improves the prediction of CHD risk when this parameter is added to models already including CRP or LDL cholesterol.

Prospective data on IL-6 and CHD risk are limited. Ridker et al observed the same predictive value of IL-6 in the Physician’s Health Study as in the Prime Study, where IL-6 remained significantly associated with CHD risk after adjustment for CRP. These results also concord with the finding that IL-6 is a marker of mortality in the elderly. The present study is the first study devoted to examining the predictive value of such parameters in nontreated patients, whereas participants in the Physician’s Health Study were treated with aspirin which decreases IL-6 level.

IL-6 is secreted by macrophages and smooth muscle cells in the atherosclerotic lesion, and so the IL-6 plasma level could reflect the extent of inflammatory reactions in the atherosclerotic vessels. This could explain why IL-6 is a predictive factor of MI-coronary death but not of angina. Indeed, most coronary thromboses responsible for fatal and nonfatal MI result from a thrombus overlying the protective fibrous caps of the fissured plaque, with a now-recognized inflammatory phenomenon playing a decisive role. In subjects with MI-coronary death, there is a more intense process of lesions in transition from clinically stable to unstable atherosclerotic plaques, whereas the absence of elevated IL-6 in patients with angina corresponds to an anatomical aspect of severely stenotic plaques, which tend to be fibrotic and stable with low inflammatory components.

In both the present study and the Physician’s Health Study, a high IL-6 level was present in individuals several years before the occurrence of the ischemic event. Because plaque rupture is an acute phenomenon, it suggests that the inflammatory process at the origin of fissuration or rupture could appear a relatively short time before the acute clinical event. However, recent studies have recently shown that considerable time elapses between the onset of plaque rupture and the onset of MI. Indeed, most fissures reseal and incorporate thrombus at the same time but do not produce clinical symptoms. Therefore, these data suggest that subjects suffering MI-coronary death are likely to have an intensive and perhaps prolonged inflammatory reaction in the artery wall.

Besides its expression and secretion by arterial macrophages present in the atherosclerotic plaque, IL-6 is also known to be produced by adipose tissue. This observation explains the relationship between plasma IL-6 levels and anthropometric measurements such as BMI and markers associated with the insulin resistance syndrome. The higher adipose tissue mass in cases rather than in controls (as noted by their respective BMI [Table 1]) can partly explain the IL-6 increase in cases compared with controls, but the moderate difference in BMI disappears in multivariate analysis and cannot entirely explain IL-6 difference. Furthermore, BMI was similar in subjects with MI-coronary death and in those with angina, whereas IL-6 was higher in MI-coronary death than in angina cases. It can be hypothesized that IL-6 is expressed to a greater extent by cells in subjects with MI-coronary death than in subjects with angina, possibly because of a different gene-environment interaction or greater genetic susceptibility in CHD subjects to have a strong immunological activation in response to a proinflammatory stimulus. A retrospective case-control study on MI (ECTIM study) established an association between an IL-6 genetic polymorphism and MI, which concords with our hypothesis.

CRP has been measured in several prospective studies of fatal and nonfatal MI. A meta-analysis of 14 available prospective studies of CRP has given a combined risk ratio of 1.9 (95% CI, 1.5 to 2.3) in individuals in the top third compared with those in the bottom third of baseline measurements, a relative risk similar to that observed in the present study (1.92; 95% CI, 1.14 to 3.22; data not shown). As in several prospective studies, fibrinogen was a CHD factor independently of lipid and nonlipid risk factors. The difference between MI-coronary death and stable angina had already been observed for CRP and fibrinogen in one case-control study but not in another. This suggests that the chronic inflammatory component of atherosclerotic lesions might be less pronounced in subjects with angina and much more intense in subjects with plaques prone to instability and consequently likely to induce MI-coronary death.

There are potential limitations to our study. First, we cannot exclude the possibility that protein degradation appeared during storage and affected the results, even if plasma were stored at very low temperature (−196°C). However, inflammatory marker levels measured in the present study are similar to those reported in previous ones that used fresh plasma samples, and the analysis of longitudinal stability of several risk factors including CRP and fibrinogen in plasma kept at −70°C for 5 years has shown no sample degradation over time. Furthermore, even if protein degradation appeared in our study, this effect could not have led to any systematic bias, because samples from case and control subjects were handled identically throughout the procedure from blood drawing to analytical analysis. Also, first clinical events were as precisely documented as possible, but it was not possible to distinguish various case subgroups in the analysis because of low numbers. Subjects with stable and possible unstable angina were put together in the group “angina,” although the atherosclerotic process at the origin of each pathology could be different. Most subjects with angina suffered a first episode of stable angina (n=114), and no association of IL-6 with unstable angina (n=44) was statistically significant, although its mean value was intermediate between that of stable angina and MI-coronary death cases. A longer follow-up of the cohort would enable us to analyze more precisely the association of IL-6 level with the different clinical forms of CHD events.
The incidence of CHD was higher in Northern Ireland than in France. Predicted risk of CHD as estimated from logistic regression equations using classical risk factors could not explain the much higher level of CHD incidence experienced in Northern Ireland as opposed to France. We found that different levels of apolipoprotein (apo) A1 and LDL cholesterol between the 2 countries explain approximately 7% and 10% (present study) of relative coronary risk. Now the difference in IL-6 between the 2 populations seems to explain a higher proportion of relative risk (13%) than apoA1 and LDL cholesterol. However, if apoA1 and LDL interact with arterial cells and probably have a direct role in the atherosclerotic process, the causal role of IL-6 appears more hypothetical. Either it is only a marker of inflammation within the atherosclerotic lesion or it has a direct role in the pathogenesis of atherosclerosis through autocrine, paracrine, and endocrine mechanisms. Observational cohort studies cannot test these hypotheses, and more mechanistic experimental studies are needed to answer these questions.

In conclusion, levels of IL-6, CRP, and fibrinogen are associated with incident acute coronary events, but not angina among healthy men, independently of traditional risk factors for CHD. From a clinical perspective, it is important to recognize that the simultaneous measurement of lipids, particularly LDL cholesterol, and IL-6 improves the prediction of risk of future MI-coronary death compared with that associated with lipids or IL-6 alone. Because treatment such as statin decreases CRP, its anti-inflammatory properties could be additionally assessed by testing levels of IL-6 rather than of CRP. Finally, IL-6 could be used clinically as a CHD risk marker, especially as fully automated measurements of IL-6 are now available.

Appendix

The PRIME Study Group

The PRIME Study is organized under an agreement between INSERM and the Merck, Sharpe, and Dohme-Chibret Laboratory, with the following participating laboratories: Strasbourg MONICA Project, Department of Epidemiology and Public Health, Faculty of Medicine, Strasbourg, France (D. Arveiller, B. Haas); Toulouse MONICA Project, INSERM U558, Department of Epidemiology, Paul Sabatier-Toulouse Purpan University, Toulouse, France (J. Ferrières, J.B. Rudauds); Lille MONICA Project, INSERM U508, Pasteur Institute, Lille, France (P. Amouyal, M. Montayre); Department of Epidemiology and Public Health, Queen’s University of Belfast, Northern Ireland (A. Evans, J. Yarnell); Department of Atherosclerosis, SERLIA-INSERM U325, Lille, France (G. Luc, J.M. Bard, L. Elkahil, J.-C. Fruchart); Laboratory of Hematology, La Timone Hospital, Marseilles, France (I. Juhan-Vague); Laboratory of Endocrinology, INSERM U326, Toulouse, France (B. Perret); Vitamin Research Unit, University of Bern, Switzerland (F. Gey); Trace Element Laboratory, Department of Medicine, Queen’s University, Belfast, Northern Ireland (D. McMaster); DNA Bank, INSERM U525/SC7, Paris, France (F. Cambien); and Coordinating Center, INSERM U258, Paris-Villejuif, France (P. Ducimetière, P.Y. Scarabin, A. Bingham).

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References

3. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of initial rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89:36–44.
interleukin-6 and C-reactive protein levels with mortality in the elderly.


C-Reactive Protein, Interleukin-6, and Fibrinogen as Predictors of Coronary Heart Disease: The PRIME Study

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on behalf of the PRIME Study Group

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