Chapter 3

C-reactive protein and angiographic characteristics of stable and unstable coronary artery disease – Data from the prospective PREVEND cohort


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Chapter 3

Abstract

Background
High sensitive-C-reactive protein (hs-CRP) is associated with coronary risk, which may be explained by an association with (unstable) coronary artery disease (CAD). Until now, histopathological and angiographic studies have failed to consistently demonstrate a strong relationship. However, most of these studies were limited by a cross-sectional design. Our aim was to prospectively evaluate the association between hs-CRP and plaque instability. Therefore, firstly, we investigated the relation between hs-CRP measured long before coronary angiography (CAG) and angiographic characteristics of stable and unstable CAD. In addition, we investigated the association with coronary events during follow up in the total PREVEND population.

Methods and results
Of the population based Prevention of REnal and Vascular ENdstage Disease (PREVEND) study, 8,139 subjects without previous documented CAD were followed for the incidence of CAG and coronary events from 1997-2003. For the qualitative angiographic analysis, 216 CAGs were available. Mean time to CAG was 37±19 months. The 864 coronary vessels were graded as follows: 436 coronary vessels as normal, 175 as non-obstructive CAD, 179 as stable obstructive CAD and 74 as unstable obstructive CAD. Multilevel ordinal regression analysis was performed to study associations between baseline clinical variables and angiographic findings. Hs-CRP contributed significantly to the multivariate model after adjustment for age, gender, smoking, lipids and blood pressure. In 8,139 subjects, two-hundred-and-one (2.5%) first coronary events occurred during follow up. Cox survival analysis showed age- and sex- adjusted hazard ratios for hs-CRP 1-3 mg/L and >3 mg/L of, respectively, 1.26 (95% CI 0.67-2.40) and 3.16 (95% CI 1.26-3.16), relative to hs-CRP <1 mg/L.

Conclusions
In the prospective PREVEND study of subjects without previous documented CAD, hs-CRP levels at baseline were associated with angiographic characteristics and clinical consequences of plaque instability during follow up. This observation supports the concept that hs-CRP significantly contributes to coronary atherogenesis.
Introduction

High sensitive-C-reactive protein (hs-CRP) has been shown to be an independent predictor of future coronary risk. Elevated levels of hs-CRP are present in many patients with an acute ischemic event, and have been associated with a poor prognosis. It has been suggested that elevated hs-CRP in these patients reflects the extent of plaque burden, or presence of multiple unstable coronary plaques, associated with an adverse outcome. Conflicting evidence exists on the active biological role of hs-CRP in atherogenesis. Until now, angiographic, histopathological and other studies have failed to consistently demonstrate a strong relationship between hs-CRP levels and coronary plaque burden. Correlations with extent of stable coronary plaque burden were small or absent. Correlations with unstable plaques were weak and diminished after adjustment for other risk factors, or were only present in patients presenting with acute coronary syndromes, with the exception of one study. However, most of these studies have been based on a cross-sectional design. Hs-CRP and other variables were measured simultaneously with coronary angiography (CAG) for stable or unstable angina, or were derived from post-mortem serum evaluation. The hs-CRP levels may have been elevated due to the acute phase responses present in patients with ischemic symptoms, which limits any conclusion on an association of causality between hs-CRP and coronary plaque burden. Therefore, we aimed to investigate whether in subjects without clinical evidence of atherosclerosis, levels of hs-CRP and other clinical variables, measured at time of inclusion in a prospective population based cohort study, are associated with angiographic characteristics of stable and unstable coronary artery disease (CAD), as documented by a first CAG during 5.5 years of follow up. This aim was studied in PREVEND participants who underwent CAG during follow up. Additionally, in the total PREVEND population, we prospectively evaluated the association between hs-CRP and the occurrence of a first coronary event.

Methods

Study population

The Prevention of REnal and Vascular ENdstage Disease (PREVEND) study is a population-based cohort study in Groningen, the Netherlands, which primary aim is to assess the value of urinary albumin excretion in relation to cardiovascular and renal risk. During the period 1997–1998, all inhabitants of the city of Groningen, The Netherlands aged between 28 and 75 years were asked to answer a short questionnaire and to send in a morning urine sample. Insulin treatment and pregnancy were exclusion criteria. Altogether 40,856 subjects responded. All subjects with a urinary albumin excretion (UAE) of at least 10 mg/L (n=7,768) and a random sample of subjects with UAE less than 10 mg/L were invited to an outpatient clinic. The screening program was completed by 8,592 subjects. Collected baseline data at the
outpatient clinic included medical history, including the presence of a malignancy and liver disease, but no information was obtained on the presence of rheumatoid arthritis or other inflammatory diseases. Further collected baseline data included demographics, use of medications, biometric data, urine- and blood collections and laboratory measurements. In case of flu and/or a febrile temperature, blood collection was postponed to a later time when participants had recovered. Of 8,592 subjects included, 8,139 participants were without previous documented CAD and were included in the current analysis. Previous documented CAD was defined as history of myocardial infarction, revascularisation procedure or obstructive coronary artery disease, prior to inclusion in the PREVEND study. A history of myocardial infarction was based on a subjects’ medical history, including structured questionnaire, and the information on previous CAD was complemented by review of the medical report. For details on the PREVEND study design we refer to earlier publications. The PREVEND study was approved by the medical ethics committee and conducted in accordance with the guidelines of the declaration of Helsinki. All participants gave written informed consent.

**Coronary angiography**

During 1997-2003, all CAGs performed in the only two hospitals in the Groningen region, namely the University Medical Center Groningen and the Martini Hospital Groningen, were collected in order to perform qualitative angiographic analysis. Of 240 performed first CAGs during 5.5 years of follow up, 216 CAGs were available for angiographic analysis (90%). CAGs for all indications were included in the analysis. These indications were reviewed by a clinical event committee and divided into stable angina, non-ST-elevation acute coronary syndrome (defined as chest pain with positive cardiac markers (troponin or creatinin kinase) and/or dynamic ST-segment changes), and ST-elevation myocardial infarction (defined as chest pain and ST-elevation over 1 mm in at least 2 contiguous leads).

**Qualitative angiographic analysis**

Qualitative coronary angiographic analysis was performed by a senior cardiologist (RT), who had no knowledge of the clinical indications for CAG or of the patients’ clinical status. By angiographic analysis, the severity of CAD was assessed in all four coronary vessels, namely the left main stem, left anterior descending artery, left circumflex artery and right coronary artery. Severity of CAD was graded as follows: normal coronary arteries (absence of any coronary plaque), non-obstructive CAD (less than 50% stenosis), stable obstructive CAD (at least 50% stenosis) and unstable obstructive CAD. Plaques were considered unstable if they caused at least 50% stenosis and had two or more of the following morphologic features: an intraluminal filling defect consistent with thrombus, defined as abrupt vessel cut-off with persistence of contrast, or an intraluminal filling defect in a patent vessel within or adjacent to a stenotic region with surrounding homogeneous contrast opacification; plaque ulceration, defined by the presence of contrast and hazy contour beyond the vessel lumen; plaque irregularity, defined by irregular margins or overhanging edges; and
impaired flow. Plaques were considered stable if less than two of these morphologic features were present. In case 2 or more coronary plaques were found in a coronary vessel, the most severe angiographic plaque was registered. An interobserver agreement of 95% was found in a random sample of 44 CAGs (20%) which were analysed by a second senior cardiologist (FZ) unaware of the prior analyses. To evaluate whether the group of patients with normal coronary arteries would be representative for PREVEND participants without a CAG or coronary event during follow up, the baseline characteristics of both groups were compared.

**Coronary events**
All 8,139 participants were followed for 5.5 years for the occurrence of coronary events, defined as myocardial infarction or cardiovascular death. The vital status of all participants was evaluated through the municipal register until December 31st 2003. Causes of death were obtained from the Central Bureau of Statistics according to ICD-10 codes (I01-I99 for cardiovascular disorders). Information related to myocardial infarctions (ICD-9 410, 411) were obtained from the national hospital information system (Prismant, Utrecht, the Netherlands). All cardiac events were reviewed by a clinical event committee.

**Analytical methods and definitions**
Systolic and diastolic blood pressure measurements were calculated as the mean of the last two out of ten consecutive measurements with an automatic Dinamap XL model 9300 series device (Johnson-Johnson Medical INC, Tampa, Florida). Fasting serum total cholesterol was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York, U.S.A.). HDL-cholesterol was determined by MEGA (Merck, Darmstadt, Germany). High sensitive C-reactive protein (hs-CRP) was measured by nephelometry with a threshold of 0.18 mg/L and intra- and interassay coefficients of variation of <4.4 and <5.7% respectively (BNII, Dade Behring, Marburg, Germany). The urinary albumin excretion was measured as the mean of two 24h-urine collections. Urinary albumin concentrations were determined by nephelometry with a threshold of 2.3 mg l$^{-1}$ and intra- and inter-assay coefficients of variation of less than 2.2% and 2.6%, respectively (Dade Behring, Marburg, Germany). Renal dysfunction was defined as creatinin clearance <60 ml/min/1.73 m$^2$. Smoking status included current and past smoking. Ever smoking is defined as current or past smoking. Antihypertensive medication included diuretics, betablockers, ACE-inhibitors and angiotensin receptor blockers.

**Statistical analysis**

**Baseline characteristics**
Continuous data were given as means (standard deviation). In case of a skewed distribution the median (interquartile range) are presented. Differences between
groups were evaluated by Chi-square tests or analysis of variance. Differences in hs-CRP levels between subjects with stable and unstable CAD were tested after logarithmic transition by one-way ANOVA with Bonferroni correction. P-values were two-sided and needed to be <0.05 to be significant. SPSS version 11.0 software (SPSS, Chicago, IL, USA) was used for these analyses.

**Association between hs-CRP and coronary plaque characteristics in PREVEND participants who underwent CAG during follow up**

**Cox survival analysis** Cox proportional hazard models were fitted to evaluate associations between clinical variables at baseline and time to CAG showing an obstructive coronary plaque, and an unstable obstructive coronary plaque, respectively, in 216 patients (at a patient level). The proportional hazard assumption was assessed for every predictor variable using graphical approaches. Proportional hazard was assumed when the log-log-survival curve was found constant over time. Hs-CRP was log transformed for this analyses because the relationship between hs-CRP levels and the endpoint was not of linear nature. A stepwise model was used, introducing a variable if p<0.15, first entering the variables with the highest Wald statistics. If p>0.10, a variable was excluded from the model. The variables introduced in the model were age, sex, smoking status, total cholesterol, diastolic blood pressure, HDL cholesterol, hs-CRP, diabetes, microalbuminuria and waist circumference. Moreover, a variable was introduced which represented one of the factors reported in the PREVEND database which may have influenced hs-CRP levels, namely renal dysfunction, malignancy, liver disorder, antihypertensive or lipid lowering medication or aspirin. Event-free survival time for participants was defined as the period between inclusion in the study and CAG. SPSS version 11.0 software (SPSS, Chicago, IL, USA) was used for these analyses.

**Multilevel ordinal logistic regression analysis** Multilevel ordinal logistic regression analysis is appropriate for hierarchical data to take into account the clustering of angiographic plaques per vessel in a patient. We performed this analysis in order to evaluate associations between clinical variables at baseline and severity of CAD on a vessel level, after adjustment for the patient level. In our dataset, each subject (higher level; n=216) has four coronary vessels (lower level; n=864). The multilevel ordinal logistic model evaluates the association of baseline clinical variables with the type of angiographic plaque per vessel, expressed as outcome variable in 4 categories (no CAD, non-obstructive CAD, obstructive stable CAD and obstructive unstable CAD). Multilevel ordinal logistic regression analysis was performed in all patients in whom all clinical variables of interest had been measured. The modelling strategy was to first enter demographic variables (p<0.05). Secondly, other clinical variables were entered and were retained in the model in case of significance (p<0.05). These included smoking status, total cholesterol, diastolic blood pressure, HDL cholesterol, hs-CRP, diabetes, microalbuminuria, waist circumference and time between inclusion to PREVEND and performance of CAG. Moreover, as was done for the Cox survival analysis, a variable was introduced which represented one of the factors reported in the PREVEND database which may have influenced hs-CRP levels, namely renal...
dysfunction, malignancy, liver disorder, antihypertensive or lipid lowering medication or aspirin. The proportional odds assumption was satisfied for all clinical variables, except for smoking status. From the 3 logits the cumulative response probabilities of the categorical outcome variable (angiographic types of plaque) were estimated (2nd order PQL estimation). MlwiN, version 2.0, was used for the multilevel analysis.

**Association between hs-CRP and the occurrence of a first coronary event in the total PREVEND population**

**Cox survival analysis** Probability weighted Cox proportional hazard models were fitted to evaluate independent predictors of time to a first coronary event taking into account adjustment for the non-random inclusion of subjects with and without elevated UAE at study entry. The proportional hazard assumption was assessed using graphical approaches. Hs-CRP was introduced as a categorical variable after adjustment for age and sex. Event-free survival time for participants was defined as the period from the date of the outpatient clinic baseline assessment to the date of first coronary event, or death from any cause until 31 December 2003, or 31 December 2002 until which date information regarding specific causes of death follow up information was available. If a person had moved away from the city of Groningen or to an unknown destination, the person was censored on the last available contact date. STATA version 10.0 software (STATA, College Station, Texas, USA) was used for this analysis.

**Results**

**Baseline characteristics**

In 216 PREVEND participants a first CAG was available for angiographic analysis. Mean time between inclusion into PREVEND and performance of first CAG was 37±19 months. Severity of CAD in 216 patients and in their 864 coronary vessels, respectively, is shown in table 1. Of 216 patients, 35 patients had normal coronary arteries. In 42 patients, non-obstructive CAD was found. In 139 patients, obstructive CAD was present, of whom at least one unstable plaque was present in 60 patients. Of 864 coronary vessels, 436 vessels were free from CAD. In 175 vessels non-obstructive CAD was found. In 179 stable coronary plaques were found, while in 74 vessels unstable CAD was present. Unstable plaques contained thrombi in 84%, impaired flow in 56%, irregularity in 80% and ulceration in 31%. Baseline characteristics according to severity of CAD are shown in table 2. Age, male gender, blood pressure, total cholesterol, HDL cholesterol, smoking status at baseline, and clinical presentation were significantly different between the groups, while hs-CRP levels showed a trend \( P_{\text{trend}} = 0.079 \). The hs-CRP levels of subjects with stable and unstable CAD were not significantly different \( (2.54 \text{ and } 2.39 \ (p=1.00)) \). Patients with unstable CAD had an adverse risk profile at baseline, while only few were receiving lipid lowering and/or antihypertensive medication. Microalbuminuria was not significantly different between the groups. All patients with normal coronary
arteries had undergone CAG for symptoms compatible with stable angina. Indications for CAG in patients with obstructive unstable plaques were non-ST-elevation acute coronary syndrome or ST-elevation myocardial infarction in 75% of cases. Regarding the baseline characteristics, the reference group (35 patients with normal coronary arteries) was comparable to the control group (7,916 PREVEND participants without CAG or cardiac event during follow up) (table 3).

**Table 1. Angiographic characteristics in 216 PREVEND participants and in their 864 coronary vessels.**

<table>
<thead>
<tr>
<th>Angiographic characteristics (in 216 patients)</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 unstable obstructive plaque</td>
<td>60/216</td>
<td>(28)</td>
</tr>
<tr>
<td>≥1 stable obstructive plaque</td>
<td>79/216</td>
<td>(37)</td>
</tr>
<tr>
<td>≥1 non-obstructive plaque</td>
<td>42/216</td>
<td>(19)</td>
</tr>
<tr>
<td>Without coronary artery disease</td>
<td>35/216</td>
<td>(16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Angiographic characteristics (in 864 coronary vessels)</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 unstable obstructive plaque</td>
<td>74/864</td>
<td>(9)</td>
</tr>
<tr>
<td>≥1 stable obstructive plaque</td>
<td>179/864</td>
<td>(20)</td>
</tr>
<tr>
<td>≥1 non-obstructive plaque</td>
<td>175/864</td>
<td>(20)</td>
</tr>
<tr>
<td>Without coronary artery disease</td>
<td>436/864</td>
<td>(51)</td>
</tr>
</tbody>
</table>

Association between hs-CRP and coronary plaque characteristics in PREVEND participants who underwent CAG during follow up

**Cox survival analysis** In 200 out of 216 PREVEND participants of whom the first CAG was available for angiographic analysis, all clinical variables of interest had been measured at baseline and were included in the analyses. Multivariate Cox regression analyses showed that hs-CRP levels at a subject level were associated with time to CAG showing an obstructive coronary plaque, and an obstructive unstable coronary plaque, respectively (table 4). Of all variables introduced in the model, the variables mentioned in table 4 significantly added to the model. There was no interaction with hs-CRP.

**Multilevel ordinal regression analysis** Additionally, the multilevel ordinal logistic regression analysis in 800 vessels of these 200 patients showed that hs-CRP, age, sex, smoking status, total cholesterol, diastolic blood pressure and HDL cholesterol at baseline were significantly associated with the angiographic outcome variable, measured at CAG during follow up. The other variables introduced were not associated with the angiographic outcome variable. There was no interaction with hs-CRP. The multilevel regression model allows the estimation of absolute probabilities and odds ratios for an angiographic type of plaque (i.e. no CAD, non-obstructive CAD, obstructive stable CAD and obstructive unstable CAD) in the presence of certain clinical variable. In figure 1, the absolute probabilities and odds ratios for the presence
Table 2. Baseline characteristics of 216 PREVEND participants according to angiographic characteristics of coronary artery disease*.

|                          | Reference Group (normal coronary arteries) | Non-obstructive coronary artery disease | Stable obstructive coronary artery disease | Unstable obstructive coronary artery disease | Ptrend  
|--------------------------|--------------------------------------------|----------------------------------------|--------------------------------------------|----------------------------------------------|--------
|                          | n=35                                       | n=42                                   | n=79                                       | n=60                                         |        
| Age, mean (SD), y        | 53 (12)                                    | 60 (10)                                 | 59 (10)                                    | 60 (9)                                       | 0.007  
| Male gender, No. (%)     | 13 (37)                                    | 23 (55)                                 | 61 (77)                                    | 41 (68)                                      | 0.001  
| Blood pressure, mean (SD), mm Hg |                          |                                        |                                            |                                              |        
| Systolic                 | 128 (19)                                   | 144 (24)                                | 140 (21)                                   | 142 (21)                                     | 0.004  
| Diastolic                | 74 (10)                                    | 80 (10)                                 | 79 (8)                                     | 81 (9)                                       | 0.001  
| Smoking status, No. (%)  |                                           |                                        |                                            |                                              | 0.045  
| Current                  | 15 (42)                                    | 13 (31)                                 | 38 (48)                                    | 31 (52)                                      |        
| Past                     | 11 (31)                                    | 16 (38)                                 | 29 (37)                                    | 24 (40)                                      |        
| Diabetes, No. (%)        | 2 (6)                                      | 2 (5)                                   | 2 (3)                                      | 5 (8)                                        | 0.593  
| Waist circumference, cm  | 89 (11)                                    | 94 (13)                                 | 98 (12)                                    | 95 (11)                                      | 0.003  
| Cholesterol, mean (SD), mmol/L |              |                                        |                                            |                                              |        
| Total                    | 5.9 (1.1)                                  | 6.0 (1.1)                               | 6.3 (0.9)                                  | 6.6 (1.2)                                    | 0.003  
| HDL                      | 1.27 (0.37)                                | 1.17 (0.35)                             | 1.11 (0.31)                                | 1.09 (0.35)                                  | 0.012  
| Albuminuria, median (interquartile range), mg/24h | 9.1 (6.4-17.9)                           | 10.7 (8.4-41.5)                         | 14.0 (8.3-33.8)                             | 15.0 (7.1-28.1)                              | 0.953  
| hs-CRP, median (interquartile range), mg/L | 1.59 (0.70-2.58)                         | 1.98 (1.18-3.53)                        | 2.54 (1.14-5.24)                            | 2.39 (1.24-7.19)                             | 0.079  
| Medication, No. (%)      |                                           |                                        |                                            |                                              |        
| Lipidlowering            | 3 (9)                                      | 5 (12)                                  | 10 (13)                                    | 5 (8)                                        | 0.934  
| Antihypertensive†         | 4 (11)                                     | 14 (33)                                 | 24 (30)                                    | 12 (20)                                      | 0.600  
| ACE inhibitors/angiotensin receptor blockers | 3 (9)                                     | 4 (10)                                 | 9 (11)                                     | 3 (5)                                        | 0.610  
| Aspirin                  | 0 (0)                                      | 2 (5)                                   | 8 (10)                                     | 5 (8)                                        | 0.078  
| Clinical presentation, No. (%)     |              |                                        |                                            |                                              | < 0.001  
| Stable angina            | 35 (100)                                   | 38 (90)                                 | 44 (56)                                    | 15 (25)                                      |        
| Non-ST-elevation acute coronary syndrome | 0 (0)                                     | 2 (5)                                   | 18 (23)                                    | 29 (48)                                      |        
| ST-elevation myocardial infarction | 0 (0)                                     | 2 (5)                                   | 17 (21)                                    | 16 (27)                                      |        

*Baseline characteristics were measured during inclusion in PREVEND cohort study. Mean time between inclusion and coronary angiography during follow up was 37±19 months. †including ACE inhibitors and angiotensin receptor blockers. Abbreviations: HDL, high density lipoprotein; hs-CRP, high sensitivity-C-reactive protein.
SI conversion factor: to convert mmol/L to mg/dL, divide values for total cholesterol and HDL cholesterol by 0.0259.
Table 3. Comparison of baseline characteristics of the reference group without coronary artery disease (n=35) versus a control group of PREVEND participants (n=7,916) who remained free from coronary angiography or coronary event during follow up.

<table>
<thead>
<tr>
<th></th>
<th>Reference group (normal coronary arteries at coronary angiography) (n=35)</th>
<th>PREVEND control group (n=7,916)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>53 (12)</td>
<td>49 (12)</td>
<td>0.054</td>
</tr>
<tr>
<td>Male gender, No. (%)</td>
<td>13 (37)</td>
<td>3836 (49)</td>
<td>0.181</td>
</tr>
<tr>
<td>Blood pressure, mean (SD), mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>128 (19)</td>
<td>128 (20)</td>
<td>0.812</td>
</tr>
<tr>
<td>Diastolic</td>
<td>74 (10)</td>
<td>74 (10)</td>
<td>0.811</td>
</tr>
<tr>
<td>Smoking status, No. (%)</td>
<td>Current 15 (42)</td>
<td>2684 (34)</td>
<td>0.272</td>
</tr>
<tr>
<td></td>
<td>Ever 26 (74)</td>
<td>5482 (70)</td>
<td>0.540</td>
</tr>
<tr>
<td>Diabetes, No. (%)</td>
<td>2 (6)</td>
<td>25 (3)</td>
<td>0.435</td>
</tr>
<tr>
<td>Cholesterol, mean (SD), mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.9 (1.1)</td>
<td>5.6 (1.1)</td>
<td>0.141</td>
</tr>
<tr>
<td>HDL</td>
<td>1.27 (0.37)</td>
<td>1.33 (0.40)</td>
<td>0.319</td>
</tr>
<tr>
<td>Albuminuria, median (interquartile range), mg/24h</td>
<td>9.1 (6.4-17.9)</td>
<td>9.23 (6.3-16.8)</td>
<td>0.833</td>
</tr>
<tr>
<td>hs-CRP, median (interquartile range), mg/L</td>
<td>1.59 (0.70-2.58)</td>
<td>1.21 (0.54-2.83)</td>
<td>0.274</td>
</tr>
<tr>
<td>Medication, No. (%)</td>
<td>Lipidlowering 3 (9)</td>
<td>366 (5)</td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td>Antihypertensive 4 (11)</td>
<td>28 (11)</td>
<td>0.821</td>
</tr>
</tbody>
</table>

Abbreviations: HDL, high density lipoprotein; hs-CRP, high sensitivity- C-reactive protein. 
SI conversion factor: to convert mmol/L to mg/dL, divide values for total cholesterol and HDL cholesterol by 0.0259.

Table 4a. Univariate- and multivariate predictors for the presence of an obstructive plaque (by Cox survival analysis).

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>(95% CI)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Male versus female sex</td>
<td>1.38</td>
<td>(0.96-2.00)</td>
<td>0.084</td>
<td>1.15</td>
</tr>
<tr>
<td>Hs-CRP, per log mg/L</td>
<td>1.22</td>
<td>(1.02-1.48)</td>
<td>0.027</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Table 4b. Univariate- and multivariate predictors for the presence of an obstructive unstable plaque (by Cox survival analysis).

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>(95% CI)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Age, per year</td>
<td>1.03</td>
<td>(1.00-1.05)</td>
<td>0.049</td>
<td>1.03</td>
</tr>
<tr>
<td>Total cholesterol, per mmol/L</td>
<td>1.32</td>
<td>(1.03-1.69)</td>
<td>0.029</td>
<td>1.38</td>
</tr>
<tr>
<td>Diastolic blood pressure, per mmHg</td>
<td>1.03</td>
<td>(1.00-1.06)</td>
<td>0.077</td>
<td>1.03</td>
</tr>
<tr>
<td>Hs-CRP, per log mg/L</td>
<td>1.38</td>
<td>(1.06-1.60)</td>
<td>0.017</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Abbreviation: hs-CRP, high sensitivity- C-reactive protein.
of a stable and unstable plaque are shown. E.g. males have a higher probability of stable and unstable coronary artery disease compared to females. With increasing age, the probabilities of stable and unstable plaques increase from 7% to 18% and from 3% to 9%, respectively. Smoking was only associated with non-obstructive coronary artery disease. With increasing total cholesterol levels, the probabilities of stable and unstable plaques increase from 13% to 26% and from 3% to 9%, respectively. Increasing hs-CRP levels are associated with an increase in the probabilities of stable and unstable coronary plaques from 19% to 26% and from 5% to 8%, respectively. Figure 1 shows that the highest probabilities for stable and unstable coronary plaques are related to age and high total cholesterol, and the lowest to elevated hs-CRP.

*except for hs-CRP: the 5th, 50th and 95th percentiles are presented for hs-CRP due to skewness of the distribution. †at the mean of other covariates.
Association between hs-CRP and the occurrence of a first coronary event in the total PREVEND population

Cox survival analysis. During 5.5 years of follow up, 201 (2.5%) coronary events occurred. In 7,722 (95%) subjects, hs-CRP had been measured and in 184 of these subjects a coronary event occurred. Coronary events occurred in 34 (1.0%) of 3,320 subjects with hs-CRP<1 mg/L; in 75 (2.9%) of 2,554 subjects with hs-CRP 1-3 mg/L; and in 75 (4.1%) of 1,848 subjects with hs-CRP>3 mg/L. Cox survival analysis showed that hs-CRP was significantly associated with the occurrence of a first coronary event. Age- and sex- adjusted hazard ratios for hs-CRP 1-3 mg/L and >3 mg/L were, respectively, 1.26 (95% CI 0.67-2.40) and 3.16 (95% CI 1.26-3.16), relative to hs-CRP <1 mg/L.

Discussion

Principal findings

In the PREVEND population based cohort study, in patients without previous documented CAD, hs-CRP levels at baseline were independently associated with angiographic characteristics of stable and unstable CAD at a first CAG during 5.5 years of follow up. Our results support the concept that hs-CRP significantly contributes to coronary atherogenesis. Furthermore, our results imply that the increased coronary risk due to hs-CRP is, at least in part, associated with coronary plaque burden. The association between hs-CRP and plaque instability was corroborated by the significant association between hs-CRP and the occurrence of a first coronary event.

Hs-CRP and coronary plaque burden

In the last decade, inflammation parameter hs-CRP has been proven to be an independent predictor of coronary risk. Although hs-CRP may be related to various systemic processes, such as insulin resistance and obesity, infections, and a prothrombotic state, recent studies have suggested an active biological role in atherogenesis. Hs-CRP has been found present in advanced atherosclerotic plaques. Hs-CRP has been shown to promote the uptake of oxidized LDL cholesterol. Furthermore, hs-CRP has pro-inflammatory and protrombotic effects on the vessel wall, implying a role in plaque vulnerability. However, the evidence on the role of hs-CRP in atherogenesis is conflicting. Angiographic and histopathological studies could not consistently demonstrate an association with the extent of coronary plaque burden and unstable coronary plaques, making the association with coronary plaque burden a controversial issue. Furthermore, the increased coronary risk associated with elevated hs-CRP levels was independent from the extent of atherosclerotic plaque burden. Whether hs-CRP should be regarded as marker of CAD activity in patients with stable or unstable angina is therefore not yet clear. Additionally, none of these studies have been performed in patients without clinically established atherosclerosis. However, we question the design chosen in most of these studies, since these are cross-
sectional data, which lack time dependency. The uniqueness of our study design is characterised by two aspects. First, the study population consists of patients previous documented CAD. Second, the measurements of hs-CRP and other clinical variables have been performed at a mean time period of 3 years before the performance of a first CAG. The angiographic outcome variable chosen was a combination of severity of luminal stenosis and characteristics of plaque instability, ranging from no or non-obstructive CAD to obstructive stable or unstable CAD. Unexpectedly, hs-CRP levels in subjects with stable and unstable CAD were of the same magnitude. This may be due to the fact that inflammatory processes are not only associated with plaque instability, but also play a role in plaque growth. Furthermore, our small sample size may have played a role. Since subjects with unstable CAD had an adverse coronary risk profile at baseline, we included established coronary risk factors and use of medication in the regression analyses, in order to evaluate whether these factors could have influenced the association between hs-CRP and the outcome variable. By use of multilevel regression analysis, it turned out that hs-CRP was independently associated with angiographic characteristics of stable and unstable coronary plaque burden. Therefore, our results confirm the hypothesis that hs-CRP is a marker of CAD activity in a population without prior documented CAD.

Beside the uniqueness of our study design, another new aspect of our study is that we were able to estimate the absolute probability on a certain type of coronary plaque by use of the multilevel regression model. These probabilities reflect the clinical relevance of the parameter. Figure 1 illustrates that dependent on the magnitude of risk factors at baseline, the absolute probability of a stable coronary plaque could range between 7 and 34%, while the probability of an unstable obstructive plaque could range between 3 and 15%. The ranges of the probabilities given in figure 1 show that hs-CRP is statistically and clinically associated with the angiographic outcome variable. This observation confirms that atherosclerosis is a multifactorial process, in which inflammation plays an independent role.

Our data are restricted to subjects who underwent CAG during follow up, but seem generalizable to the whole study population, since the patients with normal angiographic findings were comparable to subjects who remained free from CAG or major adverse cardiac event during follow up. We realise that the reference group is a somewhat more homogenous sample due to the selection of subjects with a CAG when compared to the control group, which is reflected by the wider dispersion of hs-CRP levels present in the control group. The association between hs-CRP and coronary plaque characteristics as indicators for plaque instability was corroborated by the significant association between hs-CRP and occurrence of a first coronary event in the total PREVEND study population. This is of importance, since a coronary event most frequently results from coronary plaque instability.

**Hs-CRP, coronary plaque burden and causality**

A first question that remains to be answered is whether our results imply a causal relationship with coronary atherogenesis. Our results contribute to some of the criteria for causality as have been defined by Hill. First, in the association between
hs-CRP and angiographic CAD, hs-CRP was measured before the performance of CAG, thereby fulfilling the criterion of temporality. Second, the association found was independent from other risk factors, thereby contributing to the criterion of strength. Furthermore, elevated hs-CRP levels were associated with a higher probability of angiographic characteristics of stable and unstable CAD, supporting a dose-response relationship. Our results are in line with another prospective study, which included patients with a first CAG and showed that hs-CRP was associated with progression of CAD, thereby contributing to the criterion of consistency. Additionally, our results confirm recent studies that have suggested that hs-CRP may play an active biological role in atherogenesis and plaque vulnerability, thereby contributing to the criteria of plausibility and coherence.

Our study design does not allow to comment the other criteria. It is not clear yet whether lowering hs-CRP levels will result in a reduction of CAD, although recent trials have shown promising results. Furthermore, we did not study analogous factors or specificity of the effect. Therefore, our results favour some of the criteria supporting a causal relationship, but do not provide a definite answer.

Hs-CRP, coronary plaque burden and coronary risk
A second question that remains to be answered is whether our results imply that the increased coronary risk associated with hs-CRP is due to its association with coronary plaque burden. Our results support the association with coronary plaque burden. However, morphologic coronary plaque characteristics and clinical outcome are not always closely related. Although autopsy studies have reported a heterogeneity in types of unstable coronary plaques in patients with a fatal myocardial infarction, in some patients the coronary arteries were characterised by an absence of unstable plaque characteristics. Most unstable plaques are found in patients with acute myocardial infarction and unstable angina. However, unstable plaques are also found in patients with symptoms compatible with stable angina, albeit less frequently. Furthermore, a time delay up to several weeks has been demonstrated between the occurrence of plaque rupture and first symptoms of acute myocardial infarction. Even, it has been shown that in many cases ruptured plaques do not cause symptoms at all, but heal silently. Therefore, whether plaque instability leads to a clinical ischemic event may depend on the interaction between morphologic coronary plaque characteristics and systemic factors, such as factors involved in inflammation, coagulation and fibrinolysis, lipids and blood pressure, physical activity, and diet. Since it is known that hs-CRP is associated with some of these processes, the impact of hs-CRP on coronary risk may depend on its association with plaque burden as well as on its association with the systemic processes involved in atherothrombosis. Our results therefore support that the increased coronary risk due to hs-CRP is at least in part dependent on its association with coronary plaque burden.

Limitations
In PREVEND, diabetes was not associated with the angiographic outcome variables. This observation may be explained by the fact that only very few diabetes patients
were included. Furthermore, only type 2 diabetes patients and mostly female subjects, not using insulin were included, reflecting a low coronary risk when compared to diabetes patients in general. In the multilevel ordinal model, smoking status was only associated with non-obstructive CAD, in contrast to obstructive CAD. It should be studied whether this observation can be confirmed in future studies. High-sensitive CRP may also be associated with plaque instability in other vascular regions, but the focus of the current manuscript has been on coronary artery disease. We included subjects who underwent CAG for stable and unstable presentations of coronary disease during follow up, to be able to include a wide range of types and severity of coronary plaques. Unfortunately, subgroups were too small to allow a stratified analysis. In our analyses we were able to adjust for most, but unfortunately not all, factors which could have influenced hs-CRP levels at time of study entry. Coronary angiography, although very useful in coronary risk stratification, can not be regarded as a true gold standard for identifying advanced CAD and unstable plaques, as it only shows the intravascular lumen, and not the diseased vessel wall. Coronary angiography is limited by the fact that early atherosclerosis may result in positive remodelling of the coronary artery, which may be missed by CAG since narrowing of the coronary lumen only occurs in more advanced atherosclerosis. Coronary angiography is not suitable to detect unstable characteristics of non-obstructive lesions, which is illustrated by the observation that acute coronary events may occur due to rapid progression of lesions that have been shown to be non-obstructive on a previous CAG. Nevertheless, for an evaluation of CAD in a prospective cohort study CAG is at this moment in time the most suitable technique, although non-invasive techniques such as multidetector computed tomography coronary angiography will become more important in the near future. Furthermore, the clinical impact of severity of CAD as detected by CAG is very well documented.38

**Conclusion**

This substudy of the PREVEND study shows that in subjects without previous documented CAD hs-CRP is associated with angiographic characteristics of stable and unstable coronary plaque burden.
Reference list


C-Reactive protein and coronary artery disease


