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DOI
10.1136/hrt.2009.170134

Publication date
2009

Document Version
Final published version

Published in
Heart

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Heart 2009 95: 1682-1687 originally published online July 8, 2009
doi: 10.1136/hrt.2009.170134

Inflammatory biomarkers and the prediction of coronary events among people at intermediate risk: the EPIC-Norfolk prospective population study

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Accepted 30 June 2009
Published Online First 8 July 2009

ABSTRACT

Objective: To evaluate the role of the inflammatory biomarkers C-reactive protein (CRP), myeloperoxidase, paraoxonase, secretory phospholipase A2 group IIA (sPLA2), lipoprotein-associated phospholipase A2, fibrinogen, macrophage chemoattractant protein-1 and adiponectin, in predicting the risk of coronary heart disease (CHD) among people estimated to be at intermediate risk according to the Framingham Risk Score (FRS).

Design: Prospective case–control study nested in EPIC-Norfolk cohort.

Setting: Norfolk, UK.

Patients: Apparently healthy men and women aged 45–79 years.

Main outcome measures: Risk of future coronary artery disease.

Results: For participants predicted to be at intermediate risk by the FRS, the highest c statistics were observed for FRS plus CRP (0.61, 95% CI 0.57 to 0.65) and for FRS plus sPLA2 (0.56, 95% CI 0.52 to 0.6). Net correct reclassification of cases and controls for each marker was assessed for people across the entire risk spectrum and again for people at intermediate risk only. The largest differences were observed for CRP, 12.0% net reclassification improvement in the entire risk spectrum and 28.4% net reclassification improvement in the intermediate-risk group and for sPLA2, the net reclassification improvement was 6.4% in the entire risk spectrum and 16.3% in the intermediate-risk group.

Conclusions: The discriminatory potential of inflammatory biomarkers was substantially different when analysed across the entire risk spectrum compared with the subgroup of people at intermediate risk.

The identification of people at risk for coronary heart disease (CHD) is important for clinical decision-making for diagnosis, treatment and prognosis. The Framingham Risk Score (FRS) estimates a person's risk of a CHD event over the next 10 years, based on an algorithm that incorporates the traditional cardiovascular risk factors age, sex, smoking, diabetes mellitus, blood pressure, antihypertensive therapy use, total or low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C).[1] People at low risk of CHD are those who do not have known CHD and have <10% risk of CHD according to FRS, intermediate-risk subjects are those without known CHD who have a 10-year CHD risk between 10% and 20% and high-risk subjects are those with prevalent CHD or a CHD risk equivalent or 10-year CHD risk >20%. In the US National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines, treatment decisions are based on these risk categories.[2]

Current guidelines dictate that people at high risk should receive medical treatment and people at low risk should not. For people at intermediate risk, decision-making about the initiation of preventive therapy is not straightforward and is often based on additional arguments. Numerous biomarkers, including C-reactive protein (CRP), have been proposed to enhance current risk score algorithms. Although the screening of entire populations for raised levels of inflammatory markers does not seem justified at this time,[3] guidelines suggest that inflammatory markers such as CRP may be useful in guiding therapeutic decision-making for people at intermediate risk.[4] Consequently, inflammatory markers can be considered clinically relevant only if they can correctly identify people prone to develop CHD among those estimated to be at intermediate risk. Whereas a range of studies has evaluated the predictive role of CRP compared with traditional risk factors,[5–10] surprisingly few have provided these analyses in people at intermediate risk.[11] Data on the role of other inflammatory markers among people at intermediate risk are scarce. In addition, conclusions are often based on subjective interpretations of the effect of these novel markers on the c statistic (also known as the area under the receiver operating characteristic (ROC) curve), which are characterised as "only marginal"[5] and "negligible"[9] and statistical accountability for such conclusions is seldom provided. The clinical utility of these "novel" biomarkers has been debated extensively. Arguments against their introduction have focused on their limited impact on the c statistic.[12] Proponents have argued against sole reliance on the c statistic in this assessment.[13 14]

It was our objective to evaluate the role of various inflammatory biomarkers in predicting the risk of CHD among apparently healthy people. We performed these analyses across the entire risk spectrum and also in the subgroup of people estimated to be at intermediate risk according to the FRS. Analyses were performed for CRP, myeloperoxidase (MPO), paraoxonase (PON), secretory phospholipase A2 group IIA (sPLA2), lipoprotein-associated phospholipase A2 (Lp-PLA2), fibrinogen, macrophage chemoattractant protein-1 (MCP-1) and adiponectin.

METHODS

Study design

We performed a nested case–control study among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study, a

prospective population study of 25 663 men and women aged 45–79 years, resident in Norfolk, UK, who completed a baseline questionnaire survey and attended a clinic visit.[15] Participants were recruited from age–sex registers of general practices in Norfolk as part of the 10-country collaborative EPIC study designed to investigate dietary and other determinants of cancer. Additional data were obtained in EPIC-Norfolk to enable the assessment of determinants of other diseases.

The design and methods of the study have been described in detail.[15] In short, eligible participants were recruited by mail. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire. Non-fasting blood samples were obtained by vein puncture and transferred to plain and citrate bottles. Blood samples were processed for assay at the Department of Clinical Biochemistry, University of Cambridge, or stored at −80°C. All subjects have been flagged for death certification at the UK Office of National Statistics, with vital status ascertained for the entire cohort. In addition, participants admitted to hospital were identified using their unique National Health Service number by data linkage with the East Norfolk Health Authority (ENCORE) database, which identifies all hospital contacts throughout England and Wales for Norfolk residents. Coronary artery disease (CAD) was defined as codes 410–414 according to the International Classification of Diseases 9th revision. Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD as underlying cause. Previous validation studies in our cohort indicate high specificity of such case ascertainment.[16] The study was approved by the Norwich District Health Authority ethics committee and all participants gave signed informed consent.

Participants

We have previously described this nested case–control study.[17 18] We excluded all people who reported a history of heart attack/stroke or use of lipid-lowering drugs at the baseline clinic visit. Cases were people who developed fatal or non-fatal CHD during follow-up until November 2003 (mean follow-up 6 years). Controls were study participants who remained free of any cardiovascular disease during follow-up. We matched two controls to each case by age (within 5 years), sex and time of enrolment (within 3 months).

Biochemical analyses

Serum levels of total cholesterol, HDL-C and triglycerides were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK). LDL-C levels were calculated with the Friedewald formula.[19] Assays to measure concentrations of CRP,[20] MPO,[18] PON,[21] sPLA2,[22] and Lp-PLA2[23] have all been described previously. Fibrinogen was measured with a polymerisation method as originally described by Clauss.[24] Levels of MCP-1 were measured by a multiplex cytokine assay system (Bio-Plex; Bio-Rad Laboratories, Hercules, California, USA), according to the manufacturer's protocol. Adiponectin concentrations were determined by an ELISA (B-Bridge International, San Jose, California, USA). Samples were analysed in random order to avoid systematic bias. Researchers and laboratory personnel were blinded to identifiable information and could identify samples by number only.

Statistical analysis

Baseline characteristics were compared between cases and controls taking into account the matching for sex, age and enrolment time—that is, using conditional logistic regression for dichotomous variables and a mixed effect model for continuous variables. The FRS was calculated as published.[1] Because the inflammatory markers were not normally distributed, they were log-transformed before being used in statistical analyses. We calculated the area under the ROC curve for all people and again for the subgroup of people predicted to be at intermediate risk by the FRS. We calculated FRS and entered it as continuous variable in a regression model with CHD as outcome. The area under the ROC curve was calculated on the expected values. These analyses were performed for the FRS and for the combination of FRS plus each of the inflammatory markers analysed. For each combination of FRS plus an inflammatory marker, we also quantified whether the c statistic differed statistically significantly from the c statistic for the FRS only. Using a bootstrapping approach, we designed 1000 samples drawn randomly from the database, which allowed us to calculate a standard error for each c statistic. The absolute difference between the c statistics of both models was subsequently related to their 95% confidence interval. Next, we calculated the −2 log likelihood, Bayes information criterion and Hosmer–Lemeshow goodness-of-fit test for the FRS and for each combination of FRS with an inflammatory marker. These analyses were performed for the whole cohort and for the subgroup predicted to be at intermediate risk by the FRS.

Finally, we constructed reclassification tables displaying the number of cases and controls predicted to be at low, medium and high risk by the FRS only and by a model based on FRS plus an inflammatory marker. For all inflammatory markers, analyses were performed on log-transformed values. The effects of reclassification using biomarkers were assessed using recently published methods that estimated the net reclassification improvement (NRI),[25] which expands and improves on previously published reclassification methods.[9 26] This method provides a more rigorous statistical approach to assess the improvement in reclassification by including new biomarker information in prediction models.[27] The analyses used continuous variable information with evaluation of the effects on risk category reclassification for those cases and controls during the follow-up interval. Reclassification to a higher-risk group was considered upward movement/improvement in classification for those experiencing an event. On the other hand, reclassification downward was considered a failure for people who developed an event. Conversely, among those who did not experience an event, reclassification upward was considered disadvantageous and reclassification downward was considered advantageous.[27] Improvement in reclassification was estimated by taking the sum of differences in proportions of subjects reclassified upward minus the proportion reclassified downward for people who developed events and the proportion of people moving downward minus the proportion moving upward for those who did not develop events. Using this method, the overall reclassification sum is the NRI.[27] Statistical analyses were performed using SPSS software (version 12.0.1, Chicago, Illinois, USA). A p value <0.05 was considered to indicate statistical significance.

RESULTS

Table 1 shows the baseline characteristics for cases and controls, along with the levels of inflammatory markers. As expected, cases were more likely than controls to be smokers and have diabetes mellitus. Cases had higher body mass index, total cholesterol levels, LDL-C levels and blood pressure than controls, whereas HDL-C levels were lower. Plasma levels of

CRP, MPO, sPLA2, Lp-PLA2 and fibrinogen were significantly higher among cases than controls, whereas adiponectin levels were significantly lower. PON levels were lower in cases than controls, but not statistically significantly (p = 0.08). MCP-1 levels did not differ significantly between cases and controls.

Analyses among all people in the dataset revealed that the c statistic was 0.59 (95% CI 0.567 to 0.61) for the model based on FRS only (table 2). In general, the models based on FRS plus an inflammatory marker yielded higher c statistics, the highest being for FRS plus CRP (0.65, 95% CI 0.59 to 0.64), FRS plus sPLA2 (0.61, 95% CI 0.58 to 0.63) and FRS plus fibrinogen (0.6, 95% CI 0.58 to 0.62). The model with FRS plus MCP-1 yielded a similar c statistic as the one for FRS alone. Of all the inflammatory markers analysed, only CRP resulted in a statistically significant increase of the c statistic compared with the model based on FRS only (mean difference 0.027, p = 0.005).

When analyses were restricted to the subset of people predicted to be at intermediate risk by the FRS, the c statistics for all inflammatory markers dropped substantially compared with those based on the entire dataset. The highest c statistics were again observed for FRS plus CRP (0.61, 95% CI 0.57 to 0.65) and for FRS plus sPLA2 (0.56, 95% CI 0.52 to 0.6). Again, only the addition of CRP resulted in a statistically significant increase of the c statistic compared with the model based on FRS only (mean difference 0.08, p<0.001).

Across the entire risk spectrum, the −2 log likelihood for FRS alone was 3284.6. All models with an additional inflammatory marker had a lower -2 log likelihood, the lowest ones being for FRS plus CRP (3242.7) and FRS plus sPLA2 (3253.1). When analyses were restricted to people at intermediate risk alone, FRS yielded a −2 log likelihood of 1253.6. As expected, the addition of an inflammatory marker resulted in lower −2 log likelihoods, the lowest ones again being for sPLA2 (1242.9) and CRP (1225.9). Consequently, the lowest Bayes information criteria were for sPLA2 (1.28) and CRP (1.29). The same was true for analyses based on people at intermediate risk. Analyses using the Hosmer–Lemeshow goodness-of-fit test showed that the models including FRS plus CRP (p = 0.009), PON (p = 0.006) and MCP-1 (p = 0.01) showed evidence of significant lack of fit (table 3).

In the current dataset, 921 people developed a CHD event during follow-up. Table 4 shows the percentage of people reclassified into a higher- or lower-risk category using an inflammatory marker plus FRS, compared with using the FRS only. For instance, when CRP was used in addition to the FRS, 20.8% people who developed CHD during follow-up were correctly reclassified to a higher risk category—that is, from low-risk to intermediate- or high-risk category, or from intermediate-risk to high-risk category. However, 18.7% people who did develop CHD during follow-up were incorrectly reclassified to a lower-risk category—that is, from high-risk to intermediate- or low-risk, or from intermediate-risk to low-risk categories. Thus, there was a net benefit of reclassification of 2.1% with addition of CRP across the entire risk spectrum in cases. However, when we similarly assessed reclassification among people at intermediate risk only, addition of CRP resulted in net correct reclassification of 12.9%. For the intermediate-risk group, fibrinogen also yielded a substantial rate of correct reclassification (10.3%).

We further explored the reclassification among 1629 people who did not develop CHD during follow-up. When CRP was used in addition to the FRS, 26.8% people who did not develop CHD during follow-up were correctly reclassified to a lower-risk category—that is, from higher-risk to intermediate- or low-risk,

Table 1 Baseline characteristics

Characteristics	Controls (n = 1629)	Cases (n = 921)	p Value
Age (years), mean (SD)	65 (8)	65 (8)	Matched
Male sex, % (n)	63.6 (1036)	64.4 (593)	Matched
Body mass index (kg/m^2), mean (SD)	26.2 (3.4)	27.2 (3.9)	<0.001
Smoking, % (n)			<0.001
Current	8.3 (135)	15.4 (142)	
Previous	51.0 (830)	52.2 (481)	
Never	40.8 (664)	32.4 (298)	
Diabetes mellitus, % (n)	1.5 (24)	6.1 (56)	<0.001
Systolic blood pressure (mm Hg), mean (SD)	139 (18)	144 (19)	<0.001
Diastolic blood pressure (mm Hg), mean (SD)	84 (11)	86 (12)	<0.001
Total cholesterol (mmol/l), mean (SD)	6.2 (1.1)	6.5 (1.2)	<0.001
LDL-cholesterol (mmol/l), mean (SD)	4.1 (1.0)	4.3 (1.0)	<0.001
HDL-cholesterol (mmol/l), mean (SD)	1.4 (0.4)	1.3 (0.4)	<0.001
Triglycerides (mmol/l), median (IQR)	1.6 (1.1–2.2)	1.8 (1.3–2.6)	<0.001
C-reactive protein (mg/dl), median (IQR)	1.5 (0.7–3.1)	2.2 (1.0–4.9)	<0.001
Myeloperoxidase (pmol/l), median (IQR)	552 (354–870)	625 (390–970)	<0.001
Paraoxonase (U/l), median (IQR)	43.0 (26.6–90.5)	39.7 (23.9–86.7)	0.08
Type II secretory phospholipase A2 (ng/ml), median (IQR)	8.5 (5.9–12.8)	9.5 (6.5–15.0)	<0.001
Lipoprotein-associated phospholipase A2 (U/l), median (IQR)	49.5 (40.3–60.0)	52.0 (43.5–62.3)	<0.001
Fibrinogen (g/l), median (IQR)	3.0 (2.5–3.5)	3.1 (2.6–3.7)	<0.001
MCP-1 (pg/ml), median (IQR)	51.2 (38.4–67.6)	51.1 (38.2–69.3)	0.7
Adiponectin (μg/ml), median (IQR)	9.4 (6.9–13.2)	8.9 (6.5–12.6)	0.009
Framingham Risk Score, % (n)			<0.001
Low risk	21.3 (347)	14.4 (133)	
Intermediate risk	42.4 (690)	34.5 (318)	
High risk	36.3 (592)	51.0 (470)	

For all inflammatory markers, analyses were performed on log-transformed values.
HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MCP-1, macrophage chemoattractant protein-1.

Table 2 c Statistics

	c Statistic (95% CI)	Difference compared with FRS only		
		Mean	SD (95% CI)	p Value
FRS				
All	0.59 (0.57 to 0.61)			
Intermediate	0.54 (0.5 to 0.57)			
FRS, CRP				
All	0.65 (0.59 to 0.64)	0.03	0.01 (0.01 to 0.05)	0.005
Intermediate	0.61 (0.57 to 0.65)	0.08	0.022 (0.03 to 0.12)	<0.001
FRS, MPO				
All	0.6 (0.57 to 0.62)	0.007	0.006 (−0.005 to 0.02)	0.3
Intermediate	0.54 (0.50 to 0.58)	0.004	0.01 (−0.015 to 0.02)	0.7
FRS, PON				
All	0.59 (0.57 to 0.61)	0.001	0.002 (−0.003 to 0.004)	0.6
Intermediate	0.54 (0.50 to 0.58)	0.004	0.01 (−0.016 to 0.02)	0.7
FRS, sPLA2				
All	0.61 (0.58 to 0.63)	0.02	0.009 (−0.0006 to 0.03)	0.058
Intermediate	0.56 (0.52 to 0.6)	0.02	0.02 (−0.015 to 0.06)	0.2
FRS, Lp-PLA2				
All	0.59 (0.57 to 0.61)	0.0008	0.004 (−0.007 to 0.009)	0.8
Intermediate	0.54 (0.50 to 0.58)	0.007	0.012 (−0.02 to 0.03)	0.6
FRS, fibrinogen				
All	0.6 (0.58 to 0.62)	0.01	0.008 (−0.005 to 0.03)	0.2
Intermediate	0.55 (0.52 to 0.59)	0.02	0.02 (−0.01 to 0.05)	0.3
FRS, MCP-1				
All	0.59 (0.57 to 0.61)	0.0001	0.0002 (−0.0003 to 0.0005)	0.8
Intermediate	0.54 (0.5 to 0.57)	−0.0004	0.003 (−0.006 to 0.005)	0.9
FRS, adiponectin				
All	0.59 (0.57 to 0.61)	0.001	0.003 (−0.005 to 0.007)	0.7
Intermediate	0.6 (0.52 to 0.6)	0.02	0.02 (−0.009 to 0.06)	0.2

CRP, C-reactive protein; FRS, Framingham Risk Score; Lp-PLA2, lipoprotein-associated phospholipase A2; MCP-1, macrophage chemoattractant protein-1; MPO, myeloperoxidase; PON, paraoxonase; sPLA2, secretory phospholipase A2-type II.

or intermediate-risk to low-risk categories. However, 16.9% people who did not develop CHD during follow-up were incorrectly reclassified to a higher-risk category—that is, from low-risk to intermediate- or high-risk, or intermediate-risk to high-risk categories. Thus, there was a net benefit of reclassification of 9.9% with addition of CRP across the entire risk spectrum in controls. However, when we similarly looked at reclassification among the intermediate-risk group, addition of CRP yielded net correct reclassification was 15.5%. For the intermediate-risk cohort the other notable net correct reclassification of controls was for sPLA2 (12.2%).

We then assessed the NRI for each marker for the entire risk spectrum and again for people at intermediate risk only. The largest differences were seen for CRP—12.0% NRI in the entire group and 28.4% NRI in the intermediate-risk group. Using sPLA2, the percentage of net correct reclassification was 6.4% in the entire risk spectrum and 16.3% in the intermediate-risk group.

DISCUSSION

We assessed the value of a number of inflammatory biomarkers—namely, CRP, MPO, PON, sPLA2, Lp-PLA2, fibrinogen, MCP-1 and adiponectin in the prediction of future CHD risk among apparently healthy men and women. CRP and sPLA2 performed best in correctly reclassifying people into clinically relevant risk categories. In addition, we observed that the various biomarkers behave differently when analysed across the entire risk spectrum compared with those subjects at intermediate risk only.

According to the Rose paradox, the majority of population-attributable risk occurs in the large group of people in the centre of the risk spectrum.[28] As a consequence, measures aimed at reducing risk factors at a population level are likely to improve public health by reducing population rates of CHD.[29] However, such measures are beyond the scope of doctors treating individual patients. Current guidelines focus on high-risk populations because relative risk reductions in these subjects result in large morbidity and mortality benefits. Preventive strategies aimed at all people in the intermediate-risk category would weigh too heavily on both the infrastructure and budget of healthcare institutions. More accurate risk prediction in people at intermediate risk is therefore warranted.

A substudy in the MONICA-Augsburg cohort suggested that CRP enhanced CHD risk prediction as assessed by the FRS, especially in intermediate-risk groups.[11] The addition of CRP to traditional risk factors has been shown to reclassify up to 20% of people at "intermediate risk" into higher- or lower-risk categories.[14] The recently published Reynolds Risk Score, which incorporates CRP and family history of premature CHD, reclassified 40–50% of all women estimated to be at intermediate risk by ATP-III criteria into higher- or lower-risk categories.[26] However, this study did not quantify NRI which incorporates the effects of upward, neutral and downward reclassification of cases and non-cases, leading to a net reclassification that provides a more accurate estimate than that obtained with other approaches. In our study, adding CRP to FRS led to an NRI of around 12% for the whole population and 28% for those in the intermediate-risk group. These results are consistent with the recent study by Wilson *et al*[27] which reported an NRI of 11.8% when CRP was added to traditional risk factors. However, this study did not show results restricted to the intermediate-risk FRS group. It should be noted, however, that unlike these cohort studies our study has a

Table 3 Global model fit statistics

	−2 Log likelihood	Hosmer–Lemeshow goodness-of-fit test	
		χ^2	Sigma
FRS			
All	3284.6	18	0.02
Intermediate	1253.6	12.9	0.1
FRS, CRP			
All	3242.7	20.3	0.009
Intermediate	1225.9	10.8	0.2
FRS, MPO			
All	3272	7.8	0.4
Intermediate	1252.3	4.8	0.8
FRS, PON			
All	3283.3	21.5	0.006
Intermediate	1252.5	5.0	0.8
FRS, sPLA2			
All	3253.1	8.5	0.4
Intermediate	1242.9	4.6	0.8
FRS, Lp-PLA2			
All	3280.2	8.7	0.4
Intermediate	1251.8	5.0	0.8
FRS, fibrinogen			
All	3263.6	8.2	0.4
Intermediate	1248.4	3.1	0.9
FRS, MCP-1			
All	3284.5	19.0	0.01
Intermediate	1253.5	4.9	0.8
FRS, adiponectin			
All	3283	13.8	0.09
Intermediate	1247.2	4.3	0.9

CRP, C-reactive protein; FRS, Framingham Risk Score; Lp-PLA2, lipoprotein-associated phospholipase A2; MCP-1, macrophage chemoattractant protein-1; MPO, myeloperoxidase; PON, paraoxonase; sPLA2, secretory phospholipase A2-type II.

case–control design. This approach leads to enrichment with cases, which may impact on net reclassification improvement.

Our finding that adding CRP to the FRS resulted in a statistically significant increase in the c statistic contrasts with other studies, which have shown only small, inconsistent or relatively moderate increments in the c statistic when CRP was added to the FRS or a set of established risk factors.[6 25 30] For instance, a recent systematic review of 31 prospective studies showed that CRP does not perform better than the Framingham risk equation for discrimination. The improvement in risk stratification or reclassification from addition of CRP to models based on established risk factors was small and inconsistent.[30]

In our study, we observed that the discriminatory characteristics of inflammatory biomarkers are substantially different when analysed among people at intermediate risk compared with the full risk spectrum. Our results imply that the clinical utility of such an approach cannot be extrapolated from studies performed across the entire risk spectrum. A number of recent studies have concluded that "novel" biomarkers are of limited use in CHD risk prediction, but those conclusions are not supported by the analyses provided. For instance, a recent study concluded that 10 biomarkers do not add predictive value over and above established risk factors.[31] The number of CHD cases in this study was relatively small and the analyses were not restricted to the relevant clinical subgroup—that is, people at intermediate risk. Similarly, a large analysis in the Reykjavik study concluded that CRP was a relatively moderate predictor of CHD.[5] That study also analysed the predictive value of biomarkers across the entire risk spectrum without providing data for the subgroup of people at intermediate risk.

There is a striking discrepancy between the performances of several inflammatory markers on the various statistical tests used, but CRP performed best in most analyses. For instance, CRP was the only biomarker that resulted in a statistically significant increase in the c statistic compared with FRS only. CRP also performed best in analyses of global model fit, including the lowest −2 log likelihood. sPLA2 also performed well in improving the c statistic compared with FRS only, although the differences were not statistically significant. This biomarker yielded the second lowest −2 log likelihood, but did not perform well on the Hosmer–Lemeshow goodness of fit test. Adding sPLA2 to the FRS resulted in the highest number of people at intermediate risk being correctly reclassified, followed by fibrinogen, Lp-PLA2, adiponectin and MPO.

Table 4 Reclassification by inflammatory biomarkers

		Reclassification for cases (%)			Reclassification for controls (%)			Net reclassification improvement
Biomarker	Risk category	Higher correctly	Lower incorrectly	Net correctly	Higher incorrectly	Lower correctly	Net correctly	Cases and controls combined (%)
CRP	All	20.8	18.7	2.1	16.9	26.8	9.9	12.0
	Intermediate	38.0	25.1	12.9	25.5	41	15.5	28.4
Myeloperoxidase	All	12.6	13.1	−0.5	13.5	16.4	2.9	2.4
	Intermediate	20.1	22.0	−1.9	17.1	25.4	8.3	6.2
Paraoxonase	All	5.5	4.7	0.8	5.1	2.2	−2.9	−2.1
	Intermediate	7.5	6.9	0.6	14.2	9.8	−4.4	−3.8
sPLA2	All	18.6	19	−0.4	17.2	24	6.8	6.4
	Intermediate	33.3	29.2	4.1	25.6	37.8	12.2	16.3
Lp-PLA2	All	8.5	6.7	1.8	8.9	8.8	−0.1	1.7
	Intermediate	14.5	9.7	4.8	11.6	15.6	4.0	8.8
Fibrinogen	All	17.5	14.4	3.1	16.1	17	0.9	4.0
	Intermediate	29.2	18.9	10.3	23.3	25	1.7	12.0
MCP-1	All	0.3	0.0	0.3	0.3	0.3	0.0	0.3
	Intermediate	0.9	0.0	0.9	0.3	0.4	0.1	1.0
Adiponectin	All	5.6	5.1	0.5	5.7	7.2	1.5	2.0
	Intermediate	8.8	8.5	0.3	5.6	2.7	7.1	7.4

Data are the number of people reclassified into a higher- or lower-risk category by using an inflammatory marker, compared with using the Framingham Risk Score only. CRP, C-reactive protein; Lp-PLA2, lipoprotein-associated phospholipase A2; MCP-1, macrophage chemoattractant protein-1; sPLA2, secretory phospholipase A2-type II.

Limitations

Several aspects of our study merit discussion. First, the c statistics were lower than seen in most other studies. This is because analyses were based on a prospective case–control set nested in the EPIC-Norfolk cohort. Controls were matched to cases by sex and age, thus making it impossible for these important risk factors to contribute to the c statistics. FRS is based on hard outcomes whereas the EPIC-Norfolk outcomes are a combination of hard and soft outcomes. This may have inflated our risk estimates. However, the c statistics for the FRS in the entire EPIC-Norfolk cohort were 0.71 for both men and women, which is consistent with other general population cohorts.[32] We used a nested case–control study to assess the role of inflammatory markers in risk prediction. This study design allowed us to study relative risk prediction only. In order to assess the contribution of risk factors to the prediction of absolute risk in populations, a prospective study with population denominators is required. Moreover, our study has a case–control design which by definition leads to enrichment with cases. Because NRI quantifies the combined effect of reclassification in cases and controls combined, our design may have had an impact on the NRI.

CONCLUSION

We found that a number of inflammatory markers, especially CRP, may be useful in the more accurate prediction of future CHD risk among apparently healthy men and women, compared with the FRS which is based on established risk factors only. The discriminatory potential of inflammatory biomarkers was substantially different when analysed across the entire risk spectrum compared with the subgroup of people at intermediate risk. Because novel biomarkers may have a role in clinical decision-making among people at intermediate risk, they need to be evaluated among people in this group in prospective cohort studies and not across the entire risk spectrum.

Acknowledgements: EN is Director of Research of Centre National de la Recherche Scientifique. We thank Hervé Durand (INSERM) for excellent technical assistance.

Funding: EPIC-Norfolk is supported by program grants from the Medical Research Council UK and Cancer Research UK and with additional support from the European Union, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency and the Wellcome Trust. PJT is supported by the British Heart Foundation (PG2005/014). Part of this work was supported by the Institut National de la Santé et de la Recherche Médicale.

Competing interests: None.

Ethics approval: Approval from the Norwich District Health Authority ethics committee.

The funding sources had no role in study design, conduct, analysis and decision to submit the manuscript for publication.

Provenance and peer review: Not commissioned; externally peer reviewed.

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