The Value of C-Reactive Protein in Cardiovascular Risk Prediction

The Rotterdam Study

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Background: Epidemiologic studies have shown that C-reactive protein (CRP) is a risk factor for coronary heart disease. Whether routine measurement of CRP has a role in the prediction of future coronary disease in everyday clinical practice has not yet been investigated.

Methods: Within the Rotterdam Study, a population-based cohort study of 7983 men and women 55 years and older, we conducted a nested case-control study to investigate the value of CRP in coronary disease prediction. Data are based on 157 participants who experienced a myocardial infarction during follow-up and 500 randomly selected controls. High-sensitivity CRP and traditional cardiovascular risk factors were measured at baseline.

Results: The age- and sex-adjusted relative risk of myocardial infarction for subjects in the highest quartile of the population distribution of CRP compared with the lowest quartile was 2.0 (95% confidence interval, 1.1-3.4). After additional adjustment for traditional cardiovascular risk factors, the increase in risk largely disappeared (odds ratio, 1.2; 95% confidence interval, 0.6-2.2). Adding CRP to a coronary disease risk function based on risk factors that are routinely assessed in clinical practice or to the Framingham risk function did not improve the area under the receiver operating characteristic curve of these risk functions. Sensitivity and specificity of both risk functions, computed after dichotomizing the estimated disease probabilities using prespecified cutoff points, hardly improved when CRP was added.

Conclusion: Measurement of CRP in elderly people has no additional value in coronary disease risk prediction when traditional cardiovascular risk factors are known.

Arch Intern Med. 2003;163:1323-1328
whether additional assessment of levels of CRP can improve our ability to discern those who will and those who will not have a myocardial infarction in the future.

METHODS

POPULATION

For the present study, we used a nested case-control design. All subjects included in the study were participants in the Rotterdam Study, a population-based cohort study comprising 7983 men and women. The aim of the Rotterdam Study is to investigate the incidence of and risk factors for chronic disabling diseases. From 1990 until 1993, all inhabitants of a well-defined suburb of the city of Rotterdam, the Netherlands, 55 years and older were invited to participate in an extensive home interview and 2 visits to the research center. The overall response rate was 78%. The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus University, Rotterdam, and written informed consent was obtained from all participants. A more detailed description of the Rotterdam Study and the collection of data can be found elsewhere.9

SELECTION OF CASES AND CONTROLS

Only subjects without a history of myocardial infarction at baseline were included in the study. Incident cardiovascular events were reported by general practitioners in the research district. Research assistants verified all information by checking medical records at the general practitioners' offices. In addition, they obtained letters and, in case of hospitalization, discharge reports from medical specialists. Subsequently, 2 research physicians independently coded all reported events; codes were assigned according to the International Classification of Diseases, 10th Revision. Codes on which the research physicians disagreed were discussed to reach consensus. Finally, a medical expert in the field reviewed all events and verified whether the research physicians had correctly applied the coding rules. In case of disagreement between the medical expert and the research physicians, the expert's judgment was considered final. Follow-up was complete until January 1, 1998. During follow-up, 203 participants who had visited the research center and for whom blood samples had been drawn experienced a first myocardial infarction. Because of logistic reasons, complete data were not different from the total case population. Per case, we selected 3 controls who had never experienced a myocardial infarction and had not died during follow-up. Complete data were available for 500 controls.

CLINICAL CHARACTERISTICS

A trained interviewer visited all subjects at home and collected information using a computerized questionnaire. The obtained information included current health status, medical history, drug use, smoking behavior, and family history of cardiovascular disease. A family history of early myocardial infarction was defined as the occurrence of a myocardial infarction in parents, children, or siblings of the participant before or at the age of 65 years. Additionally, during 2 visits to the research center, established cardiovascular risk factors were measured. Body mass index was computed as weight in kilograms divided by the height in meters squared. Two blood pressure measurements were taken with a random-zero sphygmomanometer after 5 minutes of rest with the subject in a sitting position. We defined hypertension as a systolic blood pressure of

160 mm Hg or higher and/or diastolic blood pressure of 100 mm Hg or higher and/or the use of antihypertensive medication. A venipuncture was performed, applying minimal stasis, using a 21-gauge butterfly needle with tube (Surflo winged infusion set; Terumo Europe NV, Leuven, Belgium). Glucose was enzymatically determined by the hexokinase method (Boehringer Mannheim, Mannheim, Germany). Diabetes mellitus was defined as the use of blood glucose–lowering medication and/or a nonfasting serum glucose level equal to or greater than 200 mg/dL (11.1 mmol/L). We determined serum total cholesterol using an automated enzymatic procedure. High-density lipoprotein cholesterol (HDL-C) was measured similarly, after precipitation of the non–HDL-C fraction with phosphotungstate-magnesium. A 12-lead resting electrocardiogram (ECG) was recorded and analyzed by the Modular ECG Analysis System (MEANS). The program provides a rhythm and contour interpretation and has been extensively evaluated.18 A history of myocardial infarction before entering the study was considered present in case of a self-report of myocardial infarction confirmed by the ECG or additional clinical information. For the assessment of left ventricular hypertrophy, the MEANS interpretation was used.

C-REACTIVE PROTEIN

Nonfasting blood was collected in tubes containing 0.129 mol/L of sodium citrate. The ratio of blood to sodium citrate was 9:1. Plasma was collected after centrifugation for 10 minutes at 3000 rotations per minute at 4°C. Subsequently, platelet-free plasma was obtained by centrifugation for 10 minutes at 10,000 rotations per minute and was immediately frozen in liquid nitrogen and stored at −80°C. All tubes were stored on ice before and after blood sampling. C-reactive protein was measured by sensitive immunologic methods using an in-house enzyme immunoassay (n=516 subjects; Dako, Glostrup, Denmark) or a nephelometric method (n=160; Dade-Behring, Marburg, Germany). It has previously been demonstrated that these 2 methods show high agreement.5 In the present study, CRP was measured by both methods in 80 subjects. The high agreement was confirmed (mean difference between methods for values of CRP ≤10 mg/L, 0.02 mg/L). C-reactive protein levels of more than 10 mg/L were measured in 3.2% of the controls and 10.8% of the cases. The population distribution of CRP was highly skewed. Outliers (>3 SDs of the control distribution of log-transformed CRP; n=1) were excluded, since they may indicate the presence of an active inflammatory disease.

CORONARY DISEASE RISK FUNCTIONS

We used 2 risk functions to predict coronary disease risk. Risk function 1 was based on cardiovascular risk factors that are routinely assessed in clinical practice by medical history and physical examination (ie, age, sex, current smoking, body mass index, hypertension, diabetes mellitus, and family history of myocardial infarction before the age of 65 years) and lipid measurements (total cholesterol and HDL-C). Risk function 2 was computed as described by the Framingham risk function, taking into account age, sex, current smoking, systolic blood pressure, diabetes mellitus, total cholesterol level, HDL-C level, and left ventricular hypertrophy present on the ECG.5

STATISTICAL ANALYSES

For subjects for whom data on continuous clinical characteristics were incomplete (n=17), we imputed the population mean. We computed baseline means and proportions of traditional cardiovascular risk factors (age, sex, current smoking, body mass index, hypertension, diabetes mellitus, family history of early


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myocardial infarction, total cholesterol level, and HDL-C level) for cases and controls. For CRP, we computed the median and interquartile range. Multivariate odds ratios (ORs) for myocardial infarction associated with the higher quartiles of the control distribution of CRP compared with the lowest quartile were computed by logistic regression analysis. Since age squared was a highly significant predictor of myocardial infarction, it was included in the analyses. We used 3 different models. Model 1 was adjusted for age, age squared, and sex. Since likely sources of inflammation include various components of cigarette smoke and adipose tissue, model 2 additionally included variables that indicated smoking behavior and body mass index. Model 3 was adjusted for all traditional cardiovascular risk factors. Subsequently, we computed a categorical variable ranging from 1 to 4 to indicate 4 situations for each traditional cardiovascular risk factor: (1) CRP level low (not in the highest quartile of the population distribution) and traditional risk factor absent, (2) CRP level high and risk factor absent, (3) CRP level low and risk factor present, and (4) CRP level high and risk factor present. Traditional cardiovascular risk factors that are measured on a continuous scale were considered to be present if they were in the highest quartile of the population distribution. Taking situation 1 (CRP level low and risk factor absent) as the reference, we then computed multivariate ORs for myocardial infarction associated with situations 2, 3, and 4 for each of the traditional cardiovascular risk factors.

Using the logistic regression model, we computed probabilities of myocardial infarction for each subject as predicted by the 2 coronary disease risk functions described herein. We then extended these risk functions by including levels of CRP. Using each risk function as a diagnostic test, we constructed receiver operating characteristic (ROC) curves, which indicate the probability of a true-positive result (sensitivity) as a function of the probability of a false-positive result (1–specificity) for all possible threshold values of the diagnostic test. Differences in the predictive value of the risk functions were estimated by comparing the areas under the ROC curve (AUCs), taking into account correlation between the areas. Subsequently, for each risk function we considered subjects to have a positive test result if they were in the upper 20% of the risk function–specific population distribution of the predicted disease probabilities. Using this cutoff point, we estimated the sensitivity (percentage of correctly classified cases) and specificity (percentage of correctly classified controls) of each of the risk functions. The procedure was repeated at a cutoff point of 10%. In all models, we included a variable that indicated the method used to measure CRP. All analyses were performed using SPSS statistical software version 9.0 (SPSS Inc, Chicago, Ill).

RESULTS

As expected, incident cases of myocardial infarction had a more adverse cardiovascular risk profile than controls (Table 1). Geometric mean levels of CRP were 2.18 mg/L (interquartile range, 1.04-5.09 mg/L) and 1.68 mg/L (interquartile range, 0.82-3.02 mg/L) for cases and controls, respectively. Table 2 shows that, after adjustment for age, age squared, and sex, the relative risk of myocardial infarction for subjects in the highest quartile of CRP compared with subjects in the lowest quartile was increased 2-fold (model 1; OR, 2.0; 95% confidence interval [CI], 1.1-3.4). The relative risk of myocardial infarction increased across quartiles of CRP (P for trend=.01). Adjustment for current smoking and body mass index did not substantially change the risk estimates (model 2; OR, 1.9; 95% CI, 1.1-3.3; P for trend=.02). However, the increase in risk largely disappeared when additional cardiovascular risk factors were added to the model (model 3; OR, 1.2; 95% CI, 0.6-2.2; P for trend=.30).

Table 3 gives the relative risk of myocardial infarction for combinations of high or low levels of CRP and the presence or absence of one of the traditional cardiovascular risk factors, with subjects with neither a high CRP level nor the presence of that particular risk factor taken as the reference group. Although CIs were wide and overlapping, the risk of myocardial infarction associated with diabetes mellitus and high levels of total cholesterol was highest when levels of CRP were also high. There seemed to be no added effect of high levels of CRP on the risk associated with other traditional cardiovascular risk factors.

Of both risk functions, the AUC was largest using the risk function based on risk factors that are routinely assessed in clinical practice by medical history, physical examination, and lipid measurements (risk function 1; AUC, 0.773; SE, 0.021; Table 4). The AUC hardly changed when CRP was added to the risk function (AUC, 0.777; SE, 0.021; ΔAUC, 0.004; P for change=.28). For comparison, adding lipid levels to a risk function based on medical history and physical examination alone caused a change in AUC of 0.047 (P for change=.001). The AUC (SE) of the Framingham risk function (risk function 2) was 0.746 (0.021). Adding CRP to the risk function did not improve the AUC (AUC, 0.748; SE, 0.021; P for change=.055).

Table 5 shows that when subjects in the upper 20% of the risk function–specific distribution of predicted disease probabilities were considered to have a positive test result, risk function 1 had the best combination of sensitivity (44.6%) and specificity (88.6%) in predicting myocardial infarction. Sensitivity and specificity did not improve after inclusion of CRP level (43.9% and 88.4%, respectively). Using the upper 10% of the population distribution as the cutoff point, sensitivity and specificity of risk function 1 were 21.7% and 94.8%, respectively.
Table 2. Odds Ratios (95% Confidence Intervals) for Myocardial Infarction According to Baseline Levels of C-Reactive Protein*

<table>
<thead>
<tr>
<th>Model</th>
<th>CRP− RF−</th>
<th>CRP+ RF−</th>
<th>CRP− RF+</th>
<th>CRP+ RF+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.2 (0.7-2.1)</td>
<td>1.5 (0.9-2.7)</td>
<td>2.0 (1.1-3.4)</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>1.2 (0.7-2.1)</td>
<td>1.5 (0.8-2.6)</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>0.9 (0.5-1.7)</td>
<td>1.0 (0.5-1.9)</td>
<td>1.2 (0.6-2.2)</td>
</tr>
</tbody>
</table>

*Model 1 was adjusted for age, age squared, and sex. Model 2 was additionally adjusted for current smoking and body mass index. Model 3 was additionally adjusted for hypertension, diabetes mellitus, a family history of early myocardial infarction, total cholesterol level, and high-density lipoprotein cholesterol level.

Table 4. AUC for Risk Functions With and Without CRP

<table>
<thead>
<tr>
<th>Variable</th>
<th>CRP (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic risk*</td>
<td>0.642 (0.026)</td>
</tr>
<tr>
<td>Risk function 1†</td>
<td>0.773 (0.021)</td>
</tr>
<tr>
<td>With CRP</td>
<td>0.777 (0.021)</td>
</tr>
<tr>
<td>Risk function 2‡</td>
<td>0.746 (0.021)</td>
</tr>
<tr>
<td>With CRP</td>
<td>0.748 (0.021)</td>
</tr>
</tbody>
</table>

*Indicated by age, age squared, and sex.
†Includes age, age squared, sex, current smoking, body mass index, hypertension, diabetes mellitus, a family history of early myocardial infarction, total cholesterol level, and high-density lipoprotein cholesterol level.
‡Based on the Framingham risk function.

This study shows that CRP level predicts the incidence of myocardial infarction in an elderly population but not independently of more traditional cardiovascular risk factors that are routinely assessed in a clinical setting. The results of this study suggest that in clinical practice measurement of CRP concentrations will not improve the ability to predict cardiovascular risk. The Rotterdam Study is a prospective population-based study with nearly 8000 participants in which extensive information has been collected on cardiovascular risk factors. For the present study, we used data from a relatively large nested sample of 157 cases of myocardial infarction and 500 controls. This is the first study, to our knowledge, in which the clinical value of CRP measurement in addition to the assessment of traditional cardiovascular risk factors has been investigated.

The present study suggests that CRP does not predict cardiovascular risk independently of other risk factors. It is possible that at an elderly age the risk of cardiovascular disease associated with CRP is lower than at a younger age, a phenomenon that is also seen for other risk factors such as cholesterol. In the Cardiovascular Health Study (with participants ≥65 years), high levels of CRP did not increase the risk of cardiovascular disease in men, and the risk in women was only increased when subclinical cardiovascular disease was present. Moreover, other studies have shown that the predictive value of CRP for ischemic heart disease and mortality was attenuated with age. The results of the present...
study indicate that measurement of CRP has no additional predictive value in men and women older than 55 years; whether this conclusion also applies to younger populations remains to be investigated.

Two studies by Ridker et al\(^2,3\) found that subjects with high levels of both total cholesterol and CRP have a higher risk of myocardial infarction compared with subjects in whom only total cholesterol or CRP levels are elevated. Although in the present study the risk of myocardial infarction was also highest when both total cholesterol and CRP levels were elevated, the results should be interpreted with caution, since CIs were wide and overlapping. The conclusion by Ridker et al\(^2,3\) that CRP and lipid levels have additional value in risk stratification does not by itself justify incorporation of CRP measurement in standard clinical practice. Information provided by all traditional cardiovascular risk factors should be taken into account when the predictive value of CRP is assessed.

Recently, it has been found that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition (“statin”) therapy may cause a reduction in cardiovascular events in subjects who have relatively low lipid levels in combination with high levels of CRP.\(^17\) However, whether measurement of CRP can play a role in making treatment decisions needs further confirmation.

Several aspects of this study need to be addressed. First, although the AUC provides a good measure of the overall diagnostic value of a coronary disease risk function, it may have limited sensitivity to detect the additive effect of new cardiovascular risk factors. In clinical practice, a threshold predicted probability is used to inform the physician about whether a patient is at low or high risk of myocardial infarction. The clinical relevance of such a threshold is determined by the number of false-positive and false-negative results.\(^18\) Therefore, we additionally computed the sensitivity (percentage of true-positive results) and specificity (percentage of true-negative results) of all prediction models choosing different cutoff points. The results confirmed that there was only, if any, a small increase in sensitivity and specificity of the risk functions when CRP level was added. Second, some of the established cardiovascular risk factors, such as diet, physical activity, and socioeconomic status, were not included in the analyses. However, since we wanted our study to resemble clinical practice, we specifically aimed at including only those risk factors that are routinely used and that can be assessed within several minutes.

Etiologic research into CRP as a cardiovascular risk factor has made and is still making an important contribution to our understanding of the processes involved in atherosclerosis. The present study indicates, however, that as a diagnostic tool, measurement of CRP concentrations in men and women older than 55 years has no additional clinical value. Further research should clarify whether and in which subjects CRP measurement may be useful in clinical risk prediction.

### Table 5. Sensitivity and Specificity for Coronary Disease Risk Functions\(^*\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases, No.</th>
<th>Controls, No.</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Cases, No.</th>
<th>Controls, No.</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic risk†</td>
<td>49</td>
<td>78</td>
<td>31.2</td>
<td>84.4</td>
<td>26</td>
<td>34</td>
<td>16.6</td>
<td>93.2</td>
</tr>
<tr>
<td>Risk function 1‡</td>
<td>70</td>
<td>57</td>
<td>44.6</td>
<td>88.6</td>
<td>34</td>
<td>26</td>
<td>21.7</td>
<td>94.8</td>
</tr>
<tr>
<td>With CRP</td>
<td>69</td>
<td>58</td>
<td>43.9</td>
<td>88.4</td>
<td>36</td>
<td>24</td>
<td>22.9</td>
<td>95.2</td>
</tr>
<tr>
<td>Risk function 2§</td>
<td>61</td>
<td>66</td>
<td>38.9</td>
<td>86.8</td>
<td>32</td>
<td>28</td>
<td>20.4</td>
<td>94.4</td>
</tr>
<tr>
<td>With CRP</td>
<td>62</td>
<td>65</td>
<td>39.5</td>
<td>87.0</td>
<td>32</td>
<td>28</td>
<td>20.4</td>
<td>94.4</td>
</tr>
</tbody>
</table>

Abbreviation: CRP, C-reactive protein.

\(^*\)The number of cases are those cases (of 157) that were correctly identified as cases. The number of controls are those controls (of 500) that were wrongly identified as cases.

†Indicated by age, age squared, and sex.

‡Includes age, age squared, sex, current smoking, body mass index, hypertension, diabetes mellitus, a family history of early myocardial infarction, total cholesterol level, and high-density lipoprotein cholesterol level.

§Based on the Framingham risk function.

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Accepted for publication August 30, 2002.

This study was supported by grant 980-10-005 from the Netherlands Organization for Health Research and Development (ZON-MW, Den Haag).

We thank all participants, research physicians, and research assistants of the Rotterdam Study for their contributions. We thank Piet Meijer, PhD, at the Gaubius Laboratory (Leiden, the Netherlands) and Numico Research (Wageningen, the Netherlands) for conducting the laboratory analyses for CRP.

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