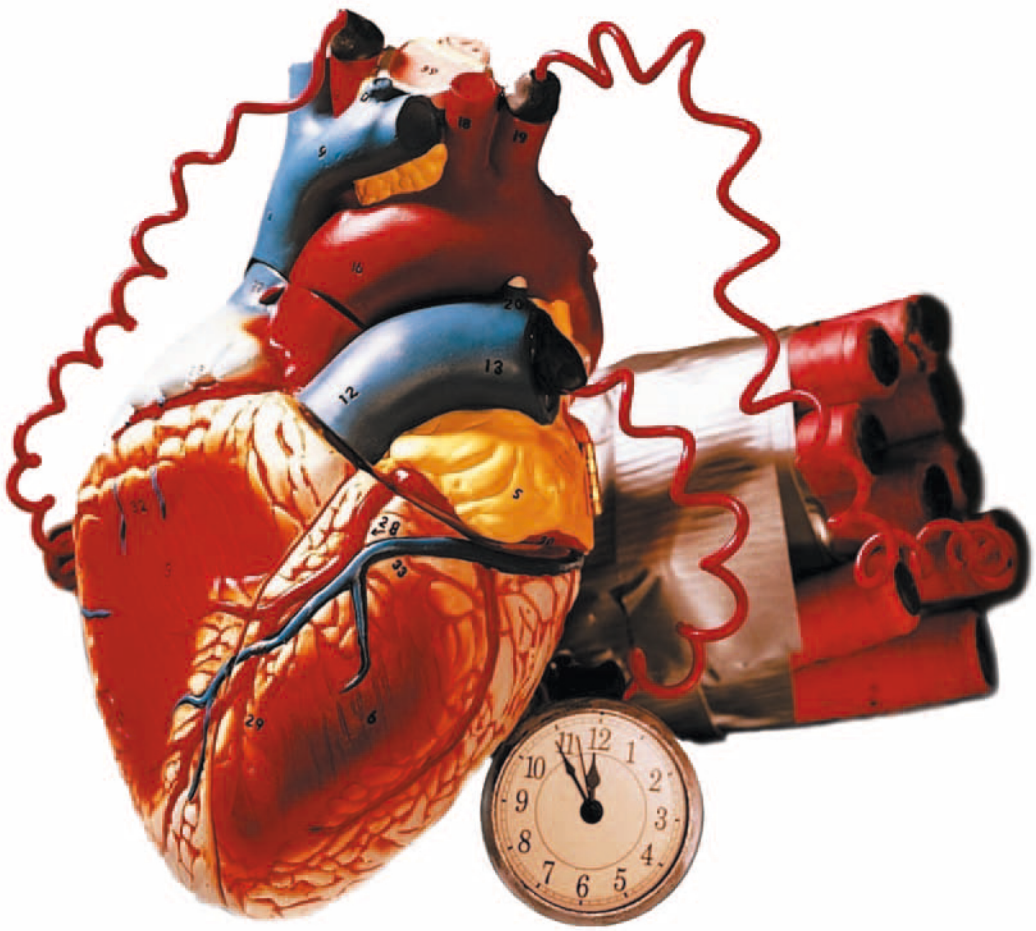
Inflammation and Cardiovascular Disease

A population-based approach



Irene M. van der Meer

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Acknowledgments

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Inflammation and Cardiovascular Disease

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Ontsteking en hart- en vaatziekten

Een studie onder de algemene bevolking

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Chapter 2.2
van der Meer IM, Bots ML, Hofman A, Iglesias del Sol A, van der Kuip DA, Witteman JC. Predictive value of non-invasive measures of atherosclerosis for incident coronary heart disease. The Rotterdam Study. *Submitted.*

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Chapter 1

Introduction

In western countries, the discouragement of smoking and the detection and treatment of hypertension, hypercholesterolemia, diabetes mellitus, and obesity take a prominent place in the primary and secondary prevention of cardiovascular disease (CVD). However, half of all patients with CVD do not have any of these established cardiovascular risk factors.[1] Because CVD remains the leading cause of death, much research is devoted to gaining insight into other factors that contribute to the initiation and progression of CVD.

In recent years, it has been recognized that atherosclerosis is an inflammatory disease.[2] In epidemiological research, the inflammatory mediator C-reactive protein has emerged as an important 'new' cardiovascular risk factor because it is a strong predictor of future CVD, independent of the traditional cardiovascular risk factors, and can be easily measured in blood.[3] Other processes playing a role in atherosclerosis are those involved in programmed cell death (apoptosis),[4] but this area has not yet received much attention in population-based studies.

The aim of the studies described in this thesis was to search for and provide more insight into factors that 1) are involved in the etiology of atherosclerosis and its clinical consequences and 2) can improve our ability to identify subjects who are at an increased risk of future CVD. The studies are based on data from the Rotterdam Study, a population-based cohort study composed of 7,983 men and women aged 55 years and over who live in a well-defined suburb of the city of Rotterdam, the Netherlands.[5] Among other things, data were collected on the presence and severity of atherosclerosis, which were measured non-invasively at multiple sites in the arterial tree, and on the occurrence of cardiovascular events during follow-up.

In chapter 2 of this thesis, studies are described in which risk factors for progression of extracoronary atherosclerosis and the predictive value of extracoronary atherosclerosis for future coronary heart disease are being examined. The studies described in chapter 3 aim at investigating the role of inflammatory processes in the etiology of CVD and the added value of C-reactive protein in the prediction of future myocardial infarction. Chapter 4 focuses on mediators of apoptosis as risk factors for atherosclerotic disease. Finally, in the general discussion in chapter 5, the main findings of this thesis are considered in the context of current scientific knowledge, relevant methodological aspects are discussed, and suggestions are made for future research in this field.

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Chapter 2

Measures of extracoronary atherosclerosis

Chapter 2.1

Risk factors for progression of extracoronary atherosclerosis

ABSTRACT

Background: Studies investigating determinants of atherosclerotic disease progression are relatively rare. Moreover, although atherosclerotic disease can be assessed non-invasively in different vascular beds, previous studies have not considered progression of atherosclerosis at more than one site. The present study was designed to identify risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree.

Methods: The Rotterdam Study is a population-based cohort study in 7,983 men and women aged 55 years and over. Carotid plaques and intima-media thickness were assessed by ultrasound, aortic atherosclerosis by X-ray, and lower extremity atherosclerosis by the ankle-arm index. Data on progression of atherosclerosis over an average period of 6.5 years were available for 3,409 participants. Associations of established cardiovascular risk factors with mild, moderate, and severe progression of atherosclerosis were investigated using multinomial regression analysis.

Results: Age, smoking, total cholesterol, and systolic blood pressure and/or hypertension were strong, independent predictors of moderate and severe progression of atherosclerosis at multiple sites. Diabetes mellitus only predicted severe progression of atherosclerosis. Associations of gender with progression of atherosclerosis were remarkably modest.

Conclusion: Age, smoking, total cholesterol, and systolic blood pressure and/or hypertension strongly predict progression of extracoronary atherosclerosis in the elderly, but gender remarkably does not. These results emphasize the need for prevention of progression of extracoronary atherosclerotic disease in men and women alike.

Various non-invasive methods are available to detect the presence and severity of atherosclerotic disease. Carotid atherosclerosis assessed by ultrasound, aortic atherosclerosis as shown on abdominal X-ray, and lower extremity atherosclerosis assessed by the ankle-arm index (AAI) are all important predictors of cardiovascular disease.[1-4] Moreover, they are strongly associated with the presence and amount of coronary calcification[5] and with cardiovascular risk factors.[6-8]

While the amount of atherosclerosis at one point in time is a reflection of lifelong accumulation of atherosclerotic lesions, changes in the extent of atherosclerosis with time give important information about whether, and at what rate atherosclerotic disease advances. Several studies have reported associations of cardiovascular risk factors with progression of atherosclerosis.[7,9,10] However, none of these studies has yet investigated whether the associations of cardiovascular risk factors with progression of atherosclerosis are consistent across different vascular beds.

Within the Rotterdam Study, a prospective, population-based cohort study among men and women aged 55 years and over, various non-invasive methods were used to assess progression of atherosclerosis over an average period of 6.5 years. We investigated associations of cardiovascular risk factors with progression of atherosclerosis measured at multiple sites in the arterial tree.

METHODS

Study population

The Rotterdam Study is a prospective population-based cohort study of 7,983 men and women aged 55 years and over. Its aim is to investigate the incidence and determinants of chronic disabling diseases. At phase one (baseline; 1990-1993), all inhabitants of a suburb of the city of Rotterdam aged 55 years and over were invited to participate in an extensive home interview and two visits to the research center. The overall response rate was 78%. Phase three was conducted in a similar way from 1997-1999. Between phases one and three, 25% of the participants had died and 0.4% were lost to follow-up. Because at phase two, only a minor part of the atherosclerosis measurements were performed, data from this phase were not included in the present study. The Medical Ethics Committee of the Erasmus University Medical Center approved the Rotterdam Study, and written informed consent was obtained from all participants.[11]

Of the 7,983 participants of the Rotterdam Study, 6,505 had never experienced a myocardial infarction, stroke, or revascularization procedure before phase three. For 6,145 of these participants, the extent of atherosclerosis was assessed at baseline at least at one site in the arterial tree. For 3,506 participants, information on at least one measure of atherosclerosis at phase three of the Rotterdam Study was also available. Subjects with missing data on more than two of the cardiovascular risk factors that are investigated in the present study were excluded (n = 97), resulting in a study population of 3,409 participants.

Clinical characteristics

At baseline, a trained investigator visited all participants at home and collected information using a computerized questionnaire. The obtained information included current health status, medical history, drug use, and smoking behavior. Additionally, during two visits to the research center, established cardiovascular risk factors were measured and non-fasting blood samples were obtained.[12] Obesity was defined as body mass index (BMI) ≥ 30.0 kg/m^2, and/or waist circumference ≥ 102 cm in men or ≥ 88 cm in women.[13] We defined hypertension as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg and/or use of blood pressure lowering medication with indication hypertension at phase one of the Rotterdam Study. Hypercholesterolemia was defined as serum total cholesterol ≥ 6.5 mmol/l and/or the use of cholesterol-lowering medication at phase one. We defined diabetes mellitus as a random or postload serum glucose level ≥ 11.1 mmol/l and/or the use of blood glucose lowering medication.

Measures of atherosclerosis

The extent of atherosclerosis was assessed at both phase one and phase three of the Rotterdam Study by measuring carotid plaques, carotid intima-media thickness (IMT), aortic atherosclerosis, and lower extremity atherosclerosis (as indicated by the AAI). Progression of atherosclerosis was divided into four categories (no, mild, moderate, severe), except for progression of carotid plaques (three categories: no, moderate, severe) because of the sample distribution of this measure of progression of atherosclerosis. To be able to compare the different associations of a particular cardiovascular risk factor with progression of the different measures of atherosclerosis, we aimed at categorizing progression of atherosclerosis in such a way that a specific category (for example, the 'severe' category) included a comparable percentage of the study sample for all of the four measures.

Carotid atherosclerosis. Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer and a duplex scanner (ATL UltraMark IV, Advanced Technology Laboratories). The common carotid artery, carotid bifurcation, and internal carotid artery were examined both left and right for the presence of plaques as described before.[2] A weighted plaque score ranging from 0 to 6 was computed by adding the number of sites at which a plaque was detected, divided by the total number of sites for which an ultrasonographic image was available, and multiplied by six (the maximum number of sites). Progression of carotid plaques was computed by subtracting the plaque score at phase one from the plaque score at phase three. Subjects for whom data on the presence of plaques were not available for at least four of the six sites that were examined both at phase one and at phase three were excluded from the analyses. We defined no, moderate, and severe progression of carotid plaques as a change in plaque score of 0, 1 – 2, or ≥ 3 points, respectively. Subjects with a decrease in plaque score were added to the group with no progression, since we considered this to be mainly due to measurement error.

Common carotid IMT was determined as the average of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[2] Progression of IMT was defined as the difference in mean IMT between phase three and phase one. Subjects were divided in categories of progression of IMT based on their ranking in the sample distribution. Subjects with an increase in IMT below the 30th percentile of the distribution of all subjects with an increase in IMT were considered to have no progression of IMT. Subjects with an increase in IMT above the 30th, 60th and 90th percentile of the sample distribution were considered to have mild, moderate, and severe progression of IMT, respectively. We added subjects for whom we found a decrease in IMT to the group with no progression. Due to limited availability of ultrasonographers at the end of 1992 and in 1993, ultrasound data are missing for part of the subjects who visited the Rotterdam Study research center at phase one.

Aortic atherosclerosis. Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film.[7] For progression of aortic atherosclerosis, baseline and follow-up films were examined in pairs. Progression was scored on a graded scale (with scores 0 – 4 corresponding to 0, ≤ 1, 1 – 2.5, 2.5 – 4.9, and ≥ 5.0 cm progression, respectively). None of the participants showed a decrease in the extent of aortic atherosclerosis. We defined no, mild, moderate, and severe progression of aortic atherosclerosis as a progression score of 0, 1, 2, and > 2, respectively.

Lower extremity atherosclerosis. Systolic blood pressure at the ankles (posterior tibial artery) was measured in supine position with a random zero sphygmomanometer and an 8 MHz continuous wave Doppler probe (Huntleigh 500D, Huntleigh Technology). We computed the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm to obtain the AAI. Since arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI > 1.50 was considered invalid.[12] Progression of lower extremity atherosclerosis was computed by subtracting the AAI at phase one from the AAI at phase three. For the analyses, we used the leg with the largest decrease in AAI. Subjects with a decrease in the AAI below the 30th percentile of the sample distribution of all subjects with a decrease in the AAI were considered to have no progression of lower extremity atherosclerosis. Subjects with a decrease in AAI above the 30th, 60th and 90th percentile of the sample distribution were considered to have mild, moderate, and severe progression of lower extremity atherosclerosis, respectively. We added subjects for whom we found an increase in AAI to the group with no progression. Data on progression of carotid plaques, IMT, aortic atherosclerosis, and lower extremity atherosclerosis were available for 2,366, 2,622, 2,687, and 2,756 participants, respectively.

Statistical analyses

For subjects with missing data on clinical characteristics measured on a continuous scale, we imputed the population mean. Using multinomial logistic regression

Table 1. Baseline characteristics of the study population.

	All subjects (n = 3,409)
Age (years)	65.4 (6.8)
Gender (%men)	38.3
Current smokers (%)	20.1
Past smokers (%)	41.9
Body mass index (kg/m^2)	26.3 (3.6)
Total cholesterol (mmol/l)	6.7 (1.2)
HDL-cholesterol (mmol/l)	1.4 (0.4)
Systolic blood pressure (mmHg)	135.7 (20.7)
Diastolic blood pressure (mmHg)	73.8 (10.8)
Diabetes mellitus (%)	6.0
Cholesterol lowering medication (%)	3.4
Blood pressure lowering medication (%)	23.1
Carotid plaques (%)*	51.8
Intima-media thickness (mm)	0.76 (0.14)
Aortic atherosclerosis (%)†	59.6
Ankle-arm index‡	1.12 (0.18)
Duration of follow-up (years)	6.5 (0.4)

HDL = high-density lipoprotein. Data are means (standard deviation) for continuous variables and percentages for dichotomous variables. *As indicated by a carotid plaque score > 0. †As indicated by an aortic atherosclerosis score > 0. ‡Lowest of both legs.

analysis, we examined the association of cardiovascular risk factors with mild, moderate, and severe progression of atherosclerosis. Analyses with age as the determinant were adjusted for gender and duration of follow-up. Analyses with gender as the determinant were adjusted for age, duration of follow-up, and – to account for the large percentage of ever smokers (84.4%) in men – for current and past smoking. Analyses with all other cardiovascular risk factors as the determinant were adjusted for age, gender, duration of follow-up, current and past smoking, BMI, waist-to-hip ratio, total cholesterol and high-density lipoprotein (HDL) cholesterol, systolic blood pressure, diastolic blood pressure, diabetes mellitus, and use of cholesterol-lowering medication and use of blood pressure lowering medication at any point in time between phase one and phase three. Analyses on the association of hypertension with progression of atherosclerosis did not include the variables systolic and diastolic blood pressure, and subjects with blood pressure lowering medication with an indication other than hypertension were excluded.

RESULTS

Table 1 shows baseline characteristics of the study population.

Current smoking was the strongest predictor of progression of carotid plaques (table 2). Age, total cholesterol, and systolic blood pressure were also strong, independent predictors of both moderate and severe progression. Men seemed to be at a higher risk of carotid plaque progression, but the odds ratio (OR) for severe progression was not statistically significant. Smoking in the past and diabetes mellitus only, but strongly so, predicted severe progression of carotid plaques. BMI and WHR had ORs in opposite directions, especially in their association with moderate progression of carotid plaques.

Table 2. Odds ratios for progression of carotid plaques associated with established cardiovascular risk factors.

	Progression of carotid plaques		
	no (n = 1,345)	**moderate (n = 826)**	**severe (n = 195)**
Age (per sd)*	1.0	1.21 (1.11 – 1.33)	1.42 (1.22 – 1.64)
Gender (men)†	1.0	1.23 (1.01 – 1.50)	1.31 (0.94 – 1.83)
Current smoking	1.0	1.57 (1.21 – 2.02)	2.95 (1.89 – 4.61)
Past smoking	1.0	1.14 (0.92 – 1.42)	1.65 (1.11 – 2.47)
Body mass index (per sd)	1.0	0.88 (0.80 – 0.98)	1.00 (0.84 – 1.19)
Waist-to-hip ratio (per sd)	1.0	1.24 (1.11 – 1.39)	1.20 (0.98 – 1.46)
Total cholesterol (per sd)	1.0	1.12 (1.02 – 1.23)	1.28 (1.09 – 1.50)
HDL-cholesterol (per sd)	1.0	0.94 (0.85 – 1.04)	0.96 (0.81 – 1.15)
Systolic blood pressure (per sd)	1.0	1.19 (1.05 – 1.35)	1.25 (1.01 – 1.55)
Diastolic blood pressure (per sd)	1.0	0.91 (0.81 – 1.03)	0.97 (0.79 – 1.19)
Hypertension (%)‡	1.0	1.44 (1.17 – 1.77)	1.29 (0.90 – 1.85)
Diabetes mellitus (%)	1.0	0.81 (0.54 – 1.21)	1.76 (1.04 – 2.99)

sd = standard deviation. Data are odds ratios ± 95% confidence intervals adjusted for age, gender,duration of follow-up, current and past smoking, body mass index, waist-to-hip ratio, total and high-density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure, diabetes mellitus, and use of blood pressure- and cholesterol-lowering medication. *Adjusted for gender and duration of follow-up. †Adjusted for age, current and past smoking, and duration of follow-up. ‡Not adjusted for systolic and diastolic blood pressure; subjects using blood pressure lowering medication with indication other than hypertension were excluded.

Age and BMI were strong predictors of mild (only BMI), moderate, and severe progression of carotid IMT (table 3). Gender (inverse association), current (borderline) and past smoking, systolic blood pressure, and hypertension (borderline) were

Table 3. Odds ratios for progression of carotid intima-media thickness associated with established cardiovascular risk factors.

	Progression of carotid intima-media thickness			
	no	**mild**	**moderate**	**severe**
	(n = 1,013)	**(n = 690)**	**(n = 690)**	**(n = 229)**
Age (per sd)*	1.0	1.02 (0.92 – 1.13)	1.16 (1.05 – 1.28)	1.34 (1.17 – 1.54)
Gender (men)†	1.0	0.99 (0.79 – 1.23)	1.09 (0.88 – 1.35)	0.70 (0.50 – 0.97)
Current smoking	1.0	1.03 (0.78 – 1.37)	1.18 (0.89 – 1.57)	1.46 (0.95 – 2.24)
Past smoking	1.0	1.04 (0.82 – 1.32)	1.10 (0.87 – 1.40)	1.46 (1.02 – 2.07)
Body mass index(per sd)	1.0	1.14 (1.02 – 1.27)	1.17 (1.05 – 1.31)	1.25 (1.06 – 1.46)
Waist-to-hip ratio (per sd)	1.0	0.97 (0.86 – 1.10)	0.89 (0.78 – 1.01)	1.01 (0.85 – 1.22)
Total cholesterol (per sd)	1.0	1.02 (0.92 – 1.13)	1.03 (0.93 – 2.14)	1.09 (0.94 – 1.27)
HDL-cholesterol (per sd)	1.0	1.02 (0.91 – 1.13)	0.93 (0.84 – 1.04)	1.01 (0.86 – 1.19)
Systolic blood pressure (per sd)	1.0	0.99 (0.86 – 1.14)	0.99 (0.86 – 1.14)	1.31 (1.07 – 1.60)
Diastolic blood pressure (per sd)	1.0	0.98 (0.86 – 1.12)	0.91 (0.79 – 1.04)	0.91 (0.75 – 1.10)
Hypertension (%)‡	1.0	0.85 (0.67 – 1.08)	0.93 (0.74 – 1.17)	1.34 (0.97 – 1.85)
Diabetes mellitus (%)	1.0	0.80 (0.52 – 1.23)	0.78 (0.51 – 1.19)	0.63 (0.33 – 1.21)

sd = standard deviation. Data are odds ratios ± 95% confidence intervals adjusted for age, gender, duration of follow-up, current and past smoking, body mass index , waist-to-hip ratio, total and high-density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure, diabetes mellitus, and use of blood pressure- and cholesterol-lowering medication. *Adjusted for gender and duration of follow-up. †Adjusted for age, current and past smoking, and duration of follow-up. ‡Not adjusted for systolic and diastolic blood pressure; subjects using blood pressure lowering medication with indication other than hypertension were excluded.

Table 4. Odds ratios for progression of aortic atherosclerosis associated with established cardiovascular risk factors.

	Progression of aortic atherosclerosis			
	no	**mild**	**moderate**	**severe**
	(n = 825)	**(n = 912)**	**(n = 765)**	**(n = 185)**
Age (per sd)*	1.0	1.42 (1.28 – 1.58)	1.56 (1.40 – 1.74)	1.84 (1.56 – 2.17)
Gender (men)†	1.0	1.28 (1.03 – 1.58)	0.96 (0.76 – 1.20)	0.99 (0.69 – 1.42)
Current smoking	1.0	1.74 (1.30 – 2.34)	2.64 (1.95 – 3.58)	2.45 (1.49 – 4.02)
Past smoking	1.0	1.31 (1.04 – 1.65)	1.48 (1.15 – 1.89)	1.69 (1.13 – 2.53)
Body mass index(per sd)	1.0	0.87 (0.78 – 0.97)	0.85 (0.75 – 0.95)	0.75 (0.62 – 0.91)
Waist-to-hip ratio (per sd)	1.0	1.04 (0.91 – 1.17)	1.10 (0.96 – 1.25)	1.05 (0.85 – 1.30)
Total cholesterol (per sd)	1.0	1.23 (1.11 – 1.37)	1.28 (1.14 – 1.43)	1.49 (1.25 – 1.78)
HDL-cholesterol (per sd)	1.0	0.83 (0.75 – 0.93)	0.81 (0.73 – 0.91)	0.94 (0.78 – 1.12)
Systolic blood pressure (per sd)	1.0	1.26 (1.09 – 1.46)	1.37 (1.17 – 1.59)	1.29 (1.03 – 1.63)
Diastolic blood pressure (per sd)	1.0	0.84 (0.73 – 0.96)	0.85 (0.74 – 0.98)	0.75 (0.60 – 0.94)
Hypertension (%)‡	1.0	1.26 (0.99 – 1.61)	1.68 (1.31 – 2.16)	1.84 (1.26 – 2.70)
Diabetes mellitus (%)	1.0	0.98 (0.63 – 1.52)	1.03 (0.65 – 1.63)	1.92 (1.07 – 3.44)

sd = standard deviation. Data are odds ratios ± 95% confidence intervals adjusted for age, gender, duration of follow-up, current and past smoking, body mass index , waist-to-hip ratio, total and high-density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure, diabetes mellitus, and use of blood pressure- and cholesterol-lowering medication. *Adjusted for gender and duration of follow-up. †Adjusted for age, current and past smoking, and duration of follow-up. ‡Not adjusted for systolic and diastolic blood pressure; subjects using blood pressure lowering medication with indication other than hypertension were excluded.

Table 5. Odds ratios for progression of lower extremity atherosclerosis associated with established cardiovascular risk factors.

	Progression of lower extremity atherosclerosis			
	no (n = 1,359)	**mild (n = 599)**	**moderate (n = 599)**	**severe (n = 199)**
Age (per sd)*	1.0	1.03 (0.93 – 1.14)	1.10 (1.00 – 1.21)	1.65 (1.43 – 1.91)
Gender (men)†	1.0	0.86 (0.70 – 1.06)	0.77 (0.62 – 0.96)	1.37 (0.98 – 1.90)
Current smoking	1.0	1.51 (1.14 – 2.00)	1.55 (1.17 – 2.05)	3.17 (2.04 – 4.93)
Past smoking	1.0	1.20 (0.95 – 1.51)	1.12 (0.89 – 1.42)	1.35 (0.90 – 2.03)
Body mass index(per sd)	1.0	0.93 (0.84 – 1.04)	0.86 (0.77 – 0.97)	0.80 (0.67 – 0.96)
Waist-to-hip ratio (per sd)	1.0	0.98 (0.86 – 1.11)	1.05 (0.92 – 1.18)	1.03 (0.83 – 1.26)
Total cholesterol (per sd)	1.0	1.05 (0.95 – 1.17)	1.15 (1.04 – 1.28)	1.37 (1.18 – 1.60)
HDL-cholesterol (per sd)	1.0	0.92 (0.83 – 1.03)	0.94 (0.85 – 1.05)	0.76 (0.64 – 0.91)
Systolic blood pressure (per sd)	1.0	1.06 (0.92 – 1.21)	1.04 (0.90 – 1.19)	1.12 (0.91 – 1.37)
Diastolic blood pressure (per sd)	1.0	1.00 (0.88 – 1.15)	0.97 (0.85 – 1.11)	0.88 (0.72 – 1.08)
Hypertension (%)‡	1.0	1.12 (0.89 – 1.41)	1.27 (1.01 – 1.60)	1.41 (1.00 – 2.00)
Diabetes mellitus (%)	1.0	0.81 (0.52 – 1.27)	0.80 (0.52 – 1.25)	1.83 (1.10 – 3.02)

sd = standard deviation. Data are odds ratios ± 95% confidence intervals adjusted for age, gender, duration of follow-up, current and past smoking, body mass index , waist-to-hip ratio, total and high-density lipoprotein (HDL) cholesterol, systolic and diastolic blood pressure, diabetes mellitus, and use of blood pressure- and cholesterol-lowering medication. *Adjusted for gender and duration of follow-up. †Adjusted for age, current and past smoking, and duration of follow-up. ‡Not adjusted for systolic and diastolic blood pressure; subjects using blood pressure lowering medication with indication other than hypertension were excluded.

significant predictors of severe, but not of mild and moderate progression of carotid IMT. Other cardiovascular risk factors did not predict progression of carotid IMT.

Table 4 shows that age, smoking habits, total cholesterol, and systolic blood pressure and hypertension were all strong predictors of mild, moderate, and severe progression of aortic atherosclerosis. Gender only predicted mild progression. For BMI and diastolic blood pressure, there was a protective effect, as well for HDL-cholesterol, but for the latter, the OR for severe progression was not statistically significant. Diabetes mellitus was a strong predictor of severe progression of aortic atherosclerosis.

Current smoking was the strongest predictor of mild, moderate, and severe progression of lower extremity atherosclerosis (table 5). Age, total cholesterol, and hypertension also predicted moderate and severe progression of lower extremity atherosclerosis. Gender was a borderline significant predictor of severe progression of lower extremity atherosclerosis, but the association with moderate progression was inverse. Diabetes mellitus only predicted severe progression. For BMI and HDL-cholesterol, there was a protective effect.

When we excluded subjects with a decrease in carotid plaque score or IMT or an increase in the AAI, results were similar to those presented in the tables. In subgroup analyses stratified by gender, we found no major differences between men and women that were consistent across the four measures of atherosclerosis. Moreover, no statistically significant interactions between risk factors were consistently found. We did not find evidence of a non-linear association for any of the associations of continuous variables with progression of atherosclerosis, apart from a modest exponential association of age with progression of carotid IMT (overall *P* value for quadratic term 0.08; for moderate and severe progression 0.07 and 0.03, respectively; adjusted for gender and duration of follow-up) and with progression of aortic atherosclerosis (overall *P* value 0.08; for mild and moderate progression 0.02 and 0.03, respectively). Addition of the quadratic term for age to the multivariate models did not change any of the ORs associated with the other cardiovascular risk factors.

Finally, figure 1 shows ORs for severe progression of all measures of atherosclerosis associated with current smoking, obesity, hypercholesterolemia, hypertension, and diabetes mellitus.

DISCUSSION

The present study shows that age, smoking behavior, total cholesterol, and systolic blood pressure and/or hypertension are strong, independent predictors of progression of atherosclerosis measured at multiple sites in the arterial tree. Diabetes mellitus is a strong predictor of severe, but not of mild or moderate progression of atherosclerosis. Gender shows a remarkably modest association with progression of extracoronary atherosclerosis.

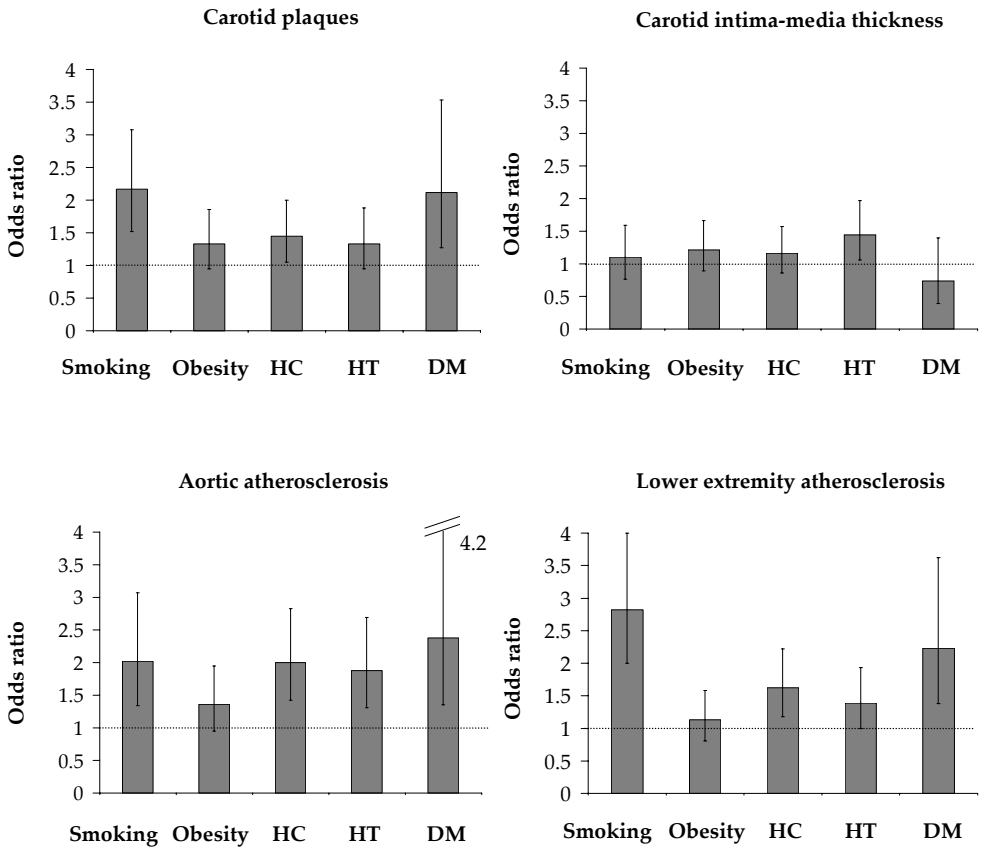

Figure 1. Associations of cardiovascular risk factors with severe progression of atherosclerosis. HC = hypercholesterolemia, HT = hypertension, DM = diabetes mellitus. Bars represent odds ratios, adjusted for age, gender, and duration of follow-up; lines represent 95% confidence intervals.

The strength of the present study, a relatively large cohort study in a very homogeneous population of elderly people, is the assessment of progression of atherosclerosis at multiple sites. Still, several methodological issues of this study need to be considered. First, subjects with the most severe atherosclerosis at baseline are more likely to have died and are therefore not included in the current study. Although this may have somewhat limited the range of baseline levels of atherosclerosis, it does not affect the validity of the risk estimates. Second, because we defined categories of progression of carotid IMT and the AAI according to percentiles, one should note that the distinction between these categories is not based on clear-cut clinical differences in progression of atherosclerosis. Moreover, our definitions of no, mild, moderate, and severe progression of atherosclerosis depend on the distribution of progression of atherosclerosis within our study population and may therefore be

different in other populations. Third, baseline levels of atherosclerosis may influence the association between cardiovascular risk factors and progression of atherosclerosis. However, due to the phenomenon of regression to the mean as a result of measurement error, adjusting for baseline atherosclerosis may introduce bias that leads to an overestimation of the risk estimates.[14] For categorical variables, no statistical model has yet been developed to adjust for baseline values without introducing bias. For continuous variables, both the ARIC Study and the Cardiovascular Health Study, investigating associations between cardiovascular risk factors and progression of IMT, showed that not adjusting for baseline levels of atherosclerosis gave similar results as adjusting for baseline atherosclerosis while at the same time taking measurement error into account.[15,16] In the present study, we did not adjust for baseline levels of atherosclerosis. Finally, although the measures of atherosclerosis that were used in the present study were strongly associated with coronary calcification,[5] differences between these measures exist. For the carotid plaque score, we measured distinct atherosclerotic lesions, and radiographically assessed calcification of the abdominal aorta has been shown to specifically represent advanced intimal atherosclerosis.[17] However, changes in IMT and AAI may also partly be due to non-atherosclerotic processes such as fibromuscular hypertrophy, which causes modest increases in IMT, and hemodynamic factors and vascular stiffness, which may influence the AAI.

While for coronary atherosclerosis, differences between men and women are pronounced,[18] we report remarkably modest and inconsistent associations between gender and progression of extracoronary atherosclerosis. This finding is in accordance with the fact that the difference between men and women is much smaller for the incidence of stroke than for the incidence of myocardial infarction.[19,20] Moreover, we have previously shown that the prevalence of peripheral arterial disease in men and women participating in the Rotterdam Study is similar.[12] It should be kept in mind that these findings could be different in populations with premenopausal women, in whom the protective effects of endogenous estrogens may lead to a slower rate of progression of atherosclerosis than in men.[21] However, since most clinical events related to atherosclerotic disease occur in postmenopausal women, the results from the present study stress the importance of prevention of progression of extracoronary atherosclerotic disease in both men and women alike.

Although we used a standardized protocol for the measurement of IMT both at baseline and at the follow-up visit, a relatively large part of the computed change in IMT over the years may be distorted by measurement error. This may explain why only few cardiovascular risk factors (smoking habits, BMI, and systolic blood pressure) were significant predictors of progression of carotid IMT, whereas associations of traditional cardiovascular risk factors with progression of carotid plaques were much stronger. Both more precise methods to measure progression of carotid IMT and the assessment of not only the presence, but also the volume of carotid plaques will further improve our ability to determine the rate of progression of carotid atherosclerosis.

This population-based study shows that age, smoking behavior, total cholesterol, and systolic blood pressure and/or hypertension are strong predictors of progression of atherosclerosis, regardless of the site of measurement. Gender is not an important risk indicator for progression of extracoronary atherosclerotic disease in men and women aged 55 years and over.

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Chapter 2.2

Atherosclerosis at multiple sites and risk of coronary heart disease

ABSTRACT

Background: Several non-invasive methods are available to investigate the severity of extracoronary atherosclerotic disease. No population-based study has yet examined whether differences exist between these measures with regard to their predictive value for coronary heart disease (CHD), or whether a given measure of atherosclerosis has predictive value independently of the other measures.
Methods: At the baseline examination of the Rotterdam Study, a population-based cohort study among subjects aged 55 years and over, carotid plaques and intima-media thickness (IMT) were measured by ultrasound, abdominal aortic atherosclerosis was measured by X-ray, and lower extremity atherosclerosis was measured by computation of the ankle-arm index. In the present study, 6,389 subjects were included; 358 cases of incident CHD occurred during follow-up.
Results: All four measures of atherosclerosis were good predictors of CHD, independently of traditional cardiovascular risk factors. Although differences were small, hazard ratios were higher for carotid plaques (1.89 (1.40 – 2.57); severe versus no atherosclerosis) and aortic atherosclerosis (1.83 (1.31 – 2.55)) than for carotid IMT (1.60 (1.09 – 2.35)) and lower extremity atherosclerosis (1.49 (1.06 – 2.10)). The hazard ratio for CHD for subjects with severe atherosclerosis according to a composite atherosclerosis score was 2.3 (1.5 – 3.5) compared to subjects with no atherosclerosis. For carotid plaques and aortic atherosclerosis, the predictive value of CHD was independent of other atherosclerosis measures.
Conclusion: Non-invasive measures of extracoronary atherosclerosis are good predictors of CHD. Risk estimates for carotid plaques and aortic atherosclerosis were highest and independent of the other atherosclerosis measures, showing the value of direct assessment of atherosclerotic plaques in CHD risk prediction.

Various non-invasive methods are available to detect the presence and severity of extracoronary atherosclerotic disease. Carotid atherosclerosis as shown on ultrasound, aortic atherosclerosis on abdominal X-ray, and lower extremity atherosclerosis reflected by the ankle-arm index (AAI) are validated measures of atherosclerosis which are routinely used in population-based studies because they are relatively cheap, non-invasive, and easy to assess. They are strongly associated with the presence and amount of coronary calcification[1] and with traditional cardiovascular risk factors.[2-4]

Several studies have shown that these measures of atherosclerosis are good predictors of coronary heart disease (CHD).[5-12] However, differences between measures with regard to their predictive value for CHD may exist, either due to factors related to the site at which atherosclerosis was measured, or to factors associated with the measurement method. No population-based study has yet compared the predictive value of several different measures of atherosclerosis or investigated whether the information provided by a given measure of atherosclerosis is independent of that provided by other atherosclerosis measures.

Within the Rotterdam Study, a population-based cohort study among men and women aged 55 years and over, we investigated several non-invasive measures of extracoronary atherosclerosis and combinations of these measures in relation to incident CHD.

METHODS

Population

The Rotterdam Study is a prospective population-based cohort study of 7,983 men and women aged 55 years and over. Its overall aim is to investigate the incidence and determinants of chronic disabling diseases. From 1990 – 1993, all inhabitants of a suburb of the city of Rotterdam aged 55 years and over were invited to participate in the study. The overall response rate was 78%. A trained investigator visited all participants at home and collected information using a computerized questionnaire. The obtained information included current health status, medical history, drug use, and smoking behavior. Additionally, during two visits to the research center, established cardiovascular risk factors were measured. The Medical Ethics Committee of the Erasmus University Medical Center approved the Rotterdam Study, and written informed consent was obtained from all participants. A more detailed description of the Rotterdam Study and the collection of data has been given elsewhere.[13,14]

Study population. Of the 7,983 participants of the Rotterdam Study, 7,393 had never experienced a myocardial infarction or revascularization procedure before the baseline examination. For 6,525 participants, the extent of atherosclerosis was assessed at at least one site of the vascular tree, and follow-up data on incident CHD were available for 6,442 of these participants. Subjects with missing data on more than two

of the established cardiovascular risk factors were excluded (n = 53), resulting in a study population of 6,389 participants.

Measures of atherosclerosis

Each of the measures of atherosclerosis included in the present study was categorized into no, mild, moderate, and severe atherosclerosis. To be able to compare the associations of the different measures of atherosclerosis with incident CHD, we aimed at categorizing the severity of atherosclerosis in such a way that especially the 'severe' category included a comparable percentage of the population for all of the four measures.

Carotid atherosclerosis. Using ultrasound, the common carotid artery, carotid bifurcation, and internal carotid artery were examined both left and right for the presence of plaques as described before.[15] A weighted plaque score ranging from 0 to 6 was computed by adding the number of sites at which a plaque was detected, divided by the total number of sites for which an ultrasonographic image was available and multiplied by six (the maximum number of sites). Subjects for whom data on the presence of plaques were not available for at least two of the six sites that were examined were excluded. Subjects with a carotid plaque score of 0, 1,2, and ≥ 3 points were considered to have no, mild, moderate, and severe carotid atherosclerosis, respectively.

The maximum common carotid intima-media thickness (IMT) was determined as the average of the maximum IMT of near- and far-wall measurements, and the average of left and right maximum common carotid IMT was computed. To indicate no, mild, moderate, and severe thickening of the carotid wall, we divided the IMT into quartiles based on the population distribution, using cut-off points of 0.88, 0.99, and 1.12 mm, respectively. Due to limited availability of ultrasonographers at the end of 1992 and in 1993, ultrasound data on carotid atherosclerosis are missing for part of the study population.

Aortic atherosclerosis. Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film.[3] The extent of abdominal aortic atherosclerosis was scored according to the length of the involved area (with scores 0 – 5 corresponding to 0, ≤ 1, 1 – 2.5, 2.5 – 4.9, 5.0 – 9.9, and ≥ 10.0 cm). Subjects with an aortic atherosclerosis score of 0, 1, 2, and ≥ 3 points were considered to have no, mild, moderate, and severe aortic atherosclerosis, respectively.

Lower extremity atherosclerosis. We obtained the AAI as described before by computing the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm.[14] The AAI is inversely related to the severity of lower extremity atherosclerosis. For the analyses, we used the lowest value of two legs. Since arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI larger than 1.50 was considered invalid. To indicate no, mild, moderate, and severe lower extremity atherosclerosis, we divided the AAI into quartiles based on the population distribution, using cut-off points of 1.21, 1.10, and 0.97, respectively.

Atherosclerosis score. To compute a composite atherosclerosis score, we made seven categories representing the severity of carotid plaques (according to the carotid plaque score, range 0 to 6), and six categories representing the severity of aortic atherosclerosis (according to the aortic atherosclerosis score, range 0 to 5). Moreover, we divided the IMT and AAI into ten equally sized categories based on the population distribution. We assigned points to each category of a given measure of atherosclerosis based on the cumulative percentage of subjects in that particular category, with a maximum number of 10 points per measure of atherosclerosis. For example, because the total percentage of subjects having a carotid plaque score of four or less was 88%, subjects with a carotid plaque score equal to four were assigned 8.8 points for this particular measure of atherosclerosis. Thus, the atherosclerosis score added up to a maximum of 40 points. For subjects with complete data on three of the four measures of atherosclerosis, we computed a weighted atherosclerosis score by multiplying the score obtained for three measures by 4/3. To indicate no, mild, moderate, and severe atherosclerosis, we divided the composite score into quartiles based on the population distribution, using cut-off points of 13.3, 18.8, and 25.4, respectively.

Data on carotid plaques, carotid IMT, aortic atherosclerosis, lower extremity atherosclerosis, and the composite atherosclerosis score were available for 5,070, 5,116, 5,234, 5,838, and 5,192 subjects, respectively.

Follow-up procedures

Follow-up started at the baseline examination and for the present study lasted until January 1st, 2000. Of all participants in the present study, 168 (2.6%) were lost to follow-up. For these subjects, the follow-up time was computed until the last date of contact. Fatal and non-fatal cardiovascular events were reported by general practitioners in the research district, with whom 85% of the participants of the Rotterdam Study were enlisted. Research assistants verified all information by checking medical records at the general practitioners' offices. All medical records of the participants under the care of general practitioners outside the study area were checked annually for possible events. Letters and, in case of hospitalization, discharge reports from medical specialists were obtained. With respect to the vital status of participants, information was also obtained regularly from the municipal health authorities in Rotterdam. After notification, cause and circumstances of death were established by questionnaire from the GPs. Two research physicians independently coded all reported events according to the International Classification of Diseases, 10th edition (ICD-10)[16] Codes on which the research physicians disagreed were discussed in order to reach consensus. Finally, a medical expert in cardiovascular disease, whose judgement was considered final, reviewed all events. Incident CHD was defined as the occurrence of a fatal or non-fatal myocardial infarction (ICD-10 code I21), a revascularization procedure (percutaneous transluminal coronary angioplasty or coronary artery bypass graft), or other forms of acute (I24) or chronic ischemic (I25) heart disease during follow-up.

Table 1. Baseline characteristics of the study population.

Characteristic	All subjects (n = 6,389)
Age (years)	69.3 ± 9.2
Gender (%women)	61.9
Current smokers (%)	21.5
Body mass index (kg/m^2)	26.3 ± 3.7
Total cholesterol (mmol/l)	6.6 ± 1.2
HDL-cholesterol (mmol/l)	1.4 ± 0.4
Systolic blood pressure (mmHg)	139.6 ± 22.3
Diastolic blood pressure (mmHg)	73.9 ± 11.6
Diabetes mellitus (%)	10.1
Cholesterol lowering medication (%)	1.6
Blood pressure lowering medication (%)	29.4
Aspirin (%)	8.7
Carotid plaques (%)*	57.8
Intima-media thickness (mm)	1.02 ± 0.21
Aortic atherosclerosis (%)†	65.3
Ankle-arm index‡	1.05 ± 0.23
Composite atherosclerosis score§	19.6 ± 8.1

HDL = high-density lipoprotein. Data are means ± standard deviation for continuous variables and percentages for dichotomous variables. *As indicated by a carotid plaque score > 0. †As indicated by an aortic atherosclerosis score > 0. ‡Lowest of left and right leg. §With a range of 0 to 40 points.

Statistical analyses

For subjects with missing data on clinical characteristics measured on a continuous scale, we imputed the population mean (n = 308 with missing data on at most 2 characteristics). For subjects with missing data on smoking habits (n = 128) or diabetes mellitus (n = 30), we made use of a missing variable indicator. Proportional hazards regression analysis was used to evaluate the predictive value for CHD of categories of the individual measures of atherosclerosis and of the composite atherosclerosis score, taking only age and gender (model 1), or also smoking habits, body mass index, total and high-density lipoprotein (HDL)-cholesterol, systolic and diastolic blood pressure, presence of diabetes mellitus, and use of aspirin, blood pressure lowering-, and cholesterol lowering medication into account (model 2). Subsequently, to investigate whether a given measure of atherosclerosis predicts CHD independently of the other measures, we included each possible combination of two measures of atherosclerosis in the fully adjusted model. Finally, for each combination of two measures of atherosclerosis, we divided the population into nine groups according to tertiles of these two measures. To compute hazard ratios for CHD, we used the group with

Table 2. Hazard ratios for incident coronary heart disease associated with carotid measures of atherosclerosis.

	Severity of atherosclerosis			
	no	**mild**	**moderate**	**severe**
Carotid plaques				
model 1	1.0	1.27 (0.86 – 1.87)	1.53 (1.09 – 2.15)	2.47 (1.84 – 3.31)
model 2	1.0	1.16 (0.79 – 1.71)	1.33 (0.94 – 1.88)	1.89 (1.40 – 2.57)
+ carotid IMT	1.0	1.21 (0.82 – 1.79)	1.34 (0.94 – 1.90)	1.82 (1.31 – 2.53)
+ aortic atherosclerosis	1.0	1.23 (0.82 – 1.84)	1.23 (0.85 – 1.79)	1.73 (1.24 – 2.42)
+ lower extremity atherosclerosis	1.0	1.19 (0.80 – 1.77)	1.29 (0.90 – 1.85)	1.94 (1.41 – 2.68)
Carotid intima-media thickness				
model 1	1.0	1.16 (0.78 – 1.71)	1.62 (1.11 – 2.37)	2.28 (1.56 – 3.31)
model 2	1.0	1.08 (0.73 – 1.60)	1.34 (0.91 – 1.95)	1.60 (1.09 – 2.35)
+ carotid plaques	1.0	1.06 (0.72 – 1.58)	1.17 (0.79 – 1.74)	1.34 (0.89 – 2.01)
+ aortic atherosclerosis	1.0	1.11 (0.73 – 1.67)	1.22 (0.81 – 1.84)	1.52 (1.00 – 2.29)
+ lower extremity atherosclerosis	1.0	1.14 (0.76 – 1.70)	1.35 (0.90 – 2.01)	1.63 (1.09 – 2.44)

IMT = intima-media thickness. All hazard rate ratios adjusted for age and gender (model 1), and subsequently (model 2) for smoking habits, body mass index, total and high-density lipoprotein (HDL)-cholesterol, systolic and diastolic blood pressure, presence of diabetes mellitus, and use of aspirin, blood pressure lowering-, and cholesterol lowering medication. Model 2 was additionally adjusted for severity of atherosclerosis as indicated by the various atherosclerosis measures.

subjects who were for both measures in the tertile with the least severe atherosclerosis as the referent group. All analyses were performed using SPSS 9.0 for Windows.

RESULTS

Baseline characteristics of the total study population are shown in table 1. The distribution of no, mild, moderate, and severe carotid plaques was 42.2%, 15.3%, 18.4%, and 24.0%, respectively. For aortic atherosclerosis, these numbers were 34.7%, 9.6%, 26.5%, and 29.2%.

During follow-up, incident CHD occurred in 358 (5.6%; 208 men and 150 women) subjects. Tables 2 and 3 show that all measures of atherosclerosis were strongly predictive of incident CHD, even after adjustment for a wide range of cardiovascular risk factors and medication use (model 2). When analyzed as continuous variables, the multivariate hazard ratios for CHD associated with 1 standard deviation increase in carotid IMT and 1 standard deviation decrease in the AAI were 1.26 (1.14 – 1.39) and

1.18 (1.04 – 1.33), respectively, in a fully adjusted model (model 2). Of the four individual measures, the hazard ratios were higher for severe carotid plaques (1.89 (1.40 – 2.57)) and aortic atherosclerosis (1.83 (1.31 – 2.55)) than for carotid IMT and lower extremity atherosclerosis, although differences were small. Hazard ratios were highest for the composite atherosclerosis score. The survival curves for different categories of the composite atherosclerosis score are shown in figure 1.

In subgroup analyses stratified by gender, the age-adjusted risk estimates for the mild, moderate, and severe categories of the composite atherosclerosis score compared to no atherosclerosis were 1.29 (0.77 – 2.15), 1.77 (1.08 – 2.91), and 2.80 (1.71 – 4.57), respectively, for men, and 1.12 (0.54-2.29), 3.37 (1.81-6.25), and 4.82 (2.57 – 9.05), respectively, for women. For each of the four separate measures of atherosclerosis, hazard ratios also tended to be higher for women than for men, especially in the 'severe' categories (data not shown).

Table 3. Hazard ratios for incident coronary heart disease associated with aortic and lower extremity atherosclerosis and with the composite atherosclerosis score.

	Severity of atherosclerosis			
	no	**mild**	**moderate**	**severe**
Aortic atherosclerosis				
model 1	1.0	1.00 (0.58 – 1.72)	2.01 (1.45 – 2.79)	2.43 (1.76 – 3.35)
model 2	1.0	0.90 (0.52 – 1.54)	1.68 (1.21 – 2.33)	1.83 (1.31 – 2.55)
+ carotid plaques	1.0	0.74 (0.40 – 1.37)	1.63 (1.15 – 2.32)	1.47 (1.01 – 2.13)
+ carotid IMT	1.0	0.92 (0.52 – 1.64)	1.69 (1.19 – 2.42)	1.67 (1.16 – 2.40)
+ lower extremity atherosclerosis	1.0	0.74 (0.40 – 1.36)	1.75 (1.25 – 2.47)	1.87 (1.32 – 2.65)
Lower extremity atherosclerosis				
model 1	1.0	1.20 (0.86 – 1.68)	1.61 (1.16 – 2.22)	1.98 (1.44 – 2.72)
model 2	1.0	1.10 (0.78 – 1.55)	1.36 (0.97 – 1.90)	1.49 (1.06 – 2.10)
+ carotid plaques	1.0	1.05 (0.73 – 1.52)	1.28 (0.88 – 1.85)	1.36 (0.93 – 1.98)
+ carotid IMT	1.0	1.10 (0.76 – 1.60)	1.35 (0.93 – 1.96)	1.47 (1.01 – 2.13)
+ aortic atherosclerosis	1.0	1.04 (0.72 – 1.49)	1.20 (0.83 – 1.72)	1.41 (0.98 – 2.03)
Composite atherosclerosis score				
model 1	1.0	1.25 (0.83 – 1.90)	2.30 (1.56 – 3.39)	3.49 (2.37 – 5.15)
model 2	1.0	1.08 (0.71 – 1.64)	1.79 (1.20 – 2.67)	2.29 (1.50 – 3.48)

IMT = intima-media thickness. All hazard rate ratios adjusted for age and gender (model 1), and subsequently (model 2) for smoking habits, body mass index, total and high-density lipoprotein (HDL)-cholesterol, systolic and diastolic blood pressure, presence of diabetes mellitus, and use of aspirin, blood pressure lowering-, and cholesterol lowering medication. Model 2 was additionally adjusted for severity of atherosclerosis as indicated by the various atherosclerosis measures.

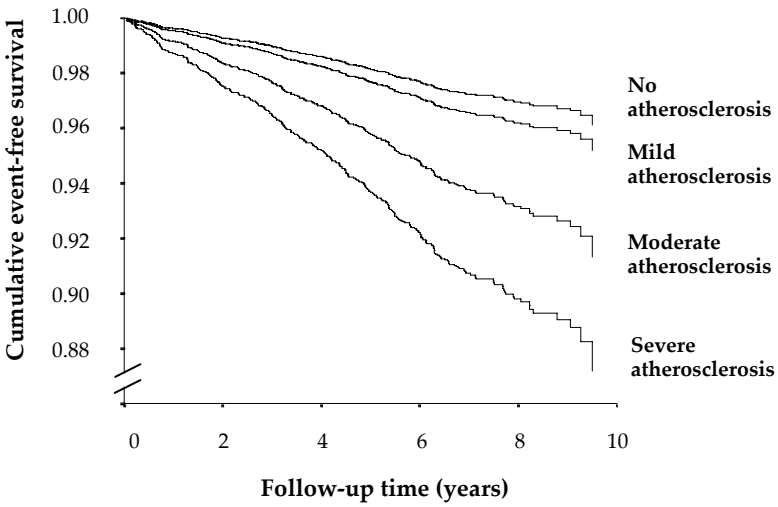

Figure 1. Survival curves for no, mild, moderate, and severe atherosclerosis as indicated by the composite atherosclerosis score.
CHD = coronary heart disease. Analyses were adjusted for age and gender

The hazard ratios associated with aortic atherosclerosis decreased, and for carotid IMT and lower extremity atherosclerosis lost statistical significance (tables 2 and 3) when carotid plaques was added to the fully adjusted models. On the contrary, the strong association between carotid plaques and CHD was independent of the other measures of atherosclerosis. When we simultaneously included carotid IMT and aortic atherosclerosis, carotid IMT and lower extremity atherosclerosis, or aortic atherosclerosis and lower extremity atherosclerosis in the model, the hazard ratios for these measures were not meaningfully different from those obtained from fully adjusted models in which only one atherosclerosis measure was included.

Finally, the predictive value for CHD of all six combinations of the measures of atherosclerosis is illustrated in figure 2.

DISCUSSION

The results of the present study show that measures of atherosclerosis assessing the severity of carotid plaques, carotid IMT, aortic atherosclerosis, or lower extremity atherosclerosis are all good predictors of incident CHD. Although differences with carotid IMT and lower extremity atherosclerosis were small, risk estimates for carotid plaques and aortic atherosclerosis were highest and independent of the other measures of atherosclerosis.

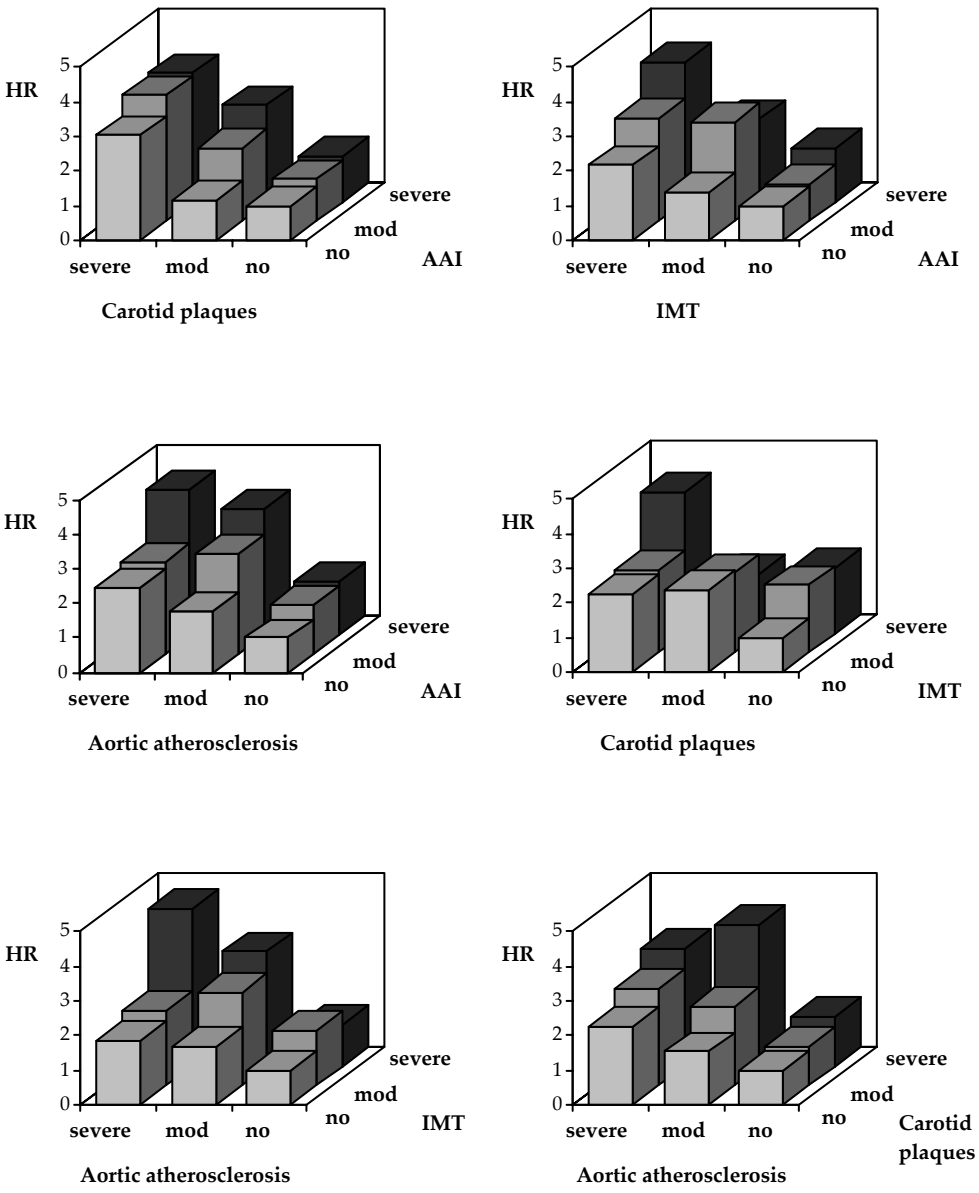

Figure 2. Hazard ratios for coronary heart disease for combinations of measures of atherosclerosis. HR = hazard ratio, mod = moderate, IMT = intima-media thickness, AAI = ankle-arm index (reflecting lower extremity atherosclerosis). Analyses were adjusted for age and gender.

The Rotterdam Study comprises a large and well-defined study population for which extensive information on traditional cardiovascular risk factors at baseline and on the incidence of CHD during follow-up has been collected. A great strength of the present study is that severity of atherosclerosis was measured at multiple sites in the arterial tree, making it possible to compare multiple measures of atherosclerosis with regard to their predictive value for CHD within one study population. Nevertheless, several methodological aspects of this study need to be considered. Although the severity of atherosclerosis was measured in a large number of subjects, not all participants had complete data on all four of the measures of atherosclerosis that are considered in the present study. Although we cannot exclude the possibility that health-related issues have also played a role, missing data are predominantly due to logistic reasons, and we therefore believe that they will not have affected our results. Furthermore, no data were available on the presence and severity of coronary atherosclerosis. It would be interesting to see how the hazard ratios for CHD associated with measures of extracoronary atherosclerosis as reported in the present study relate to those associated with coronary atherosclerosis. Several population-based studies now use electron-beam computed tomography to measure coronary atherosclerosis, but to date, no prospective population-based data have yet been published on coronary atherosclerosis and incident CHD.

Only few population-based studies have reported on the presence and severity of carotid plaques[5,17] or aortic atherosclerosis[6,12] in relation to incident CHD. The present study shows that the hazard ratios for CHD for these measures, which are based on the relatively crude, but direct assessment of the presence of atherosclerotic plaques, were somewhat higher than those for carotid IMT and lower extremity atherosclerosis. Moreover, the predictive value of both measures, but especially of carotid plaques, was independent of other measures of atherosclerosis, whereas hazard ratios associated with carotid IMT and lower extremity atherosclerosis lost statistical significance when carotid plaques was additionally included in the analyses. Because differences in IMT may partly be due to non-atherosclerotic processes such as fibromuscular hypertrophy, it has previously been suggested that presence, area, or volume of carotid plaques may be better indicators of future CHD risk than carotid IMT.[18,19] Furthermore, the fact that the AAI is an indirect measure of lower extremity atherosclerosis, which may also be influenced by hemodynamic factors and vascular stiffness, may explain why the hazard ratios for CHD associated with lower extremity atherosclerosis were lower than those associated with the other measures of atherosclerosis. Thus, although the carotid plaque and aortic atherosclerosis scores are more subjective and less commonly measured than carotid IMT and the AAI, they are strong predictors of CHD, likely because they directly assess the presence of atherosclerotic lesions. This is consistent with previous data from our group showing that associations with coronary calcification as assessed by electron beam computed tomography, which is also a direct measure of atherosclerotic lesions, were strongest for carotid plaques and aortic atherosclerosis.[1]

The predictive value for CHD of each of the individual measures of atherosclerosis was independent of a wide variety of traditional cardiovascular risk factors and of medication use. For purposes of CHD risk stratification, the measurement of extracoronary atherosclerotic disease as an indication of the presence of unknown or unmeasured cardiovascular risk factors, such as genetic predisposition or inflammation,[20,21] may therefore be a meaningful addition to the measurement of traditional cardiovascular risk factors. Although the measurement of carotid IMT is unlikely to be of use as a screening tool in the general population,[22] measurement of the AAI has been suggested to be useful in persons above the age of 50 or in persons at high risk.[23] However, the present study shows that compared to the other measures, lower extremity atherosclerosis is a relatively weak predictor of CHD. Future research, which should also include electron beam computed tomography, is needed to establish the clinical value of the various measures of atherosclerosis either in the general population or in populations at high risk of CHD.

In conclusion, non-invasive measures of extracoronary atherosclerosis are good predictors of CHD, independently of traditional cardiovascular risk factors. Risk estimates for carotid plaques and aortic atherosclerosis were highest and independent of the other atherosclerosis measures, showing the value of direct assessment of atherosclerotic plaques in CHD risk prediction.

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Chapter 3

Inflammatory mediators and cardiovascular disease

Chapter 3.1

Inflammatory mediators and atherosclerosis

ABSTRACT

Background: Inflammatory mediators and soluble cell adhesion molecules (sCAMs) predict cardiovascular events. It is not clear whether they reflect severity of underlying atherosclerotic disease.

Methods: Within the Rotterdam Study, we investigated associations of C-reactive protein (CRP), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) with non-invasive measures of atherosclerosis. Levels of CRP were assessed in a random sample of 1,317 participants, levels of IL-6 and sCAMs in a subsample of 714 participants.

Results: In multivariate analyses, log-transformed CRP (β = -0.023, 95% CI -0.033 to -0.012) and IL-6 (β = -0.025 (-0.049 to -0.001)) were inversely associated with the ankle-arm index. Only CRP was associated with carotid intima-media thickness (β = 0.018 (0.010 to 0.027)). Compared to the lowest tertile, the odds ratio for moderate to severe carotid plaques associated with levels of CRP in the highest tertile was 2.0 (1.3 to 3.0). sICAM-1 levels were strongly associated with carotid plaques (OR 2.5 (1.5 to 4.4; highest versus lowest tertile)). sVCAM-1 was not significantly associated with any of the measures of atherosclerosis.

Conclusion: This study indicates that CRP is associated with severity of atherosclerosis measured at various sites. Associations of the other markers with atherosclerosis were less consistent.

Over the past years, it has been recognized that inflammation may contribute to all stages of the atherosclerotic process.[1] The acute phase protein C-reactive protein (CRP), which is mainly regulated by the cytokine interleukin-6 (IL-6), is a sensitive marker of inflammation. In several prospective studies, high levels of CRP and IL-6 predicted coronary events, both in patients with stable or unstable angina and in healthy subjects.[2-4] Other molecules that have been suggested to contribute to atherosclerosis and its clinical outcomes are the endothelial cell adhesion molecules (CAMs), which facilitate the emigration of leukocytes into the vessel wall - an important feature of atherosclerotic plaque formation.[5] Indeed, high levels of soluble intercellular adhesion molecule-1 (sICAM-1) have been shown to predict myocardial infarction in the Physicians' Health Study and the Atherosclerosis Risk in Communities study.[6,7]

The presence of CRP, IL-6, ICAM-1, and vascular cell adhesion molecule-1 (VCAM-1) has been demonstrated in atherosclerotic lesions.[8-10] Moreover, levels of CRP and IL-6 were positively related to peripheral and coronary artery disease in selected patient populations,[11,12] although Folsom et al. recently concluded that CRP is not a strong marker of prevalent atherosclerosis.[13] sICAM-1 showed a positive relationship with peripheral arterial disease (PAD),[14] established by angiography or ultrasound, whereas sVCAM-1 was related to the extent of atherosclerosis, established angiographically in multiple vascular beds in patients with PAD.[15] In various populations, sCAMs were positively related to common carotid intima-media thickness (IMT).[6,16,17]

Most of the studies on inflammatory mediators or adhesion molecules and atherosclerosis were based on patient series. In the Rotterdam Study, a population-based cohort study of men and women aged 55 years and over, we investigated whether plasma levels of CRP, IL-6, sICAM-1, and sVCAM-1 are associated with severity of atherosclerosis, measured at various sites of the arterial tree.

METHODS

Study population

The Rotterdam Study is a prospective population-based cohort study comprising 7,983 men and women aged 55 years and over. Its overall aim is to investigate the incidence of and risk factors for chronic disabling diseases. From 1990 – 1993, all inhabitants of a suburb of the city of Rotterdam aged 55 years and over were invited to participate in an extensive home interview and two visits to the research center. The overall response rate was 78%. The Medical Ethics Committee of the Erasmus University Rotterdam approved the Rotterdam Study, and written informed consent was obtained from all participants. For the current study, levels of CRP were assessed in a randomly selected age- and gender-stratified sample of 1,317 participants. Levels of IL-6, sICAM-1 and sVCAM-1 were assessed in a random subsample of 714

participants. A more detailed description of the Rotterdam Study and the collection of data has been given elsewhere.[18,19]

Measures of atherosclerosis

Using a random zero sphygmomanometer, sitting blood pressure was measured at the right upper arm. The average of two measurements obtained at one occasion was used. Systolic blood pressure at the ankles (posterior tibial artery) was measured in supine position with a random zero sphygmomanometer and an 8 MHz continuous wave Doppler probe (Huntleigh 500D, Huntleigh Technology, Bedfordshire, UK). The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm was computed to obtain the ankle-arm index (AAI). For the analyses, we used the lowest value of two legs. Since arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI larger than 1.50 was considered invalid.[19]

Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer and a duplex scanner (ATL UltraMark IV, Advanced Technology Laboratories, Bethel, Washington, USA). Common carotid IMT was determined as the average of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[20]

Carotid plaques were defined as a focal widening relative to adjacent segments, with the protrusion into the lumen composed of either only calcified deposits or a combination of calcification and noncalcified material.[20] A plaque score ranging from 0 to 1 was derived by dividing the number of sites (left and right side of common carotid artery, carotid bifurcation, and internal carotid artery) with a detectable plaque by the total number of sites (with a maximum of six) for which an ultrasonographic image was available. To indicate the presence of moderate or severe carotid plaques, we used cut-off points of 0.5 and 0.75, respectively.

Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. The extent of abdominal aortic atherosclerosis was scored according to the length of the involved area (with scores 0 – 5 corresponding to 0, ≤ 1, 1 – 2.5, 2.5 – 4.9, 5.0 – 9.9, and ≥ 10.0 cm).[21] A calcification score of 3 was considered moderate, scores 4 and 5 were considered severe.

Inflammatory mediators and CAMs

Due to logistic reasons, CRP was measured by two sensitive immunological methods, using an in-house enzyme immunoassay (n = 603 subjects; DAKO Denmark) or a nephelometric method (n = 714; Dade-Behring Marburg). These two methods show a high level of agreement.[22] CRP was measured by both methods in 134 subjects. For each of these subjects with values of CRP ≤ 10 mg/l, we plotted the difference between the log-transformed results of the two methods against the mean of the two methods.[23] The plot showed no systematic relationship between the difference and the mean of the paired measurements and the two methods showed good agreement. The mean difference in CRP was 0.036 mg/l. To ascertain that differences in the

Table 1. Baseline characteristics of the study population.

Variable	All subjects (n = 1,317)	Subsample (n = 714)
Age (years)	71.2 ± 8.9	71.6 ± 9.0
Gender (% men)	46.3	47.2
Body mass index (kg/m^2)	26.3 ± 3.6	26.4 ± 3.6
Systolic blood pressure (mmHg)	138.8 ± 21.5	138.4 ± 21.0
Total cholesterol (mmol/l)	6.6 ± 1.2	6.6 ± 1.2
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.3 ± 0.4
Current smokers (%)	19.3	19.9
Diabetes mellitus (%)	9.5	8.3
History of myocardial infarction (%)	15.1	14.9
Carotid plaques (%)*	27.2	25.0
Aorta calcification (%)†	32.3	30.9
C-reactive protein (mg/l)‡	1.82 (0.85 – 3.68)	1.73 (0.81 – 3.68)
Interleukin-6 (pg/ml)‡		1.91 (1.25 – 3.13)
sICAM-1 (ng/ml)		223.7 ± 69.9
sVCAM-1 (ng/ml)		542.6 ± 184.5

HDL = high-density lipoprotein, sICAM-1 = soluble intercellular adhesion molecule-1, sVCAM-1 = soluble vascular cell adhesion molecule-1. Data are means ± standard deviations for continuous variables and percentages for dichotomous variables. *Defined as a plaque score (range 0 to 1) ≥ 0.5. †Defined as a calcification score (range 0 to 5) ≥ 3. ‡For data with a skewed distribution, the median and interquartile range are shown.

distribution of CRP for the two methods had not influenced the results, we standardized the two distributions of CRP by computing Z-scores (value minus mean, divided by the standard deviation of the mean). We repeated all analyses using the standardized data, and found similar results as the ones reported here.

IL-6, sICAM-1 and sVCAM-1 were measured using commercially available ELISA (IL-6: Quantikine® HS IL-6; sICAM-1 and sVCAM-1: Parameter®; all assays from R&D Systems Europe, Oxon, United Kingdom). Outliers (values above three standard deviations of the population distribution) of log-transformed CRP (n = 4) and IL-6 (n = 9) and of sICAM-1 (n = 13) and sVCAM-1 (n = 13) were excluded, since they may indicate the presence of an active inflammatory disease. In the study population, 5.2% had levels of CRP > 10 mg/l.

Statistical analyses

We used log-transformed values of CRP and IL-6 to normalize the distribution of these variables. Cut-off points for tertiles were 1.11 and 2.77 mg/l for CRP, 1.44 and 2.43 pg/ml for IL-6, 190.1 and 231.4 ng/ml for sICAM-1, and 451.8 and 574.8 ng/ml for sVCAM-1. We estimated partial correlations between inflammatory mediators and

sCAMs. Mean values of the AAI and IMT were computed for increasing tertiles of CRP, IL-6, sICAM-1 and sVCAM-1 by means of ANCOVA. We tested linearity using linear regression analysis with the AAI and IMT as dependent variables. Logistic regression analysis was used to compute odds ratios (for the higher tertiles of inflammatory mediators and sCAMs compared to the lowest tertile) of having moderate to severe carotid plaques and abdominal aorta calcification. Analyses involving CRP were adjusted for the method used to measure CRP levels. All analyses were adjusted for age, gender, smoking status (current, past, or never smokers), and packyears of smoking, and additionally for body mass index (BMI) and diabetes mellitus. Analyses were done using SPSS 9.0 for Windows.

RESULTS

Baseline characteristics of the study population are shown in table 1. Baseline characteristics of the subsample of 714 participants were similar to those of the total sample.

CRP and IL-6, as well as sICAM-1 and sVCAM-1, were moderately correlated (r = 0.56 and r = 0.27, respectively; P <0.001; table 2). Both CRP and IL-6 were correlated with sICAM-1 (r = 0.20 and r = 0.21, respectively; P <0.001).

Table 2. Partial correlations between inflammatory mediators and soluble cell adhesion molecules.

	Interleukin-6*	sICAM-1	sVCAM-1
C-reactive protein*	0.56†	0.20†	0.04
Interleukin-6*		0.21†	0.09‡
sICAM-1			0.27†

sICAM-1 = soluble intercellular adhesion molecule-1, sVCAM-1 = soluble vascular cell adhesion molecule-1. Correlations are adjusted for age, gender, smoking status, and packyears of smoking. *For CRP and IL-6 log-transformed values are used. †P <0.001, ‡P = 0.02.

Increasing tertiles of CRP and IL-6 were inversely associated with the AAI. In linear regression analysis, the regression coefficients (β) were -0.026 (95% confidence interval -0.036 to -0.016) and -0.034 (-0.057 to -0.011), respectively, for log-transformed values of CRP and IL-6. After adjustment for BMI and diabetes mellitus, the associations of CRP (β = -0.023 (-0.033 to -0.012)) and IL-6 (β = -0.025 (-0.049 to -0.001)) with the AAI remained. The data presented in figure 1 are based on the fully adjusted model. sCAMs were not linearly associated with the AAI.

Table 3. Odds ratios for carotid plaques and abdominal aorta calcification associated with increasing tertiles of inflammatory mediators and cell adhesion molecules.

	Tertiles of plasma level			Tertiles of plasma level*		
	1	**2**	**3**	**1**	**2**	**3**
Carotid plaques†						
CRP	1.0	1.5 (1.0 – 2.3)	2.0 (1.3 – 2.9)	1.0	1.6 (1.0 – 2.4)	2.0 (1.3 – 3.0)
IL-6	1.0	1.4 (0.8 – 2.5)	1.7 (0.9 – 3.0)	1.0	1.4 (0.8 – 2.5)	1.5 (0.8 – 2.7)
sICAM-1	1.0	1.5 (0.9 – 2.5)	2.6 (1.5 – 4.5)	1.0	1.4 (0.8 – 2.4)	2.5 (1.5 – 4.4)
sVCAM-1	1.0	1.4 (0.8 – 2.3)	1.5 (0.9 – 2.6)	1.0	1.4 (0.8 – 2.4)	1.6 (0.9 – 2.7)
Abdominal aorta calcification‡						
CRP	1.0	1.3 (0.8 – 1.9)	1.3 (0.9 – 2.0)	1.0	1.2 (0.8 – 1.9)	1.2 (0.8 – 1.9)
IL-6	1.0	1.4 (0.8 – 2.6)	1.7 (0.9 – 3.1)	1.0	1.4 (0.8 – 2.5)	1.5 (0.8 – 2.8)
sICAM-1	1.0	1.6 (0.9 – 2.8)	1.7 (1.0 – 3.1)	1.0	1.6 (0.9 – 2.9)	1.6 (0.9 – 2.9)
sVCAM-1	1.0	1.6 (0.9 – 2.9)	1.1 (0.6 – 1.9)	1.0	1.6 (0.9 – 2.9)	1.1 (0.6 – 1.9)

CRP = C-reactive protein, IL-6 = interleukin-6, sICAM-1 = soluble intercellular adhesion molecule-1, sVCAM-1 = soluble vascular cell adhesion molecule-1. All analyses adjusted for age, gender, smoking status, and packyears of smoking. *Additionally adjusted for body mass index and diabetes mellitus. †Defined as a plaque score (range 0 to 1) ≥ 0.5. ‡Defined as a calcification score (range 0 to 5) ≥ 3.

CRP was significantly linearly associated with IMT both before (β = 0.021 (0.013 to 0.029)) and after (β = 0.018 (0.010 to 0.027); figure 2) additional adjustment for BMI and diabetes mellitus. IL-6 and sCAMs were not associated with IMT.

Compared to the lowest tertile, odds ratios (OR) for carotid plaques associated with levels of CRP and sICAM-1 in the highest tertile were 2.0 (1.3 to 2.9) and 2.6 (1.5 to 4.5), respectively (table 3). For IL-6 and sVCAM-1, there was a comparable, but not statistically significant trend. Associations of CRP and sICAM-1 with carotid plaques were very robust; the association between IL-6 and carotid plaques was somewhat weaker after adjustment for BMI and diabetes mellitus (OR 1.5 (0.8 to 2.7)).

Only sICAM-1 was borderline statistically significantly associated with abdominal aorta calcification (OR 1.7 (1.0 to 3.1); table 3). For IL-6, there was a similar trend. After adjusting for BMI and diabetes mellitus, the association of sICAM-1 with aorta calcification (OR 1.6 (0.9 to 2.9)) was slightly weaker.

To take possible changes in lifestyle and medication use in participants with a clinically manifest myocardial infarction in the past into account, we repeated all analyses with additional adjustment for past myocardial infarction and for aspirin and statin use at baseline. Associations of inflammatory mediators and adhesion molecules with measures of atherosclerosis were not meaningfully different (data not shown).

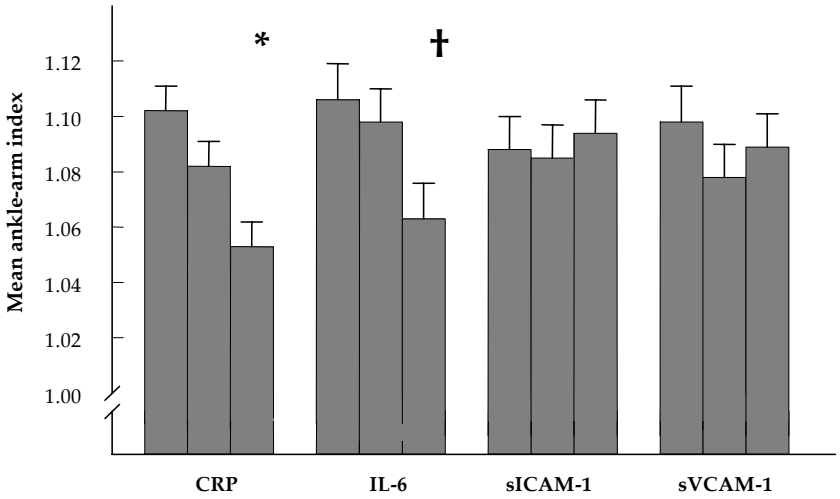

Figure 1. Mean ankle-arm index by tertiles of C-reactive protein (CRP), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1).
Bars represent means and lines represent the standard error of the mean. Means are adjusted for age, gender, smoking status, packyears of smoking, body mass index, and diabetes mellitus.
*P trend <0.001, †P trend = 0.04.

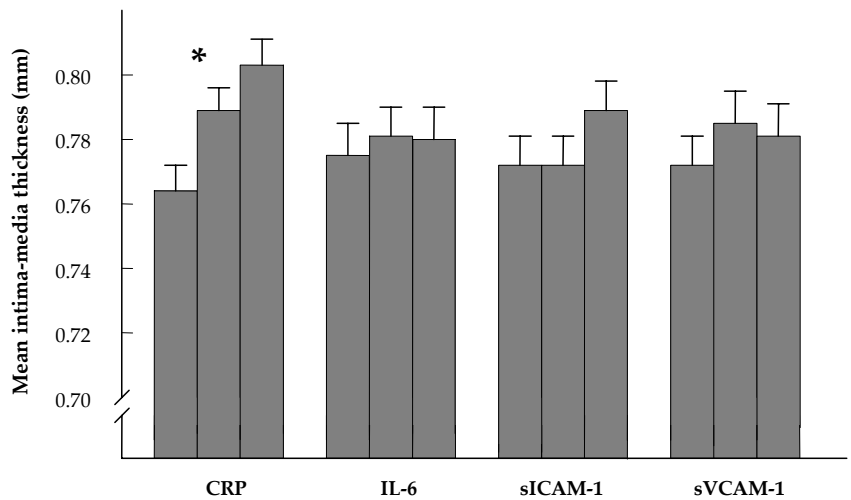

Figure 2. Mean intima-media thickness (mm) of the common carotid artery by tertiles of C-reactive protein (CRP), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1).
Bars represent means and lines represent the standard error of the mean. Means are adjusted for age, gender, smoking status, packyears of smoking, body mass index, and diabetes mellitus.
* P trend <0.001.

DISCUSSION

This population-based study in elderly subjects shows that CRP is related to severity of atherosclerosis at various sites of the arterial tree. Although the results of this study suggest that IL-6 and sICAM-1 are also associated with atherosclerosis, associations of these markers with measures of atherosclerosis were less consistent.

In this study, we examined multiple inflammatory mediators in relation to multiple measures of atherosclerosis in a population-based setting. There are several methodological issues that need to be discussed before interpreting these data. First, we used different methods and different parts of the vascular tree to assess the severity of atherosclerosis. The AAI represents the amount of atherosclerotic disease distal to the aortic bifurcation, but it may also be influenced by hemodynamic factors and vascular stiffness. Carotid IMT and plaques reflect atherosclerosis in the carotid vessel wall, although it cannot be excluded that non-atherosclerotic processes such as fibromuscular hypertrophy cause modest degrees of intima-media thickening.[24] Radiographically assessed calcification of the abdominal aorta has been shown to be highly specific, representing advanced intima atherosclerosis.[25] Second, we have presented our results both with and without additional adjustment for body mass index and diabetes mellitus. It has recently been reported that adipose tissue is an important source of cytokine production,[26] and adjusting for BMI will substantially lower the variation in levels of inflammatory mediators. Furthermore, inflammation has been suggested to be a triggering factor in the origin of diabetes mellitus,[27] which implies that diabetes mellitus may be an intermediate rather than a confounder. Adjusting for BMI and diabetes mellitus is only indicated, if one wants to examine whether the association of inflammation with atherosclerosis is independent of these variables. Third, levels of CRP, IL-6, sICAM-1 and sVCAM-1 were only measured once. Intra-individual variation, as has been reported for CRP and IL-6,[28,29] can therefore not be taken into account. However, such variation will likely result in an underestimation of the true relationship.

Many studies have found a relationship between CRP and risk of myocardial infarction in the general population.[3] In addition, levels of CRP and IL-6 were positively related to peripheral and coronary artery disease in selected patient populations.[11,12] This study is the first to show a positive association between inflammation, represented by levels of CRP and IL-6, and severity of atherosclerosis in a population-based study in the elderly.

IL-6 is the principal determinant of the hepatic synthesis of CRP. In the present study, CRP is linearly associated with carotid IMT, whereas IL-6 is not. A possible explanation for the discrepancy between CRP and IL-6 is the substantial intra-individual variation in levels of IL-6.[29] Although associations with different measures of atherosclerosis are more consistent for CRP than for IL-6, it is likely that levels of CRP reflect general inflammatory activity. However, specific actions of CRP itself may also be involved in atherogenesis.[30,31]

CRP was not associated with the presence of abdominal aorta calcification. This suggests that, once atherosclerotic plaques have reached the stage in which they are calcified, inflammation is no longer one of the main features of these plaques. Similarly, several cross-sectional studies reported a lack of association of CRP with coronary calcification as measured by electron beam tomography.[32,33]

Since the acute phase response may be induced by damage to the vascular endothelium caused by atherosclerotic processes, plasma levels of inflammatory mediators may simply represent the extent of atherosclerotic disease. Alternatively, however, increased local or systemic inflammation due to chronic infection, chronic inflammatory diseases, smoking, obesity, or impaired glucose tolerance[26,34,35] may precede (progression of) atherosclerosis.[36] Due to the cross-sectional design of this study, it is not possible to draw conclusions about the direction of the associations between inflammatory mediators and atherosclerosis.

CAMs may be important in atherosclerosis since they facilitate the emigration of leukocytes into the vessel wall.[5] Pathological studies have shown that CAMs are expressed on atherosclerotic plaques[10] and in mice, deficiency of CAMs appeared to protect against atherosclerosis.[37] Soluble CAMs are likely to result from shedding from atherosclerotic plaques[38] and they are thought to represent the severity of atherosclerosis. However, reports on adhesion molecules and atherosclerosis are not consistent. Several studies reported significant associations of only sICAM-1,[6,14] only sVCAM-1,[15,16] or both sCAMs[17] to measures of atherosclerosis.

It is not clear what accounts for the diversity of the associations found between sCAMs and atherosclerosis. The populations that have been studied varied substantially in the degree of atherosclerosis, and atherosclerosis was measured at different sites of the arterial tree. In this study, sICAM-1, but not sVCAM-1, is significantly correlated with CRP and IL-6, and the odds ratios for carotid plaques and aorta calcification associated with high levels of sICAM-1 were increased. sVCAM-1 was not related to atherosclerosis. This study adds to the understanding that sICAM-1 and sVCAM-1 are likely to have different roles in atherogenesis or progression of atherosclerosis. Moreover, whereas CRP was strongly associated with the AAI and IMT, sICAM-1 was not. More research, both at the molecular level and at the population level, is necessary to elucidate the role of CAMs in atherosclerosis.

In this study, associations with measures of atherosclerosis were more consistent for CRP than for IL-6. Since relatively cheap, automated, high-sensitivity methods for CRP measurement have become commercially available,[39] measurement of CRP will be the most useful once inflammatory markers are routinely assessed in clinical settings.

We conclude that CRP is associated with severity of atherosclerosis at various sites of the arterial tree. Associations of IL-6 and sCAMs with atherosclerosis are less consistent.

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Chapter 3.2

C-reactive protein and progression of atherosclerosis

ABSTRACT

Background: C-reactive protein (CRP) predicts myocardial infarction and stroke. Its role as a predictor of progression of subclinical atherosclerosis is not yet known. We investigated whether CRP predicts progression of atherosclerosis measured at various sites of the arterial tree.

Methods: Levels of CRP were measured in a random sample of 773 subjects aged 55 years and over participating in the Rotterdam Study. Subclinical atherosclerosis was assessed at various sites at two points in time, with a mean duration between measurements of 6.5 years.

Results: After adjustment for age, gender, and smoking habits, odds ratios associated with levels of CRP in the highest quartile compared to the lowest quartile were increased for progression of carotid (odds ratio 1.9 (95% confidence interval 1.1 – 3.3)), aortic (1.7 (1.0 – 3.0)), iliac (2.0 (1.2 – 3.3)) and lower extremity atherosclerosis (1.9 (1.0 – 3.7)). The odds ratio for generalized progression of atherosclerosis as indicated by a composite progression score was 4.5 (2.3 – 8.5). Except for aortic atherosclerosis, these estimates hardly changed after additional adjustment for multiple cardiovascular risk factors. In addition, odds ratios for progression of atherosclerosis associated with high levels of CRP were as high as the odds ratios associated with the traditional cardiovascular risk factors high cholesterol, hypertension, and smoking. Geometric mean levels of CRP increased with the total number of sites showing progression of atherosclerosis (P trend = 0.002).

Conclusion: CRP predicts progression of atherosclerosis measured at various sites of the arterial tree.

In recent years, C-reactive protein (CRP) has become established as a risk factor for cardiovascular disease. Increased levels of CRP predict future myocardial infarction[1] and stroke[2] independent of other cardiovascular risk factors, and it has been suggested that measurement of CRP, in addition to traditional risk factors, may improve our ability to predict cardiovascular disease.[3]

In selected patient groups, levels of CRP were positively associated with angiographically established coronary artery disease.[4,5] In addition, CRP has been related both cross-sectionally and prospectively to peripheral arterial disease (PAD).[5,6] In the Rotterdam Study, we found that CRP is strongly associated with atherosclerosis measured at various sites of the arterial tree.[7] Several mechanisms have been described by which CRP and other inflammatory mediators may be actively involved in atherogenesis.[8] However, not all studies found a clear association between CRP and atherosclerosis.[9]

The inflammatory activity within the atherosclerotic plaque is one of the main determinants of a plaque's vulnerability to rupture.[10] Because plaque rupture, thrombus formation, and subsequent organization and incorporation of the thrombus in the plaque are thought to be the most important cause of rapid progression of atherosclerotic plaques,[11] CRP may be a good predictor of progression of atherosclerosis. Until now, only one small study has reported an association between CRP and progression of carotid atherosclerosis.[12]

In the Rotterdam Study, a population-based cohort study of men and women aged 55 years and over, we investigated whether levels of CRP are associated with progression of atherosclerosis measured at various sites of the arterial tree.

METHODS

Study population

The Rotterdam Study is a prospective population-based cohort study composed of 7,983 men and women aged 55 years and over. Its overall aim is to investigate the incidence and determinants of chronic disabling diseases. The first phase lasted from 1990 until 1993, when all inhabitants of a suburb of the city of Rotterdam aged 55 years and over were invited to participate in an extensive home interview and two visits to the research center. The overall response rate was 78%. Phase 3 was held from 1997 until 1999. Between phase 1 and phase 3, 25% of the participants had died and 0.4% were lost to follow-up. The Medical Ethics Committee of the Erasmus University Rotterdam approved the Rotterdam Study, and written informed consent was obtained from all participants. The population used for the current study was an age- and gender-stratified randomly selected sample of 773 participants. Given the age and gender distribution, the prevalence of cardiovascular risk factors in the study population was similar to the prevalence of these risk factors in the whole Rotterdam Study population. A more detailed description of the Rotterdam Study has been given elsewhere.[13]

Clinical characteristics

A trained investigator visited all participants at home and collected information using a computerized questionnaire. The obtained information included current health status, medical history, drug use, and smoking behavior. Additionally, during two visits to the research center, blood samples were drawn, and established cardiovascular risk factors were measured, as described previously.[14] We defined hypertension as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg and/or use of antihypertensive medication. Diabetes mellitus was defined as the use of blood glucose lowering medication and/or a non-fasting serum glucose level ≥ 11.1 mmol/l. A 12-lead resting ECG was recorded and analyzed by the Modular ECG Analysis System (MEANS).[15] A history of myocardial infarction before entering the study was considered present in case of a confirmed self-report of myocardial infarction or an ECG characteristic for past myocardial infarction. Aspirin and statin (HMG-CoA reductase inhibitor) use between phases 1 and 3 were assessed using computerized pharmacy records and data from the interview at phase 3.

Measures of atherosclerosis

For each participant, the extent of atherosclerosis was assessed at both phase 1 and phase 3 of the Rotterdam Study by measuring carotid plaques, aortic and iliac calcification, and the ankle-arm index (AAI) .

Carotid atherosclerosis. Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer and a duplex scanner (ATL UltraMark IV). The common carotid artery, carotid bifurcation, and internal carotid artery were examined both left and right for the presence of plaques, defined as a focal widening relative to adjacent segments, with the protrusion into the lumen composed of either only calcified deposits or a combination of calcified and noncalcified material.[14] A plaque score ranging from 0 to 1 was computed by dividing the number of sites with a detectable plaque by the total number of sites for which an ultrasonographic image was available (with a maximum of six). Subjects for whom data on the presence of plaques were not available for at least two of the six sites that were examined were excluded. Progression of carotid atherosclerosis was defined as an increase in plaque score of more than 0.17 (= 1/6). Participants (15.1%) with a decrease in plaque score were added to the group with no progression, since we considered this to be mainly due to measurement error. Exclusion of these subjects from the analyses did not substantially change the results. Due to limited availability of ultrasonographers at the end of 1992 and in 1993, not all subjects who visited the research center could be examined for the presence of carotid plaques.

Aortic and iliac atherosclerosis. Aortic and iliac atherosclerosis were diagnosed by radiographic detection of calcified deposits in the abdominal aorta and iliac arteries on a lateral abdominal film.[16] The extent of aortic atherosclerosis was scored according to the length of the involved area. Atherosclerosis of the iliac arteries was scored as absent, present either left or right, or present on both sides. For progression of aortic and iliac atherosclerosis, baseline and follow-up films were examined in pairs.

Progression of aortic atherosclerosis was scored on a graded scale (with scores 0 – 4 corresponding to 0, ≤ 1, 1 – 2.5, 2.5 – 4.9, and ≥ 5.0 cm progression, respectively) and considered present if the score was more than one; progression of iliac atherosclerosis was scored as either absent or present. None of the participants showed a decrease in the extent of aortic and iliac atherosclerosis. All films were read by one observer, who was aware of the date of the radiographs. Before the scoring, a sample of the films was read by 2 observers simultaneously to reach agreement on the interpretation of the scoring protocol. Interobserver agreement on progression scoring (absent versus present), as previously determined at our department for 758 pairs of lateral radiographic films of the lumbar spine, reached a percentage of agreement of atherosclerotic change of 88, and a κ statistic of 0.74.[16] Progression of aortic and iliac atherosclerosis could not be evaluated for 19 and 29 participants, respectively, because the aorta or iliac arteries were not clearly depicted on the radiograph at baseline or follow-up.

Lower extremity atherosclerosis. We computed the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm to obtain the AAI.[17] The AAI is susceptible to measurement error, and we therefore considered a decline in AAI to be real if it was larger than 15%.[18] In addition, since it is not likely that an AAI which is still in the upper range of the distribution at phase 3 reflects a true difference in lower extremity atherosclerosis, we considered progression of lower extremity atherosclerosis to be present if the decline in AAI resulted in an AAI at phase 3 smaller than 0.9. Since arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI > 1.50 was considered invalid.

Composite progression score. Finally, we computed a composite progression score by adding 1 point for each measure of atherosclerosis that had shown progression during follow-up. Generalized progression of atherosclerosis was defined as a composite progression score equal to or larger than three. For logistic reasons, data were not complete for all subjects. Data on progression of carotid, aortic, iliac, and lower extremity atherosclerosis were available for 88.2%, 81.9%, 80.6%, and 80.3% of the study population, respectively. For subjects who had a missing value on one (n = 269) or two (n = 180) of the four measures, a weighted score was computed giving equal weight to each measure.

Measurement of C-reactive protein

A venipuncture was performed, applying minimal stasis, using a 21 gauge Butterfly needle with tube (Surflo winged infusion set, Terumo). Non-fasting blood was collected in tubes containing 0.129 mol/l sodium citrate at 4 °C. The ratio of blood to sodium citrate was 9:1. Plasma was collected after centrifugation for 10 minutes at 3000 rotations per minute (RPM). Subsequently, platelet-free plasma was obtained by centrifugation for 10 minutes at 10,000 RPM and was immediately frozen in liquid nitrogen and stored at –80 °C. All tubes were stored on ice before and after blood sampling. CRP was measured by sensitive immunological methods using an in-house

enzyme immunoassay (n = 334 subjects; DAKO) or a nephelometric method (n = 439; Dade-Behring). These two methods demonstrate a high level of agreement.[19] CRP was measured by both methods in 70 subjects. For each of these subjects with values of CRP ≤ 10 mg/l, we plotted the difference between the logarithmically transformed results of the two methods against the mean of the two methods.[20] The plot showed no systematic relationship between the difference and the mean of the paired measurements, and the two methods showed good agreement. The mean difference in CRP was 0.01 mg/l. To ascertain that differences in the distribution of CRP for the two methods had not influenced the results, we standardized the two distributions of CRP by computing z scores (value minus mean, divided by the SD of the mean). We repeated all analyses by using the standardized data and found results similar to the ones reported in the present study. In the study population, 3.5% had levels of CRP > 10 mg/l. Outliers (values above three standard deviations of the population distribution of log-transformed CRP; n = 3) were excluded, since they may indicate the presence of an active inflammatory disease.

Statistical analyses

When data were missing on clinical characteristics that are measured on a continuous scale (n = 10), we imputed the population mean. Using multivariate logistic regression analysis, we computed odds ratios for carotid, aortic, iliac, lower extremity, and generalized progression of atherosclerosis associated with increasing quartiles of the population distribution of CRP, with the lowest quartile as the reference. All analyses were adjusted for age, gender, and smoking status at baseline, duration of follow-up, and the method used to measure CRP levels. Analyses were additionally adjusted for baseline total cholesterol:HDL-cholesterol ratio, body mass index (BMI), diabetes mellitus, systolic blood pressure, aspirin and statin use at any time between phase 1 and phase 3, and a history of myocardial infarction. We computed age- and gender-adjusted odds ratios for progression of each measure of atherosclerosis for subjects with cholesterol levels in the highest versus the lowest quartile, for subjects with hypertension versus subjects without hypertension, and for current smokers versus non-smokers, to compare these estimates with the odds ratios for progression associated with CRP. Finally, we computed geometric mean levels of CRP and their 95% confidence intervals for increasing scores of the composite progression score. Geometric means were adjusted for baseline levels of cardiovascular risk factors. We used ANCOVA to test for linearity. Analyses were done using SPSS 9.0 for Windows.

RESULTS

Baseline characteristics of the study population are shown in table 1. The geometric mean level of CRP was 1.54 mg/l (interquartile range 0.78 – 2.93).

Odds ratios associated with levels of CRP in the highest quartile of the population distribution compared to the lowest quartile were clearly increased for progression of

Table 1. Baseline characteristics of the study population.

Variable	All subjects (n = 773)
Age (years)	67.4 ± 7.5
Gender (% men)	47.3
Body mass index (kg/m^2)	26.3 ± 3.5
Current smokers (%)	19.4
Total cholesterol (mmol/l)	6.6 ± 1.2
HDL-cholesterol (mmol/l)	1.3 ± 0.4
Hypertension (%)*	27.0
Diabetes mellitus (%)	4.4
History of myocardial infarction (%)	10.0
Progression of atherosclerosis (%)	
Carotid	25.8
Aortic	40.3
Iliac	57.9
Lower extremity	14.7
Duration of follow-up (years)	6.5 ± 0.4
C-reactive protein (mg/l)†	1.54 (0.78 – 2.93)

HDL = high-density lipoprotein. Data are means ± standard deviations for continuous variables and percentages for dichotomous variables. *Defined as systolic blood pressure ≥ 160 mmHg, diastolic blood pressure ≥ 100 mmHg, or use of antihypertensive medication. †For C-reactive protein, the median and interquartile range are shown.

carotid (odds ratio 1.9 (95% confidence interval 1.1 – 3.3)), aortic (1.7 (1.0 – 3.0)), iliac (2.0 (1.2 – 3.3)), and lower extremity atherosclerosis (1.9 (1.0 – 3.7)) after adjusting for age, gender, smoking behavior, and duration of follow-up (table 2, model 1). For iliac and lower extremity atherosclerosis, odds ratios for progression increased across quartiles of CRP, while for carotid and aortic atherosclerosis, the increase was only present for subjects with CRP levels in the highest quartile. 26.4% of the population had generalized progression of atherosclerosis as indicated by a composite progression score equal to or larger than three. The odds ratio for generalized progression of atherosclerosis associated with levels of CRP in the highest quartile was 4.5 (2.3 – 8.5).

To investigate whether the associations of CRP with progression of atherosclerosis were independent of other cardiovascular risk factors, we additionally adjusted for baseline total cholesterol:HDL-cholesterol ratio, BMI, presence of diabetes mellitus, systolic blood pressure, aspirin and statin use, and history of myocardial infarction (table 2, model 2). Odds ratios for carotid (OR 1.7 (1.0 – 3.1)), iliac (2.2 (1.3 – 3.8)), lower extremity (1.9 (0.9 – 4.1)), and generalized (4.6 (2.2 – 9.5)) progression of atherosclerosis associated with CRP levels in the highest quartile were still clearly increased. The odds ratio for progression of aortic atherosclerosis was attenuated.

The analyses were repeated in subjects without atherosclerosis at the baseline examination. In subjects with a carotid plaque score of 0, no PAD (AAI ≥ 0.9), or no generalized atherosclerosis at baseline, associations of CRP with progression of carotid plaques, lower extremity atherosclerosis, and generalized atherosclerosis, respectively, did not meaningfully change (data not shown). However, in subjects without aortic atherosclerosis at baseline, the association of CRP with progression of aortic atherosclerosis disappeared (OR highest quartile 0.5 (0.1 – 1.6)). In subjects without iliac atherosclerosis at baseline, associations with progression of iliac atherosclerosis were attenuated (OR highest quartile 1.5 (0.7 – 3.2)).

We compared odds ratios for progression of atherosclerosis associated with high levels of CRP with the odds ratios for progression associated with a high total cholesterol (highest quartile of the population distribution), hypertension, and current smoking. Figure 1 shows that the age- and gender-adjusted odds ratios for progression of atherosclerosis associated with high levels of CRP are comparable to the odds ratios associated with traditional cardiovascular risk factors. For example,

Table 2. Association of C-reactive protein with progression of atherosclerosis measured at various sites of the arterial tree.

	Quartiles of C-reactive protein (mg/l)			
Progression of	**1 (≤ 0.78)**	**2 (0.78 – 1.52)**	**3 (1.52 – 2.90)**	**4 (> 2.90)**
Carotid atherosclerosis				
Model 1	1.0	1.4 (0.8 – 2.4)	0.9 (0.5 – 1.6)	1.9 (1.1 – 3.3)
Model 2	1.0	1.3 (0.7 – 2.2)	0.8 (0.4 – 1.4)	1.7 (1.0 – 3.1)
Aortic atherosclerosis				
Model 1	1.0	1.0 (0.6 – 1.8)	0.9 (0.5 – 1.6)	1.7 (1.0 – 3.0)
Model 2	1.0	0.9 (0.5 – 1.6)	0.7 (0.4 – 1.3)	1.5 (0.8 – 2.8)
Iliac atherosclerosis				
Model 1	1.0	1.5 (0.9 – 2.5)	1.8 (1.1 – 3.0)	2.0 (1.2 – 3.3)
Model 2	1.0	1.6 (0.9 – 2.6)	1.8 (1.0 – 2.9)	2.2 (1.3 – 3.8)
Lower extremity atherosclerosis				
Model 1	1.0	0.8 (0.4 – 1.7)	1.4 (0.7 – 2.8)	1.9 (1.0 – 3.7)
Model 2	1.0	0.7 (0.3 – 1.6)	1.4 (0.6 – 3.0)	1.9 (0.9 – 4.1)
Generalized progression of atherosclerosis*				
Model 1	1.0	1.5 (0.8 – 2.8)	1.9 (1.0 – 3.5)	4.5 (2.3 – 8.5)
Model 2	1.0	1.5 (0.8 – 2.9)	1.6 (0.8 – 3.2)	4.6 (2.2 – 9.5)

Estimates are odds ratios with 95% confidence intervals. Model 1 is adjusted for age, gender, and smoking status at baseline, and duration of follow-up. Model 2 is additionally adjusted for baseline total cholesterol:high-density lipoprotein-cholesterol ratio, body mass index, diabetes mellitus, systolic blood pressure, aspirin and statin use, and a history of myocardial infarction. *As indicated by a composite progression score ≥ 3.

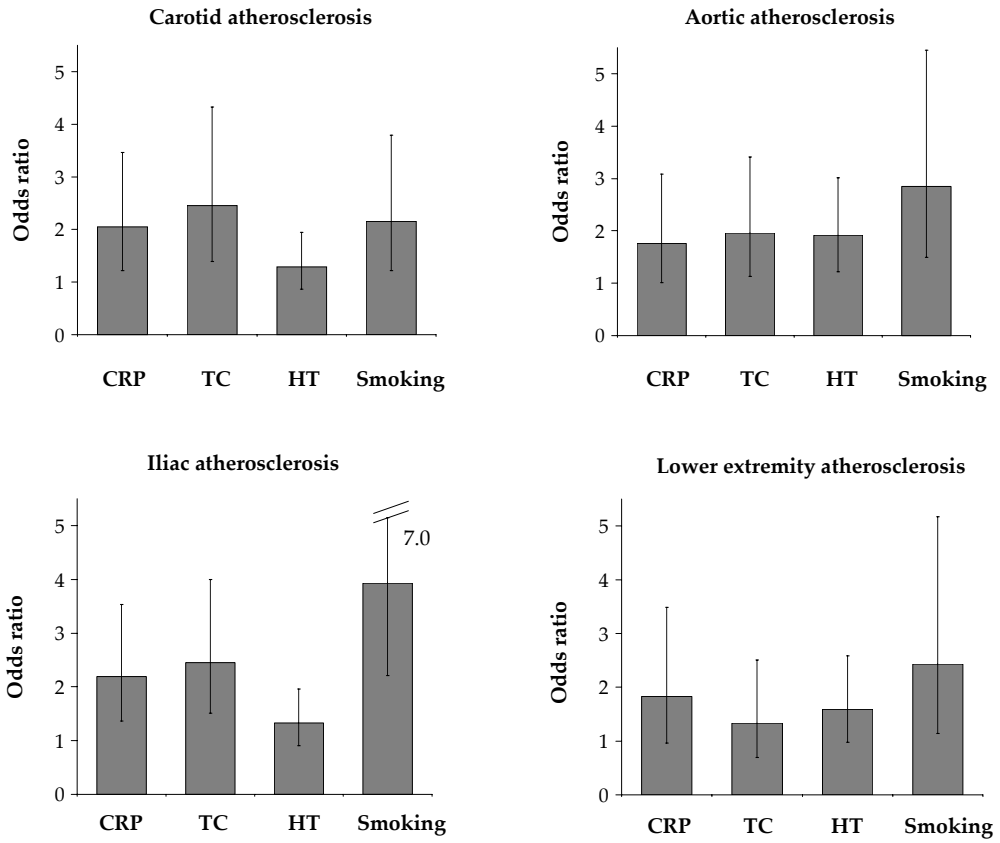

Figure 1. Odds ratios and 95% confidence intervals for progression of atherosclerosis associated with traditional cardiovascular risk factors and C-reactive protein.
CRP = C-reactive protein, TC = total cholesterol, and HT = hypertension. Odds ratios adjusted for age, gender, and duration of follow-up. For CRP and TC, the highest quartile is compared to the lowest quartile. Hypertension is defined as systolic blood pressure ≥ 160 mmHg, diastolic blood pressure ≥ 100 mmHg, or use of antihypertensive medication. Smokers are compared to non-smokers.

odds ratios for progression of carotid atherosclerosis associated with high levels of CRP, high cholesterol, hypertension, and smoking are 2.1 (1.2 – 3.5), 2.5 (1.4 – 4.3), 1.3 (0.9 – 1.9), and 2.2 (1.2 – 3.8), respectively.

Finally, we computed geometric mean levels of CRP for increasing scores of the composite progression score. Figure 2 shows that mean levels of CRP increase linearly with the number of sites at which progression of atherosclerosis is present (*P* trend = 0.002).

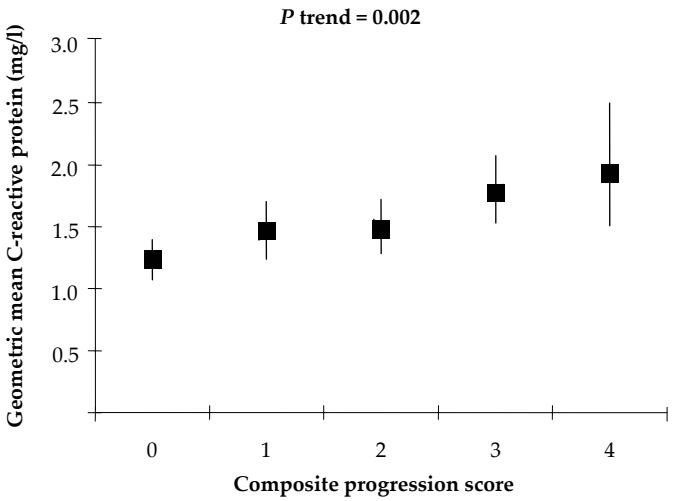

Figure 2. Geometric mean level and 95% confidence interval of C-reactive protein for each value of the composite progression score. Means are adjusted for baseline levels of traditional cardiovascular risk factors.

DISCUSSION

In this population-based study, we show that CRP predicts progression of atherosclerosis measured at various sites of the arterial tree.

Several cross-sectional studies have shown that CRP is related to atherosclerotic disease.[4,5,7] Furthermore, CRP predicted PAD in apparently healthy men,[6] and in Japanese outpatients, it was associated with progression of carotid plaques.[12] The strength of the present study is that we investigated progression of atherosclerosis at multiple sites in a relatively large, population-based study. Our study shows that CRP predicts progression of atherosclerosis as indicated by various non-invasive measures, and that the risk estimates associated with CRP are as high as those associated with more traditional cardiovascular risk factors.

Several methodological issues need to be discussed before interpreting these data. First, it should be kept in mind that all study participants survived until the follow-up measurement of atherosclerosis. Subjects with the most severe atherosclerosis at baseline are more likely to have died. Although this may have somewhat limited the range of baseline levels of atherosclerosis, it does not affect the validity of the risk estimates presented in the study. Second, we used different measures of atherosclerosis. Carotid, aortic, and lower extremity atherosclerosis have been shown to be associated with cardiovascular risk factors and cardiovascular disease risk.[14,21,22]

The use of iliac atherosclerosis is not yet very common, and more research is needed to determine its value as an indicator of atherosclerosis. Moreover, we did not study the association of CRP with progression of carotid intima-media thickness, because evaluation of the intima-media thickness as a measure of progression of atherosclerosis suggested that – at least within the Rotterdam Study – it is substantially influenced by measurement error. Third, not all subjects in our study population had complete data for all four measures of atherosclerosis. Since missing data are predominantly due to logistics and therefore random, it is not likely that they have affected our results. Fourth, levels of CRP were only measured once. However, a study in which CRP was regularly measured over a 6-month period concluded that CRP appeared to be tightly regulated, with few short-term fluctuations.[23] Furthermore, intra-individual variation in CRP would likely result in an underestimation of the true relationship.

Sudden plaque rupture, thrombus formation, and subsequent incorporation of the thrombus into the atherosclerotic plaque are thought to cause rapid progression of atherosclerosis.[11] Not only CRP, but multiple inflammatory mediators regulate a variety of pathophysiological processes that have been shown to be involved in atherosclerotic plaque rupture.[8,10] It is likely that elevated levels of CRP reflect the total amount of inflammatory activity within, and therefore the vulnerability of, the atherosclerotic plaque, suggesting that CRP can be a valuable predictor of progression of atherosclerotic disease. This idea is supported by the prospective data presented in this study.

Many studies have shown that CRP predicts myocardial infarction and stroke.[1] Part of the predictive value of CRP for these events may be explained by the association between CRP and progression of atherosclerosis as reported in the present study. Clearly, the association of CRP with progression of carotid atherosclerosis is consistent with reports about the predictive value of CRP for stroke.[1,2] Although no data were available on the progression of coronary atherosclerosis, there is a strong relationship between the various measures of extracoronary atherosclerosis and coronary atherosclerosis,[24] and the predictive value of CRP for progression of extracoronary atherosclerosis may be in line with the predictive value of CRP for myocardial infarction.

The association of CRP with progression of aortic atherosclerosis, represented by the extent of abdominal aortic calcification, was weaker than the association of CRP with progression of atherosclerosis measured at other sites. Likewise, several cross-sectional studies reported a lack of association of CRP with coronary calcification measured by electron beam tomography.[25] It is possible that, once the atherosclerotic plaque is in the process of being calcified, it is protected against progression caused by (inflammation-induced) rupture. Evidence for such a mechanism has been reviewed by Doherty et al., who argued that calcification may stabilize plaques and diminish the risk of rupture.[26] However, this hypothesis cannot explain why the association with progression of iliac atherosclerosis was attenuated and the association with progression of aortic atherosclerosis disappeared in subjects without

calcification at the baseline examination. Although calcification is an indicator of total atherosclerotic burden,[27] calcification and inflammation represent very distinct processes within the atherosclerotic plaque, which may well explain the modest associations between CRP and progression of calcification in the present study.

The present study shows that risk estimates for progression of atherosclerosis associated with CRP were as high as those associated with traditional cardiovascular risk factors. As expected in an elderly population,[28] the risk estimates for progression of atherosclerosis associated with traditional risk factors were relatively low; however, this was especially the case for hypertension and progression of carotid atherosclerosis.[29] The latter may be due to the fact that more than half of the hypertensive subjects in our study received antihypertensive treatment and were thus at a lower risk of progression of atherosclerosis, or to the relatively high age of the population.

Because a substantial part of incident myocardial infarction and stroke is unaccounted for by traditional cardiovascular risk factors, there is great need for finding novel and preferably modifiable factors that can identify subjects at high risk. CRP is a serious candidate, especially since it has recently been reported that measuring CRP levels may improve clinical risk prediction,[30] and that statin treatment positively influences clinical outcome in persons with a low cholesterol but high CRP.[31] Although more research is necessary to determine the value of CRP in everyday clinical practice, our study indicates that CRP is an important risk factor for cardiovascular disease progression.

We conclude that CRP predicts progression of atherosclerosis measured non-invasively at various sites of the arterial tree.

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Chapter 3.3

Added value of C-reactive protein in the prediction of myocardial infarction

ABSTRACT

Background: Epidemiological 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 in 7,983 men and women aged 55 years and over, 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 gender-adjusted relative risk of myocardial infarction for subjects in the highest quartile of the population distribution of CRP compared to 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 (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 pre-specified cut-off 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.

Traditional cardiovascular risk factors only explain part of the incidence of coronary heart disease, and much effort is put in finding novel risk factors that will improve existing prediction models. Over the past few years, it has repeatedly been shown that the acute phase protein C-reactive protein (CRP) is a strong predictor of cardiovascular disease.[1] Therefore, research now focuses on the usefulness of measuring CRP in addition to traditional risk factors to improve the identification of high-risk subjects.

Both the Physicians' Health Study and the Women's Health Study showed that measurement of CRP increased the predictive value of lipid parameters in determining the risk of first myocardial infarction.[2,3] On this basis, it has been suggested that measurement of CRP may be integrated in standard clinical practice.[4] However, the clinical value of CRP measurement needs to be confirmed in populations of varying ages and background. Moreover, it has not yet been investigated whether CRP has additional predictive value when not only lipid parameters are taken into account, but instead a risk function is constructed based on all traditional cardiovascular risk factors combined.

For each participant of the Rotterdam Study, a population-based cohort study in men and women aged 55 years and over, we determined coronary disease risk using information on traditional cardiovascular risk factors that are routinely assessed in general clinical practice. In addition, we computed a coronary risk profile based on the Framingham risk function.[5] Using these risk profiles, we investigated 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 of the Rotterdam Study, a population-based cohort study comprising 7,983 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 aged 55 years and over were invited to participate in an extensive home interview and two visits to the research center. The overall response rate was 78%. The Rotterdam Study was approved by the Medical Ethics Committee of the 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.[6]

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, two research physicians independently coded all reported events; codes were assigned according to the International Classification of Diseases, 10th edition. Codes on which the research physicians disagreed were discussed in order 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 judgement 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. Due to logistic reasons, complete data on traditional cardiovascular risk factors and CRP levels were available for 157 cases. Clinical characteristics of these cases were not different from the total case population. Per case, we selected three 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. Additionally, during two visits to the research center, established cardiovascular risk factors were measured. Body mass index (BMI) was computed as weight divided by height squared. Two blood pressure measurements were taken with a random-zero sphygmomanometer after 5 minutes of rest with the subject in sitting position. We defined hypertension as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg 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, 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 non-fasting serum glucose level equal to or larger than 11.1 mmol/l. We determined serum total cholesterol using an automated enzymatic procedure. High-density lipoprotein (HDL) cholesterol was measured similarly, after precipitation of the non-HDL fraction with phosphotungstate-magnesium. A 12-lead resting 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.[7,8] 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

Non-fasting blood was collected in tubes containing 0.129 mol/l sodium citrate. The ratio of blood to sodium citrate was 9:1. Plasma was collected after centrifugation for 10 minutes at 3,000 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. CRP was measured by sensitive immunological methods using an in-house enzyme immunoassay (n = 516 subjects; DAKO Denmark) or a nephelometric method (n = 160; Dade-Behring Marburg). It has previously been demonstrated that these two methods show high agreement.[9] In the present study, CRP was measured by both methods in 80 subjects. The high agreement was confirmed (mean difference between methods 0.02 mg/l, for values of CRP ≤ 10 mg/l). CRP levels > 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 standard deviations 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 two 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 (i.e. age, gender, current smoking, BMI, hypertension, diabetes mellitus, and family history of myocardial infarction before the age of 65), and lipid measurements (total and HDL-cholesterol). Risk function 2 was computed as described by the Framingham risk function, taking into account age, gender, current smoking, systolic blood pressure, diabetes mellitus, total cholesterol, HDL-cholesterol, 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, gender, current smoking, body mass index, hypertension, diabetes mellitus, family history of early myocardial infarction, total cholesterol, and HDL-cholesterol) for cases and controls. For CRP, we computed the median and interquartile range. Multivariate odds ratios (OR) for myocardial infarction associated with the higher quartiles of the control distribution of CRP compared to 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 three different models. Model 1 was adjusted for age, age squared, and gender. Since likely sources of inflammation include various components of cigarette smoke as well as adipose tissue,[10,11] model 2 additionally

Table 1. Baseline characteristics of cases of myocardial infarction and controls.

	Cases (n = 157)	Controls (n = 500)
Age (years)	70.8 ± 7.6	69.2 ± 8.4
Gender (% men)	61.1	40.6
Current smokers (%)	24.2	19.2
Body mass index (kg/m²)	26.3 ± 3.0	26.4 ± 3.8
Hypertension (%)	43.9	27.2
Diabetes mellitus (%)	17.2	7.2
Family history of early myocardial infarction (%)	28.7	13.2
Total cholesterol (mmol/l)	6.9 ± 1.1	6.6 ± 1.1
HDL-cholesterol (mmol/l)	1.2 ± 0.3	1.4 ± 0.3
C-reactive protein (mg/l)*	2.18 (1.04 – 5.09)	1.68 (0.82 – 3.02)

HDL = high-density lipoprotein. Data are means ± standard deviations for continuous variables and percentages for dichotomous variables. *For C-reactive protein, the median and interquartile range are shown.

included variables indicating 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 four situations for each traditional cardiovascular risk factor: CRP low (not in the highest quartile of the population distribution) and traditional risk factor absent; CRP high and risk factor absent; CRP low and risk factor present; and CRP 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 low and risk factor absent) as the reference, we then computed multivariate odds ratios 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 two coronary disease risk functions described above. 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, taking correlation between the areas into account.[12] 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 cut-off 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 cut-off point of 10%.

In all models, we included a variable indicating the method used to measure CRP. All analyses were performed using SPSS 9.0 for Windows.

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) and 1.68 mg/l (0.82 – 3.02) for cases and controls, respectively. Table 2 shows that, after adjustment for age, age squared, and gender, the relative risk of myocardial infarction for subjects in the highest quartile of CRP compared to subjects in the lowest quartile was twofold increased (model 1; odds ratio (OR) 2.0; 95% confidence interval 1.1 – 3.4). The relative risk of myocardial infarction increased across quartiles of CRP (*P* trend = 0.01). Adjustment for current smoking and body mass index did not substantially change the risk estimates (model 2; OR 1.9 (1.1 – 3.3); *P* trend = 0.02). However, the increase in risk largely disappeared when additional cardiovascular risk factors were added to the model (model 3; OR 1.2 (0.6 – 2.2); *P* trend 0.50).

Table 3 shows 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 nor the presence of that particular risk factor taken as the reference group. Although confidence intervals 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 appeared 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 area under the receiver operating characteristic curve (AUC) was largest using the risk function based on risk factors that are routinely

Table 2. Odds ratios for myocardial infarction according to baseline levels of C-reactive protein.

	Quartiles of C-reactive protein (mg/l)				
	1 (≤ 0.82)	**2 (0.82 – 1.68)**	**3 (1.68 – 3.02)**	**4 (≥ 3.02)**	***P* trend**
Model 1	1.0	1.2 (0.7 – 2.1)	1.5 (0.9 – 2.7)	2.0 (1.1 – 3.4)	0.01
Model 2	1.0	1.2 (0.7 – 2.1)	1.5 (0.8 – 2.6)	1.9 (1.1 – 3.3)	0.02
Model 3	1.0	0.9 (0.5 – 1.7)	1.0 (0.5 – 1.9)	1.2 (0.6 – 2.2)	0.50

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

Table 3. Odds ratios for myocardial infarction associated with C-reactive protein levels above (+) or below (-) the 75th percentile of the control distribution (3.02 mg/l) in combination with the presence (+) of absence (-) of traditional cardiovascular risk factors.

	CRP- RF-	CRP+ RF-	CRP- RF+	CRP+ RF+
Current smoking	1.0	1.4 (0.8 – 2.3)	1.5 (0.8 – 2.9)	1.3 (0.6 – 2.7)
BMI (> 28.4 kg/m^2)*	1.0	1.4 (0.8 – 2.3)	0.7 (0.4 – 1.4)	0.5 (0.2 – 1.2)
Hypertension	1.0	1.8 (1.0 – 3.2)	2.8 (1.6 – 4.6)	2.0 (1.1 – 3.8)
Diabetes mellitus	1.0	1.2 (0.8 – 2.0)	2.1 (0.9 – 4.5)	2.4 (1.0 – 6.1)
Family history of early MI	1.0	1.3 (0.8 – 2.1)	3.6 (2.0 – 6.5)	3.6 (1.7 – 7.9)
TC (> 7.3 mmol/l)*	1.0	1.2 (0.7 – 2.1)	2.8 (1.6 – 4.8)	3.2 (1.6 – 6.6)
HDL (< 1.1 mmol/l)*	1.0	1.4 (0.8 – 2.4)	2.0 (1.1 – 3.5)	2.1 (1.1 – 4.1)

CRP = C-reactive protein, RF = risk factor, BMI = body mass index, MI = myocardial infarction, TC = total cholesterol, HDL = high-density lipoprotein. All analyses adjusted for age, age squared, gender, smoking status, body mass index, hypertension, diabetes mellitus, a family history of early myocardial infarction, and total and HDL-cholesterol. *For cardiovascular risk factors measured on a continuous scale, the upper quartile of the control distribution was used to indicate presence.

assessed in clinical practice by medical history, physical examination, and lipid measurements (risk function 1; AUC = 0.773; standard error (SE) = 0.021; table 4). The AUC hardly changed when CRP was added to the risk function (AUC (SE) = 0.777 (0.021); ΔAUC = 0.004, *P* for change = 0.28). 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 <0.001). 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 (0.748 (0.021), *P* for change = 0.55).

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 (43.9% and 88.4%, respectively). Using the upper 10% of the population distribution as the cut-off point, sensitivity and specificity of risk function 1 were 21.7% and 94.8%, respectively. Sensitivity (22.9%) and specificity (95.2%) only slightly improved after inclusion of CRP. For the Framingham risk function (risk function 2), we found lower sensitivities and specificities. Adding CRP resulted in only marginally improved sensitivity and specificity for the cut-off point of 20%, but not of 10%.

Since the Framingham risk function was originally designed for a population younger than the one studied here, all analyses were repeated after restricting the study population to subjects younger than 75 years of age. Both for risk function 1 (AUC (SE) = 0.787 (0.024)) and for the Framingham risk function (AUC = 0.752 (0.025)), the AUC slightly increased. Adding levels of CRP did not improve the AUC.

Table 4. Area under the receiver-operating characteristic curve for risk functions with and without C-reactive protein.

	AUC (SE)
Basic risk*	0.642 (0.026)
Risk function 1†	0.773 (0.021)
+ C-reactive protein	0.777 (0.021)
Risk function 2‡	0.746 (0.021)
+ C-reactive protein	0.748 (0.021)

AUC = area under the curve; SE = standard error. *Basic risk is indicated by age, age squared, and gender. †Risk function 1includes age, age squared, gender, current smoking, body mass index, hypertension, diabetes mellitus, a family history of early myocardial infarction, total cholesterol, and high-density lipoprotein cholesterol. ‡Risk function 2 is based on the Framingham risk function.

DISCUSSION

This study shows that CRP 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 will not improve the ability to predict cardiovascular risk. The Rotterdam Study is a prospective population-based study with nearly 8,000 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 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 which is also seen for other risk factors such as cholesterol.[13] In the Cardiovascular Health Study (with participants aged 65 years and over) 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.[14] Moreover, other studies have shown that the predictive value of CRP for ischemic heart disease and for mortality was attenuated with age.[15,16] The results of the present study indicate that measurement of CRP has no additional predictive value in men and women over 55 years of age; whether this conclusion also applies to younger populations remains to be investigated.

In two studies by Ridker et al,[2,3] it was found that subjects with high levels of both total cholesterol and CRP have a higher risk of myocardial infarction compared to subjects in whom only total cholesterol or only CRP levels are elevated. Although in

Table 5. Sensitivity and specificity for coronary disease risk functions.

	Percentage of the distribution of predicted disease probabilities that indicates a positive test result							
	Upper 20%				Upper 10%			
	Cases§	Controls ‖	Sens. (%)	Spec. (%)	Cases §	Controls ‖	Sens. (%)	Spec. (%)
Basic risk*	49	78	31.2	84.4	26	34	16.6	93.2
Risk function 1†	70	57	44.6	88.6	34	26	21.7	94.8
+ C-reactive protein	69	58	43.9	88.4	36	24	22.9	95.2
Risk function 2‡	61	66	38.9	86.8	32	28	20.4	94.4
+ C-reactive protein	62	65	39.5	87.0	32	28	20.4	94.4

MI = myocardial infarction, Sens. = sensitivity, Spec. = specificity. *Basic risk is indicated by age, age squared, and gender. †Risk function 1includes age, age squared, gender, current smoking, body mass index, hypertension, diabetes mellitus, a family history of early myocardial infarction, total cholesterol, and high-density lipoprotein cholesterol. ‡Risk function 2 is based on the Framingham risk function. §Number of cases (out of 157) that are correctly identified as cases. ‖ Number of controls (out of 500) that are wrongly identified as cases.

the present study, the risk of myocardial infarction was also highest when both total cholesterol and CRP were elevated, the results should be interpreted with caution, since confidence intervals were wide and overlapping. The conclusion by Ridker et al. that CRP and lipids 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 HMG CoA 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, while the area under the ROC curve 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 cut-off points. The results confirmed that there was only, if any, a very small increase in sensitivity and specificity of the risk functions when CRP was added. Second, some of the established cardiovascular risk factors, such as diet, physical activity, or socio-economic 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 which are routinely used, and which 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 in men and women over 55 years of age has no additional clinical value. Further research should clarify whether, and in which subjects, CRP measurement may be useful in clinical risk prediction.

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Chapter 3.4

Genetic variation in Fcγ receptor IIa and peripheral atherosclerosis

ABSTRACT

Background: Immune processes play a substantial role in atherosclerotic disease. The role in atherosclerosis of Fcγ receptor IIa (FcγRIIa), a receptor for immunoglobulin G and for the inflammatory mediator C-reactive protein, is not yet clear. Since the R/H131 polymorphism in the FcγRIIa gene strongly influences binding to FcγRIIa, we investigated the association of the R/H131 polymorphism with peripheral atherosclerosis.

Methods and Results: Within the Rotterdam Study, a population-based cohort study, we determined FcγRIIa genotype in 430 subjects with peripheral atherosclerosis as indicated by the ankle-arm index, and 411 controls. Heterozygous and homozygous carriers of the H131 allele were protected against peripheral atherosclerosis (age- and gender-adjusted odds ratio (OR) 0.77 (0.54 – 1.12) and 0.65 (0.44 – 0.98), respectively, *P* trend = 0.04). This effect was most pronounced in subjects with modestly elevated levels of inflammation as indicated by the leukocyte count (OR 0.52 (0.29 – 0.93) and 0.45 (0.23 – 0.86), for heterozygotes and H131 homozygotes, respectively; *P* trend = 0.02).

Conclusion: This is the first study showing that the H131 allele of FcγRIIa protects against peripheral atherosclerosis.

Many studies have implicated a role for immune processes in atherosclerotic disease.[1] Fcγ receptor IIa (FcγRIIa) is a receptor for immunoglobulin (Ig) G, which has been detected in atherosclerotic lesions in all stages of development.[2] It is expressed on a wide range of cells, including monocytes, macrophages, and endothelial cells. Binding of IgG-containing immune complexes to this receptor triggers the release of a variety of mediators, including tumor necrosis factor α, matrix metalloproteinase-1, superoxide anion, and proteolytic enzymes,[3,4] which suggest an active role for FcγRIIa in atherosclerotic disease. Importantly, the inflammatory mediator C-reactive protein (CRP), a strong predictor of future cardiovascular disease,[5] has also been reported to bind to FcγRIIa.[6] Therefore, FcγRIIa may be intimately involved in some of the suggested pro-atherogenic actions of CRP, such as foam cell formation and the recruitment of monocytes into the arterial wall.[7,8]

A well-described polymorphism in the FcγRIIa gene results in a single amino acid difference – arginine (R) or histidine (H) – at position 131. The polymorphism is known to determine the receptor's functional response and IgG subclass specificity.[9] In addition, Stein *et al*. showed that binding of CRP to FcγRIIa is strongly decreased in H131 homozygotes, and to a lesser extent in RH131 heterozygotes.[10]

Within the Rotterdam Study, a population-based cohort study among men and women aged 55 years and over, we determined the R/H-131 polymorphism in the FcγRIIa gene in 430 subjects with peripheral atherosclerosis as indicated by the ankle-arm index (AAI), and 411 controls. We hypothesized that the H131 allele protects against atherosclerotic disease.

METHODS

Population

All subjects included in the study were participants of the Rotterdam Study, a population-based cohort study comprising 7,983 men and women. The aim of the Rotterdam Study is to investigate the incidence of and risk factors for chronic disabling diseases. From 1990 – 1993, all inhabitants of a well-defined suburb of the city of Rotterdam aged 55 years and over were invited to participate in an extensive home interview and two visits to the research center. The overall response rate was 78%. The Rotterdam Study was approved by the Medical Ethics Committee of the 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.[11]

Clinical characteristics

From 1990 – 1993, 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, and smoking behavior. During two visits to the research center, established cardiovascular risk factors were measured.

Blood samples were collected, and hematological parameters, including a one-time leukocyte count, were obtained by standard clinical laboratory procedures. CRP was measured using a high-sensitivity assay (Immage®, Beckman-Coulter, the Netherlands).

Using a random zero sphygmomanometer, sitting blood pressure was measured at the right upper arm. The average of two measurements obtained at one occasion was used. Systolic blood pressure at the ankles (posterior tibial artery) was measured in supine position with a random zero sphygmomanometer and an 8 MHz continuous wave Doppler probe (Huntleigh 500D, Huntleigh Technology). The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm was computed to obtain the AAI. Since arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI larger than 1.50 was considered invalid.[12]

Selection of cases and controls

For the present study, we used a nested case-control design. Cases with peripheral atherosclerosis (n = 432) and controls (n = 411) were defined by the distribution of the AAI, using the lowest value of two legs for each subject. In 6 strata defined by age (≤ 65, 65 – 75, > 75) and gender, we selected a case-control population composed of individuals for whom DNA samples were available and who were in the 15% extremes of the stratum-specific distribution of the AAI. We excluded two controls who, despite their relatively high AAI, fulfilled the criteria of the World Health Organization / Rose-questionnaire for intermittent claudication.

Genotyping

The FcγRIIa genotype was determined using allele-specific PCR reactions. For the FcγRIIa alleles, a reverse PCR primer (5'-CAGACTCCCCATACCTTGGA-3') was used to bind to a sequence in intron 4 that is unique to the FcγRIIa gene together with either H131- or R131-specific reverse primers (5'-GGAAAATCCCAGAAATTCTCTCG-3' and 5'-GGAAAATCCCAGAAATTCTCTCA-3'). We also used a forward control primer (5'-AAGGACAAGCCTCTGGTCAA-3') to be sure that the absence of a PCR product reflected the absence of the specific allele.

Statistical analyses

Baseline characteristics of cases and controls were compared using a t-test (continuous variables) or χ^2-test (dichotomous variables). Hardy-Weinberg equilibrium was tested by a χ^2-test with 1 degree of freedom. Using logistic regression analysis, we computed age- and gender-adjusted odds ratios (OR) of peripheral atherosclerosis associated with FcγRIIa genotype, taking the RR131 genotype as the reference. The analyses were repeated in strata of inflammatory status as indicated by the leukocyte count and by levels of CRP, using the median as the cut-off point. Finally, we assessed the relationship of leukocyte count and log-transformed levels of CRP with peripheral atherosclerosis. The analyses were repeated in strata of genotype. Extreme values of

Table 1. Baseline characteristics of subjects with peripheral atherosclerosis and controls.

	Cases (n = 430)	Controls (n = 411)	*P* value
Age (years)	67.3 ± 6.4	66.8 ± 6.9	NS
Gender (% men)	40.7	43.6	NS
Current smokers (%)	34.3	15.0	<0.001
Body mass index (kg/m²)	26.2 ± 4.1	26.2 ± 3.5	NS
Hypertension (%)	47.2	20.0	<0.001
Total cholesterol (mmol/l)	6.8 ± 1.3	6.5 ± 1.1	<0.001
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.4 ± 0.4	NS
Diabetes mellitus (%)	14.1	6.4	<0.001
Leukocyte count (10^9/l)	7.1 ± 1.8	6.3 ± 1.6	<0.001
C-reactive protein (mg/l)*	2.21 (1.26 – 4.00)	1.51 (0.82 – 2.82)	<0.001
Ankle-arm index†	0.79 (0 – 0.97)	1.32 (1.21 – 1.50)	<0.001

NS = not significant, HDL = high-density lipoprotein. Data are means ± standard deviations for continuous variables and percentages for dichotomous variables. *Geometric mean (interquartile range). †Median (range).

the leukocyte count and levels of CRP (≥ 3 standard deviations; n = 6 and n = 15, respectively) were excluded. Due to logistic reasons, levels of CRP were available for 682 subjects. All analyses were performed using SPSS 9.0 for Windows.

RESULTS

Baseline characteristics of cases and controls are shown in table 1. As expected, cases with peripheral atherosclerosis had a worse cardiovascular risk profile than controls. Table 2 shows FcγRIIa genotype frequencies. The study population was in Hardy-Weinberg equilibrium. The frequency (95% confidence interval) of the R131 allele was 0.48 (0.45 – 0.51) in cases and 0.43 (0.40 – 0.46) in controls.

Table 2. Genotype frequencies of the FcγRIIa polymorphism in cases and controls.

Genotype	Cases (n = 430)		Controls (n = 411)		*P* value*
	n	(%)	n	(%)	
RR131	91	(21.2)	67	(16.3)	
RH131	228	(53.0)	219	(53.3)	NS
HH131	111	(25.8)	125	(30.4)	0.04

NS = not significant. *Using RR131 as the reference group.

Figure 1 shows age- and gender-adjusted ORs of peripheral atherosclerosis for the RH131 and HH131 genotypes, with the RR131 genotype as the reference. For subjects homozygous for the H131 allele, the odds ratio for peripheral atherosclerosis was clearly decreased (OR 0.65 (0.44 – 0.98)). For heterozygotes, the reduction in risk was intermediate, although not statistically significant (OR 0.77 (0.54 – 1.12); *P* trend = 0.04). The ORs did not change after additional adjustment for current and past smoking, body mass index, hypertension, total and high-density lipoprotein cholesterol, and diabetes mellitus (OR 0.64 (0.41 – 1.00) and 0.73 (0.49 – 1.09), for H131 homozygotes and heterozygotes, respectively).

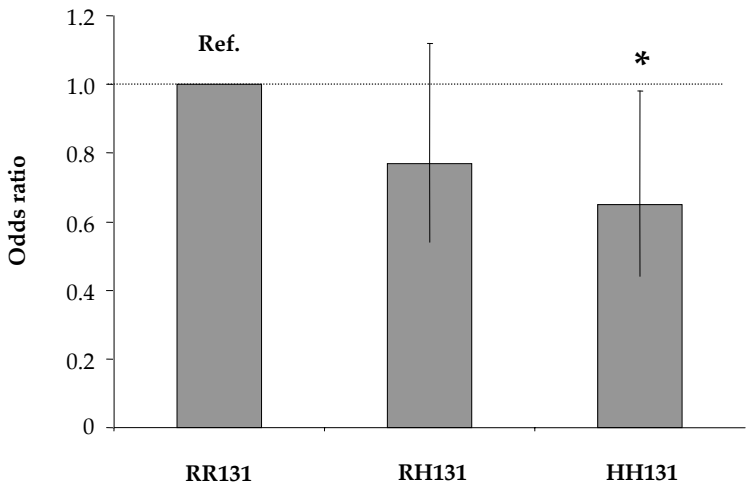

Figure 1. Odds ratios of peripheral atherosclerosis associated with FcγRIIa genotype.
Ref. = reference. Lines represent 95% confidence intervals. **P* trend = 0.04.

After stratification for inflammatory status as indicated by the leukocyte count, there was a strong association between the polymorphism and peripheral atherosclerosis in subjects with a high leukocyte count (OR 0.52 (0.29 – 0.93) and 0.45 (0.23 – 0.86), for heterozygotes and H131 homozygotes, respectively; *P* trend = 0.02), but not in subjects with a low leukocyte count. No clear differences were seen between strata of inflammation as indicated by levels of CRP. Stratification for smoking status did not influence the associations.

Leukocyte count and log-transformed levels of CRP were strongly associated with peripheral atherosclerosis (table 3). When we stratified according to genotype, the association of leukocyte count with peripheral atherosclerosis was strongest for R131

Table 3. Odds ratios (95% confidence interval) of peripheral atherosclerosis associated with markers of inflammation.

	Leukocyte count (10^9/l)*	C-reactive protein (mg/l)†
Overall	1.19 (1.08 – 1.31)	1.31 (1.08 – 1.58)
Genotype strata		
RR131	1.44 (1.12 – 1.86)	1.30 (0.82 – 2.06)
RH131	1.21 (1.06 – 1.39)	1.40 (1.07 – 1.83)
HH131	1.12 (0.92 – 1.36)	1.18 (0.79 – 1.76)

Odds ratios adjusted for age, gender, body mass index, smoking habits, total and high-density lipoprotein cholesterol, hypertension, and diabetes mellitus. *Per 1 unit increase in leukocyte count. †Per 1 log-transformed unit increase in C-reactive protein.

homozygotes, but became weaker in heterozygotes and in H131 homozygotes. Although for levels of CRP the trend across genotype strata was less pronounced, the OR of peripheral atherosclerosis associated with this inflammatory mediator was also lowest in H131 homozygotes.

DISCUSSION

This is the first study showing that the H131 allele of the FcγRIIa gene protects against peripheral atherosclerosis. The protective effect was independent of traditional cardiovascular risk factors, suggesting a role for FcγRIIa in the development of atherosclerotic disease.

The results of this study support an active role for FcγRIIa in atherosclerosis. Immune complexes containing immunogenic particles such as low-density lipoproteins (LDL), heat shock proteins, or microorganisms such as *Chlamydia pneumoniae* may activate FcγRIIa within atherosclerotic plaques.[1,4] Activation will subsequently lead to the production and secretion of mediators that are involved in atherosclerosis, such as superoxide anion, tumour necrosis factor α, and proteolytic enzymes.[3] The results from the present study suggest that the IgG subclass specificity and/or the functional response of the FcγRIIa among subjects carrying the H-131 allele results in a less atherogenic phenotype compared with RR131 homozygotes.

In addition to IgG, the acute phase reactant CRP has also been shown to bind to FcγRIIa, although this has not been unequivocally established.[6,13] CRP is capable of recruiting monocytes into the atherosclerotic plaque dependent on the presence of a specific CRP receptor.[7] Moreover, the uptake by macrophages of native LDL that has been opsonized by CRP is facilitated by FcγRIIa.[8] Our finding that subjects carrying the H131 allele of FcγRIIa, to which CRP shows only minimal binding,[10] have less peripheral atherosclerosis than subjects homozygous for the R131 allele may imply a CRP-dependent role for FcγRIIa in atherosclerotic disease. Of note, not only CRP, but

also the related protein serum amyloid P component, which has been detected in atherosclerotic lesions,[14] has been shown to bind to FcγRIIa.[15]

Several methodological issues need to be discussed. First, subjects with severe atherosclerosis may have died before they could be included in the study. However, if the reported association between the R/H131 polymorphism and atherosclerosis is genuine, subjects carrying the R131 allele will likely be underrepresented in the cases, leading to an underestimation of the risk estimates. Second, we used peripheral atherosclerosis as indicated by the AAI as the outcome in this study.[16] It will be interesting to investigate whether the H131 allele also protects against clinical cardiovascular events such as myocardial infarction and stroke.

In conclusion, the present study shows that the H131 allele of FcγRIIa protects against peripheral atherosclerosis.

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Chapter 4

Programmed cell death in atherosclerosis

Chapter 4.1

Mediators of apoptosis and coronary and extracoronary atherosclerosis

ABSTRACT

Background: Findings from laboratory studies strongly suggest a role for apoptosis, the process of programmed cell death, in cardiovascular disease. No population-based study has yet investigated whether serum levels of soluble forms of Fas, a receptor capable of inducing the apoptosis cascade, and of Fas-ligand are associated with coronary and extracoronary atherosclerosis.

Methods: Within the Rotterdam Coronary Calcification Study, a population-based cohort study, we measured coronary calcification using electron-beam computed tomography, abdominal aortic calcification by abdominal X-ray, carotid plaques and common carotid intima-media thickness (IMT) by ultrasonography, and lower extremity atherosclerosis by computation of the ankle-arm index. Levels of sFas and sFas-ligand were measured in 1,036 and 481 participants, respectively.

Results: Levels of sFas were not related to coronary or extracoronary atherosclerosis. sFas-ligand was positively associated with advanced coronary and abdominal aortic calcification (multivariate odds ratios (95% confidence intervals) for the highest category of sFas-ligand versus the lowest 2.3 (1.0 – 5.4) and 2.4 (1.0 – 6.0), respectively), but not with carotid plaques and IMT. Its association with lower extremity atherosclerosis was ambiguous. The inflammatory mediator C-reactive protein showed strong associations with measures of atherosclerosis, including coronary atherosclerosis, which largely remained after adjustment for traditional cardiovascular risk factors.

Conclusion: The results of this study do not support a role for sFas in the identification of subjects with atherosclerotic disease. Because sFas-ligand was positively associated with some, but not all of the measures of coronary and extracoronary atherosclerosis that we investigated, further studies into the relationship between sFas-ligand and CVD are warranted.

The discouragement of smoking and the detection and treatment of hypertension, hypercholesterolemia, diabetes mellitus, and obesity take a prominent place in the primary and secondary prevention of cardiovascular disease (CVD), but half of all patients with CVD do not have any of these established cardiovascular risk factors.[1] In the past few years, much epidemiological research has successfully been devoted to the relationship between inflammation and CVD, and currently, the inflammatory mediator C-reactive protein (CRP) is considered an important 'new' cardiovascular risk factor.[2] However, the search for other factors involved in the initiation and progression of CVD continues.

Apoptosis is the process of programmed cell death. Although apoptosis is an essential physiologic process required for normal development and maintenance of tissue homeostasis, it is also involved in a wide range of pathologic conditions, including CVD.[3,4] In atherosclerotic lesions, large numbers of apoptotic cells are present, as well as apoptosis-related proteins.[5-7] Moreover, findings from laboratory studies strongly suggest an active role for apoptosis in atherosclerotic disease.[8-12]

One of the so-called 'death receptors' on the cell membrane is Fas (also known as Apo-1 or CD95), which, upon ligation by Fas-ligand (CD178), rapidly induces the apoptosis cascade. Recently, it has been reported that soluble Fas (sFas) is associated with coronary and peripheral atherosclerosis in patients with end-stage renal disease.[13,14] Moreover, a small case-control study showed that levels of soluble Fas-ligand are elevated in patients with acute myocardial infarction and unstable angina pectoris.[15] In 47 patients with hypertension, levels of sFas-ligand, but not sFas, were positively associated with common carotid intima-media thickness (IMT).[16]

No study has yet investigated whether a relationship exists between serum levels of sFas and sFas-ligand and atherosclerosis in the general population. Therefore, we measured sFas and sFas-ligand in participants of the Rotterdam Coronary Calcification Study, and studied their associations, as well as associations of CRP, with coronary calcification measured by electron-beam computed tomography (EBCT), aortic calcification as shown on an abdominal X-ray, carotid plaques and common carotid IMT measured by ultrasonography, and lower extremity atherosclerosis as indicated by the ankle-arm index (AAI).

METHODS

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBCT.[17] The study is embedded in the Rotterdam Study, a population-based cohort study composed of 7,983 men and women aged 55 years and over living in a well-defined suburb of Rotterdam, the Netherlands. At baseline (1990 – 1993) and during two follow-up visits (1993 – 1995 and 1997 – 1999), a trained investigator visited all participants at home and collected information using a computerized questionnaire. The obtained information included

current health status, medical history, drug use, and smoking behavior. Additionally, during two visits to the research center, established cardiovascular risk factors and the presence and severity of extracoronary atherosclerosis (see below) were measured. A more detailed description of the Rotterdam Study and the collection of data have been given elsewhere.[18,19] Participants younger than 85 years of age who completed the second follow-up visit of the Rotterdam Study (n = 3,371) were invited to participate in the Rotterdam Coronary Calcification Scan Study and have an EBCT scan. The Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study, and all participants gave informed consent. The response rate was 61%. The median duration between the second follow-up visit to the Rotterdam Study center and the EBCT scan was 50 days. For the present study, we measured levels of sFas in a random selection of 1,036 of the 2,013 participants for whom EBCT scores are available. Levels of sFas-ligand were measured in 481 of the 1,036 participants.

Measures of atherosclerosis

Coronary calcification. Coronary calcification in the epicardial coronary arteries was assessed using EBCT scans from a C-150 Imatron scanner (Imatron, South San Francisco, California), as described in more detail before.[17] A calcification was defined as the presence of at least two adjacent pixels (area = 0.52 mm^2) with a density > 130 Hounsfield units. A coronary calcium score was obtained for each high-density lesion in the epicardial coronary arteries, according to the method by Agatston *et al.*[20] The scores for individual calcifications were added, resulting in a calcium score for the entire epicardial coronary system. A coronary calcium score > 1000 was considered to indicate advanced coronary atherosclerosis.

Abdominal aortic calcification. Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. The extent of abdominal aortic calcification was scored according to the length of the involved area (with scores 0 – 5 corresponding to 0, ≤ 1, 1 – 2.5, 2.5 – 4.9, 5.0 – 9.9, and ≥ 10.0 cm).[21] An aortic calcification score > 3 was considered to indicate advanced aortic atherosclerosis.

Carotid atherosclerosis. Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer and a duplex scanner (ATL UltraMark IV, Washington, USA), The common carotid artery, carotid bifurcation, and internal carotid artery were examined both left and right for the presence of plaques.[22] A weighted plaque score ranging from 0 to 6 was computed by adding the number of sites at which a plaque was detected, divided by the total number of sites for which an ultrasonographic image was available and multiplied by six (the maximum number of sites). Subjects for whom data on the presence of plaques were not available for at least two of the six sites that were examined were excluded. A carotid plaque score > 3 was considered to indicate advanced carotid atherosclerosis. Common carotid IMT was determined as the average of the maximum IMT of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[22]

Lower extremity atherosclerosis. We obtained the AAI as described before by computing the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm.[19] The AAI is inversely related to the severity of lower extremity atherosclerosis. For the analyses, we used the lowest value of two legs. Since arterial rigidity prevents arterial compression and will therefore lead to spurious high values of the AAI, an AAI larger than 1.50 was considered invalid. Peripheral arterial disease (PAD) was defined as an AAI < 0.9.

Data on coronary calcification, abdominal aortic calcification, carotid plaques and IMT, and lower extremity atherosclerosis were available for 100.0%, 87.3%, 95.7%, 93.0%, and 97.6% of the study population, respectively.

Determination of sFas, sFas-ligand, and CRP

Blood samples were drawn at the research center after an overnight fast and were directly put on ice. Serum samples were processed within 30 minutes, after which they were kept frozen at –80 °C. Serum levels of sFas (Biosource International, Belgium) and sFas-ligand (MedSystems Diagnostics, Austria) were determined using commercially available enzyme-linked immunosorbent assays. CRP was measured using a nephelometric method (Immage®, Beckman Coulter, the Netherlands). Intraassay coefficients of variation are < 5% for sFas and CRP, and 6.1% for sFas-ligand. Interassay coefficients of variation are 5.9 – 8.9% for sFas, and < 7.5% for CRP and sFas-ligand. Outliers (values larger than the mean ± 3 SD of the population distribution) of sFas (n = 15), sFas-ligand (n = 4; mean and SD computed in those with levels > 0 ng/ml), and CRP (n = 3) were excluded. In the study population, 4.6% had levels of CRP > 10 mg/l.

Statistical analyses

Categories for sFas and CRP were based on tertiles of the population distribution, with cut-off points of 4.2 and 5.5 ng/ml for sFas and 1.7 and 3.5 mg/l for CRP. For sFas-ligand, all participants with levels under the detection limit were included in the lower category; the upper two categories were defined by the median (0.21 ng/ml) of those with detectable levels. For subjects with missing data on clinical characteristics measured on a continuous scale (n = 23 with missing data on at most 2 characteristics), we imputed the population mean. To assess associations between cardiovascular risk factors and sFas and sFas-ligand, we computed means ± standard deviations (SD) or percentages of cardiovascular risk factors per category of these markers; associations were subsequently tested for trend using linear or logistic regression analysis when appropriate, with cardiovascular risk factors as the dependent, and a variable ranging from 1 to 3 indicating categories of sFas or sFas-ligand as the independent variable. Odds ratios for the presence of advanced coronary and aortic calcification, advanced carotid plaques, and PAD were computed for increasing categories of sFas, sFas-ligand, and CRP using logistic regression analysis, adjusting for age and gender, and subsequently for current and past smoking, body mass index, total and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, and,

Table 1. Baseline characteristics of the study population.

Characteristic	All subjects (n = 1,036)
Age (years)	70.7 ± 5.4
Gender (%men)	47.1
Current smokers (%)	15.8
Body mass index (kg/m^2)	26.9 ± 3.9
Total cholesterol (mmol/l)	5.8 ± 1.0
High-density lipoprotein cholesterol (mmol/l)	1.4 ± 0.4
Systolic blood pressure (mmHg)	144.4 ± 21.5
Diastolic blood pressure (mmHg)	76.2 ± 11.0
Diabetes mellitus (%)	12.0
Coronary calcium score*	155.0 (17.0 – 607.2)
Abdominal aortic calcification (%)†	19.5
Carotid plaques (%)‡	18.2
Peripheral arterial disease§	17.0
Maximum intima-media thickness (mm)	1.1 ± 0.2
Ankle-arm index‖	1.0 ± 0.2
C-reactive protein (mg/l)*	2.4 (1.3 – 4.2)
sFas (ng/ml)	4.9 ± 1.9
sFas-ligand (ng/ml)*¶	0.2 (0.1 – 0.7)

Data are means ± standard deviation for continuous variables and percentages for dichotomous variables. *Expressed as median (interquartile range) because of skewed distributions. †Defined as an aortic calcification score > 3. ‡Defined as a carotid plaque score > 3. §Defined as an AAI < 0.9. ‖Lowest of left and right leg. ¶In those with detectable levels (44.9%).

for analyses with sFas and sFasligand, log-transformed levels of CRP. Tests for trend were performed as described above. Finally, we computed geometric mean values of the coronary calcium score and mean values of common carotid IMT and the AAI for categories of sFas, sFas-ligand, and CRP by means of ANCOVA. Data were analyzed using SPSS 9.0 for Windows.

RESULTS

Table 1 shows baseline characteristics of the study population. Baseline characteristics were similar for the random subsample in which sFas-ligand was measured. In 55.1% of the study population, levels of sFas-ligand were below the detection limit.

Table 2 shows that levels of sFas were positively associated with age, total cholesterol, and diastolic blood pressure; the association with systolic blood pressure was borderline statistically significant. Systolic and diastolic blood pressure

Table 2. Associations of cardiovascular risk factors with sFas.

Risk factor	Categories of sFas (concentration in ng/ml)			*P* trend*
	≤ 4.20	4.21 – 5.50	≥ 5.51	
Age (years)	70.0 ± 5.1	70.7 ± 5.4	71.5 ± 5.6	<0.001
Gender (% men)	46.0	51.0	44.3	0.92
Current smoking (%)	14.0	15.8	18.2	0.16
Body mass index (kg/m^2)	26.6 ± 3.8	27.1 ± 4.3	27.0 ± 3.4	0.11
Total cholesterol (mmol/l)	5.7 ± 1.0	5.8 ± 1.0	5.9 ± 1.0	<0.01
HDL-cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.54
Systolic blood pressure (mmHg)	143.7 ± 22.6	143.0 ± 21.1	146.3 ± 20.9	0.06
Diastolic blood pressure (mmHg)	75.9 ± 11.4	76.1 ± 11.1	76.7 ± 10.5	0.04
Diabetes mellitus (%)	11.6	13.2	11.5	0.65
C-reactive protein (mg/l)†	2.4 (1.3 – 4.0)	2.5 (1.3 – 4.2)	2.4 (1.3 – 4.3)	0.82

HDL = high-density lipoprotein. Data are means ± standard deviation for continuous variables and percentages for dichotomous variables. *Adjusted for age and gender, if appropriate. †Expressed as median (interquartile range) because of its skewed distribution; *P* trend computed for log-transformed levels.

Table 3. Associations of cardiovascular risk factors with sFas-ligand.

Risk factor	Categories of sFas-ligand (concentration in ng/ml)			*P* trend*
	< 0.01	0.01 – 0.21	≥ 0.22	
Age (years)	71.0 ± 5.7	70.5 ± 5.0	71.0 ± 5.6	0.86
Gender (% men)	43.3	50.9	49.1	0.65
Current smoking (%)	15.3	13.9	17.0	0.74
Body mass index (kg/m^2)	26.8 ± 3.5	27.7 ± 4.0	26.3 ± 3.5	0.77
Total cholesterol (mmol/l)	5.9 ± 0.9	5.8 ± 0.9	5.7 ± 0.9	0.05
HDL-cholesterol (mmol/l)	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	0.73
Systolic blood pressure (mmHg)	139.9 ± 19.8	149.5 ± 23.4	145.7 ± 22.1	<0.01
Diastolic blood pressure (mmHg)	74.9 ± 10.6	76.8 ± 11.0	77.3 ± 11.5	0.06
Diabetes mellitus (%)	9.5	12.0	8.6	0.56
C-reactive protein (mg/l)†	2.3 (1.2 – 3.8)	3.2 (1.9 – 4.6)	2.3 (1.5 – 4.3)	0.66
sFas (ng/ml)	5.4 ± 1.3	5.4 ± 1.2	5.4 ± 1.4	0.90

HDL = high-density lipoprotein. Data are means ± standard deviation for continuous variables and percentages for dichotomous variables. *Adjusted for age and gender, if appropriate. †Expressed as median (interquartile range) because of its skewed distribution; *P* trend computed for log-transformed levels.

(borderline) also increased with increasing categories of sFas-ligand (table 3). The relationship between levels of sFas-ligand and total cholesterol appeared to be inverse. There was no association between sFas and sFas-ligand or between these markers and levels of CRP.

Levels of sFas were not related to the presence of advanced coronary or aortic calcification, carotid plaques, or PAD (table 4). Levels of sFas-ligand were positively associated with coronary and abdominal aortic calcification, even after adjustment for traditional cardiovascular risk factors and CRP. However, they were not associated with the presence of advanced carotid plaques or PAD. The age- and gender-adjusted associations of CRP with coronary and aortic calcification, carotid plaques, and PAD were strong, and largely remained after additional adjustment for traditional cardiovascular risk factors.

The geometric mean coronary calcium score increased non-significantly with increasing categories of sFas-ligand (figure 1; *P* trend = 0.22). For CRP, there was an increase in coronary calcium score across categories (*P* trend <0.01), but the association disappeared after additional adjustment for traditional cardiovascular risk factors (*P* trend = 0.29). Figure 2 shows that sFas and sFas-ligand were not associated with mean carotid IMT, but that there was a strong positive relationship between CRP and IMT (*P* trend <0.01), which was only somewhat attenuated (*P* trend = 0.095) after adjustment for traditional cardiovascular risk factors. Finally, the mean AAI was significantly lower in subjects with detectable levels of sFas-ligand (upper two categories) compared with subjects in whom levels are below the detection limit (figure 3). The mean AAI also significantly decreased with increasing categories of CRP. After adjustment for traditional cardiovascular risk factors, the association between CRP and the AAI disappeared, but between sFas-ligand and the AAI remained (*P* trend <0.05).

Reported associations between CRP and atherosclerosis were not meaningfully different when restricted to subjects with levels of CRP ≤ 10 mg/l. Additional adjustment for use of HMG Co A reductase inhibitors ('statins') and aspirin, which may influence levels of CRP, did not change the results.

DISCUSSION

This is the first population-based study investigating the relationship of mediators of apoptosis with coronary and extracoronary atherosclerosis. We were not able to confirm previous reports of an association between levels of sFas, which in its membrane-bound form is an important mediator of apoptosis, and coronary and extracoronary atherosclerotic disease. sFas-ligand was positively associated with advanced coronary and abdominal aortic calcification, but not with advanced carotid plaques or common carotid IMT. The continuous association of sFas-ligand with lower extremity atherosclerosis was not reflected in an association with PAD.

Table 4. Odds ratios associated with increasing categories of sFas, sFas-ligand, and C-reactive protein for advanced coronary and abdominal aortic calcification, advanced carotid plaques, and peripheral arterial disease.

	Categories of serum level				Categories of serum level*			
	1	2	3	*P* trend	1	2	3	*P* trend
Coronary calcification†								
sFas	1.0	0.9 (0.5 – 1.5)	0.9 (0.5 – 1.5)	0.66	1.0	0.8 (0.5 – 1.5)	0.8 (0.5 – 1.5)	0.52
sFas-ligand	1.0	1.6 (0.8 – 3.5)	2.2 (1.0 – 4.8)	0.05	1.0	1.7 (0.8 – 3.8)	2.3 (1.0 – 5.4)	0.05
C-reactive protein	1.0	1.6 (1.0 – 2.8)	1.9 (1.2 – 3.3)	0.01	1.0	1.4 (0.8 – 2.5)	1.6 (0.9 – 2.8)	0.09
Abdominal aortic calcification‡								
sFas	1.0	0.7 (0.4 – 1.2)	1.1 (0.6 – 1.9)	0.82	1.0	0.6 (0.4 – 1.2)	1.0 (0.5 – 1.8)	0.97
sFas-ligand	1.0	2.2 (1.0 – 5.0)	1.7 (0.8 – 3.7)	0.12	1.0	2.6 (1.0 – 6.3)	2.4 (1.0 – 6.0)	0.04
C-reactive protein	1.0	1.5 (0.9 – 2.5)	2.6 (1.5 – 4.4)	<0.001	1.0	1.3 (0.7 – 2.4)	2.3 (1.2 – 4.2)	<0.01
Carotid plaques§								
sFas	1.0	0.8 (0.5 – 1.4)	1.4 (0.9 – 2.5)	0.14	1.0	0.7 (0.4 – 1.3)	1.1 (0.6 – 2.0)	0.60
sFas-ligand	1.0	0.9 (0.4 – 1.9)	0.9 (0.4 – 1.9)	0.73	1.0	0.8 (0.3 – 1.7)	0.9 (0.4 – 2.1)	0.69
C-reactive protein	1.0	1.8 (1.1 – 3.0)	2.3 (1.4 – 3.8)	<0.001	1.0	1.7 (1.0 – 3.0)	1.8 (1.1 – 3.2)	0.03
Peripheral arterial disease‖								
sFas	1.0	1.5 (0.9 – 2.2)	1.1 (0.7 – 1.7)	0.81	1.0	1.6 (1.0 – 2.5)	1.1 (0.7 – 1.7)	0.81
sFas-ligand	1.0	1.9 (1.0 – 3.4)	1.4 (0.7 – 2.6)	0.22	1.0	1.4 (0.7 – 2.7)	1.3 (0.7 – 2.7)	0.37
C-reactive protein	1.0	1.2 (0.8 – 1.8)	1.6 (1.1 – 2.4)	0.02	1.0	1.1 (0.7 – 1.7)	1.5 (0.9 – 2.3)	0.09

All analyses adjusted for age and gender. Categories are tertiles for sFas and C-reactive protein; for sFas-ligand, the lowest category includes all participants with levels below the detection limit. *Additionally adjusted for smoking status, body mass index, total and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, diabetes mellitus, and log-transformed levels of C-reactive protein, if appropriate. †Defined as a coronary calcium score > 1000. ‡Defined as an aortic calcification score > 3. §Defined as a carotid plaque score > 3. ‖ Defined as an ankle-arm index < 0.9.

The Rotterdam Coronary Calcification Study is composed of a well-defined study population for which extensive information has been collected through home interviews and visits to the research center. A great strength of the present study is that the presence and severity of atherosclerosis was measured in the coronary arteries by means of EBCT, as well as at multiple extracoronary sites in the arterial tree. Nevertheless, several methodological issues of this study need to be considered. First, we measured soluble forms of Fas, which result from alternative splicing of the Fas gene,[23] and of Fas-ligand, which is formed when cell-bound Fas-ligand is cleaved by a metalloproteinase.[24] However, blood levels of these molecules may not represent the biological activity of the membrane-bound forms, or may even be biologically active of their own.[23] Therefore, we must be careful with the interpretation of our results. Of note, the lack of an association of the soluble form of Fas with atherosclerosis does not rule out a role for membrane-bound forms of this molecule in the etiology of CVD. Second, we used different measures to indicate the presence and severity of atherosclerosis, each with their own advantages and limitations. The coronary calcification, aortic calcification, and carotid plaque scores directly indicate the presence of atherosclerotic lesions in coronary and extracoronary arteries. Although EBCT and abdominal X-ray will fail to detect non-calcified atherosclerotic plaques, coronary calcification is a close correlate of total coronary plaque area,[25] and the presence of aortic calcification has been shown to be very specific and highly predictive of future CVD.[26,27] The IMT and AAI are indirect measures of atherosclerosis, which may also be influenced by nonatherosclerotic processes such as fibromuscular hypertrophy (IMT) or vascular stiffness (AAI). However, both are reliable measures of atherosclerosis, which are strongly associated with traditional cardiovascular risk factors and future CVD.[22,28-30]

In atherosclerotic lesions, large numbers of apoptotic cells and apoptosis-related proteins can be found.[5-7] Moreover, the results from experimental studies suggest an active role for apoptosis in the etiology of CVD. Possible mechanisms are manifold, and include the potential role of Fas-mediated cell death in the control of vessel wall inflammation,[31] but also the pro-inflammatory and procoagulant properties of apoptotic cells that are present in atherosclerotic plaques.[10,11] Moreover, apoptosis may influence the stability of the atherosclerotic plaque, as apoptotic cells are predominantly found in areas of plaque rupture.[32]

Although an inhibitory effect on apoptosis has been reported for soluble isoforms of Fas,[23] recent studies in patients with end-stage renal disease reported a strong positive association between sFas and coronary and peripheral atherosclerosis.[13,14] The discrepancy between the positive results from these studies and the lack of an association between sFas and atherosclerosis reported here may be explained by the fact that patients with end-stage renal disease comprise a highly selected patient population, in whom sFas levels were much higher (mean 28.1 ng/ml, SD 9.4) than in the population-based sample included in the present study. In a small study among hypertensive patients, levels of sFas were not associated with carotid IMT.[16] For sFas-ligand, small studies have reported associations with carotid IMT[16] and with acute

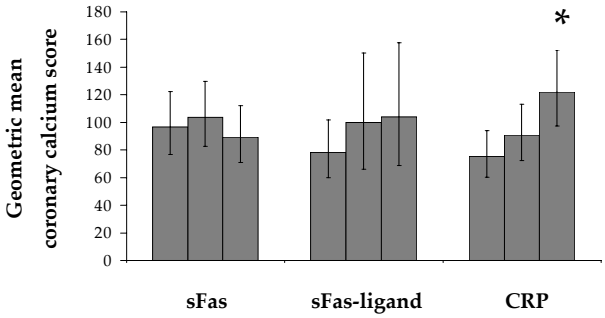

Figure 1. Geometric mean coronary calcium score for categories of sFas, sFas-ligand, and C-reactive protein (CRP). Bars represent geometric means and lines 95% confidence intervals. Geometric means are adjusted for age and gender. *P trend <0.01.

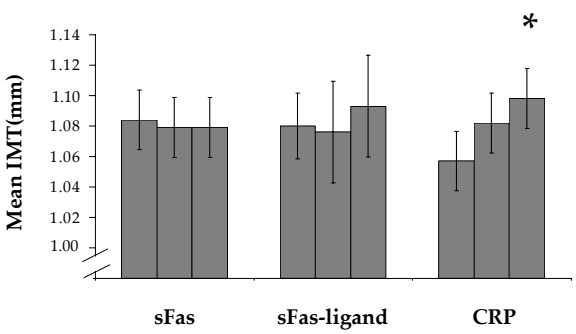

Figure 2. Mean carotid intima-media thickness (IMT) for categories of sFas, sFas-ligand, and C-reactive protein (CRP). Bars represent means and lines 95% confidence intervals. Means are adjusted for age and gender. **P* trend <0.01.

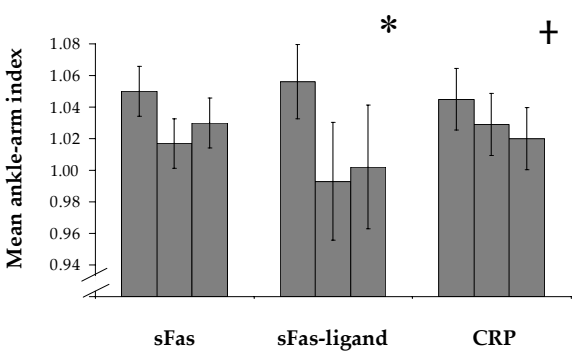

Figure 3. Mean ankle-arm index for categories of sFas, sFas-ligand, and C-reactive protein (CRP).
Bars represent means and lines 95% confidence intervals. Means are adjusted for age and gender. **P* trend <0.01. †*P* trend = 0.08.

myocardial infarction and unstable angina pectoris.[15] Although we cannot confirm the reported association between sFas-ligand and carotid atherosclerosis, which we quantified by measuring carotid plaques and common carotid IMT, the present study shows an association between sFas-ligand and coronary and abdominal aortic calcification. Associations of sFas-ligand with lower extremity atherosclerosis and PAD were not consistent.

CRP is a strong predictor of future CVD and has been proposed to be of added value in the clinical assessment of CVD risk.[2,33] Although apoptosis and inflammation may interact in the pathogenesis of atherosclerotic disease,[10,31] we found no association between levels of sFas and sFas-ligand and inflammatory status as indicated by levels of CRP. Previous studies from our group showed that CRP is associated with extracoronary atherosclerosis measured at multiple sites, which is confirmed by the data presented here.[34] Moreover, in the present study, the association between CRP and coronary calcification clearly showed a positive trend, although it was attenuated after adjustment for traditional cardiovascular risk factors. Previous reports on the association between CRP and coronary calcification measured by EBCT have been inconclusive.[35-37]

In conclusion, the results of this study do not support a role for sFas in the identification of subjects with atherosclerotic disease. Because sFas-ligand was positively associated with some, but not all of the measures of coronary and extracoronary atherosclerosis that we investigated, further studies into the relationship between sFas-ligand and CVD are warranted.

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Chapter 5

General discussion

The aim of the studies described in this thesis was to search for and provide more insight into factors that 1) are involved in the etiology of atherosclerosis and its clinical consequences and 2) can improve our ability to identify subjects who are at an increased risk of future cardiovascular disease (CVD). Each previous chapter included a discussion on the merits and limitations of the study that was described. The present chapter will provide a more general discussion of these studies in the light of current knowledge and ongoing research in the field of CVD. It includes the background for this thesis, an overview of the main findings considered in the context of current scientific knowledge, a discussion of the methodological aspects involved, and suggestions for future research.

BACKGROUND

The burden of cardiovascular disease

According to estimates from the World Health Organization, 17 million people around the globe die of CVD each year.[1] In industrialized countries including the Netherlands, CVD mortality has declined over the past 30 years as a result of a combination of public health measures (tobacco policies, health education, nutrition programs, etc.) and improvements in medical care.[2-4] However, CVD still remains the leading cause of death,[5,6] and recent data suggest that the decline is slowing down.[7] Moreover, due to the better prognosis of CVD patients resulting from improved medical care, CVD is often a cause of serious disability, which may last for a considerable number of years.

In Americans, the lifetime risk at age 40 of developing coronary heart disease (CHD) has been estimated to be one in two for men and one in three for women.[8] However, half of all patients with CHD do not have any of the traditional risk factors hypertension, hypercholesterolemia, cigarette smoking, diabetes mellitus, marked obesity, or physical inactivity.[9] Non-invasive methods to measure (subclinical) atherosclerosis are valuable tools in epidemiological research and may improve the estimation of CVD risk (chapter 2 of this thesis). In addition, inflammatory mediators have recently been identified as key players in the etiology of CVD and are expected to contribute importantly to CVD risk prediction in clinical practice (chapter 3). Research into the field of programmed cell death (apoptosis), which is still in its early stages, may also increase our understanding of CVD (chapter 4).

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study composed of 7,983 men and women aged 55 years and over who live in a well-defined suburb of the city of Rotterdam, the Netherlands. Its overall aim is to investigate the incidence and determinants of chronic disabling diseases, such as CVD, dementia, osteoporosis, and visual impairment.[10] At phase one of the Rotterdam Study (baseline; 1990 – 1993), all participants were extensively interviewed at home and paid two visits to the

research center. Among other things, information was collected about cardiovascular risk factors, blood samples were drawn, and the presence and severity of atherosclerosis were non-invasively assessed. These procedures were repeated at phases two (1993 – 1995) and three (1997 – 1999). All cardiovascular events (such as myocardial infarction, revascularization procedures, and sudden (cardiac) death) that occurred after the baseline examination were and, at the time of writing, are still being registered by general practitioners in the district, and subsequently validated by research physicians. All the studies described in this thesis are based on data from the Rotterdam Study.

MAIN FINDINGS

Measures of extracoronary atherosclerosis

Most of the studies described in this thesis make use of data indicating the presence and severity of extracoronary atherosclerosis as measured by various accurate, relatively cheap, and relatively easy non-invasive methods. These methods are discussed below.

Carotid atherosclerosis (1). Ultrasonography of the carotid arteries can be used to directly detect the presence of atherosclerotic plaques. By measuring plaques in the common carotid artery, carotid bifurcation, and internal carotid artery, a simple carotid plaque score can be constructed, as was done in the Rotterdam Study.[11] The measurement of plaque area and volume may provide even better estimates of the burden of atherosclerosis.[12] Within the Rotterdam Study, a reproducibility study on the assessment of plaques showed moderate reproducibility.[13]

Carotid atherosclerosis (2). Ultrasonography of the carotid artery wall provides an easy way to measure carotid intima-media thickness (IMT), which gives an indication of the presence of early arterial wall alterations and of more advanced atherosclerosis not yet obstructing the lumen. Besides being used in observational studies, IMT measurements are also commonly used in clinical trials to assess whether certain interventions delay the progression or even induce regression of atherosclerotic lesions.[14] In general, the reproducibility of the measurement is high, although the degree of measurement error increases proportionally with the level of IMT.[15,16] However, as atherosclerosis is a process which is confined to the intimal layer of the vessel wall (while ultrasound imaging cannot discriminate between the intimal and medial layers), it cannot be excluded that non-atherosclerotic processes such as fibromuscular hypertrophy, or the adaptive response of the vessel wall to changes in shear and tensile stress, cause modest degrees of intima-media thickening.[17,18] Although lower values of IMT will therefore be less useful in CVD research, at elevated levels IMT is thought to be a reliable indicator of atherosclerosis because of its consistent associations with atherosclerosis in other arteries, cardiovascular risk factors, and future CVD.[18]

Aortic atherosclerosis. Calcified lesions in the abdominal aorta as shown on an X-ray of the prelumbar region have been shown to indicate the presence of advanced atherosclerosis.[19] By assessing the presence and severity of these lesions, an aortic atherosclerosis score can be constructed.[20,21] The reproducibility of the assessment of aortic atherosclerosis using X-ray has been shown to be high.[21,22] Although non-calcified atherosclerotic lesions will not be detected on an abdominal X-ray, the severity of aortic calcification is a strong predictor of future CVD,[23,24] and may therefore be considered an indicator of generalized atherosclerosis. The presence and severity of calcified lesions in the iliac arteries can also be scored, but the value of iliac calcification as a measure of atherosclerosis has not yet been determined.

Lower extremity atherosclerosis. The ankle-arm index (AAI), which is the ratio between the systolic blood pressure at the ankle (posterior tibial artery) and the systolic blood pressure at the arm (brachial artery), represents the presence of atherosclerosis in the lower extremity arterial system. The method used for the assessment of the AAI has been shown to provide reliable measurements.[25] Of note, the AAI is an indirect measure of atherosclerosis, which may also be influenced by other factors such as vascular stiffness. Therefore, it is subject to debate whether the AAI can be used as a continuous measure; many studies choose the generally accepted cut-off point of an AAI < 0.9 to indicate the presence of significant peripheral arterial disease.[26]

All of the measures mentioned above have been shown to be strongly associated with the presence and severity of coronary calcification,[27] with traditional cardiovascular risk factors,[22,28-30] and with future CVD.[23,24,31-35] However, studies investigating determinants of progression of atherosclerosis are relatively rare. Moreover, no study has yet investigated whether differences in the predictive value for CVD between these measures exist, and whether a given measure of atherosclerosis can predict CVD independently of the other measures. The study described in chapter 2.1 shows that the traditional cardiovascular risk factors age, smoking, total cholesterol, and systolic blood pressure and/or hypertension are strong, independent predictors of moderate and severe progression of atherosclerosis measured at multiple sites in the arterial tree. However, gender is not, which stresses the importance of prevention of progression of extracoronary atherosclerotic disease in both men and women alike. In chapter 2.2, a study is presented showing that all four of the measures of atherosclerosis mentioned above are good predictors of future CHD. In this study, risk estimates for CHD associated with carotid plaques and aortic atherosclerosis are highest and independent of the other atherosclerosis measures, which shows the value of the relatively crude, but direct assessment of atherosclerotic plaques in CHD risk prediction.

The studies presented in chapters 2.1 and 2.2 show that non-invasive measures of extracoronary atherosclerosis are valuable tools in epidemiological studies investigating CVD. Although differences between these measures exist, they are all strongly predictive of future CVD, and can be used to assess the presence, severity, and progression of atherosclerotic disease. In recent years, promising results have also been obtained from research using newer methods such as electron-beam and

multidetector-row computed tomography,[36] which provide non-invasive and direct measurements of the amount of coronary calcification, a close correlate of total atherosclerotic plaque burden[37]. For the purpose of primary prevention, the routine use in clinical practice of the extracoronary measures of atherosclerosis above may therefore be limited, also because they may not contribute significantly to the information obtained from traditional cardiovascular risk factors, as has been shown for IMT.[38] The AAI, however, has the advantage of being a very fast, easy, and cheap way to assess the severity of atherosclerosis, and its potential use in clinical practice, especially in a non-hospital-based setting, needs further attention.

Inflammatory mediators and cardiovascular disease

C-reactive protein and atherosclerosis

Until the 1990s, the accumulation of lipids in the arterial wall was by many considered to be the main cause of atherosclerotic disease. Gradually, however, evidence emerged that a wide range of inflammatory cells and mediators play a key role in the initiation and progression of atherosclerotic lesions.[39] A marker of acute inflammatory or infectious processes, which has since long been used in the clinic, is the acute phase protein C-reactive protein (CRP). After a few epidemiological studies in high-risk subjects had suggested that even very mild elevations in CRP, which had never been considered clinically relevant, were predictive of CVD,[40,41] a crucial report was published by Ridker et al. in 1997.[42] It described a strong relationship between CRP and myocardial infarction and stroke in an apparently healthy male population. Since then, an overwhelming amount of epidemiological research has focused on this marker of inflammation as a new risk factor for CVD.[43]

The results of population-based studies investigating the relationship between CRP and the severity of extracoronary atherosclerosis have not been consistent.[44-48] Chapter 3.1 therefore presents a study investigating whether CRP is related to extracoronary atherosclerosis measured non-invasively at several sites in the arterial tree. This study shows that levels of CRP are associated with carotid plaques, carotid IMT, and the AAI. In addition, consistent with the findings of a previous small study in Japanese outpatients,[49] the study presented in chapter 3.2 shows that CRP is also an independent predictor of *progression* of atherosclerosis; the risk estimates for progression associated with CRP are as high as those associated with more traditional cardiovascular risk factors. Whereas no conclusion about cause and effect can be drawn from the cross-sectional association between CRP and atherosclerosis described in chapter 3.1, the study in chapter 3.2 suggests that inflammatory processes may be actively involved in the progression of atherosclerotic disease. However, the debate about whether inflammation is a cause or consequence of CVD has largely subsided, because it has widely been accepted that both points of view are likely to be true.[50]

Several, although not all studies have reported a lack of association between CRP and coronary atherosclerosis measured by electron-beam computed tomography.[51-53]

In the study presented in chapter 4.1, in which the associations of CRP with extracoronary atherosclerosis are confirmed, the association between CRP and coronary calcification clearly shows a positive trend, which is somewhat attenuated after adjustment for traditional cardiovascular risk factors. Although differences of opinion exist, it has been argued that calcification stabilizes plaques and diminishes the risk of plaque rupture.[54] Furthermore, it has been suggested that CRP does not merely reflect the burden of atherosclerosis, but rather gives an indication of plaque vulnerability.[55] It is therefore possible that inflammation becomes a less important feature of atherosclerotic plaques once they have reached the stage in which they are calcified. Recently, it has been proposed that coronary calcium scores and CRP are complementary for CVD risk prediction, because they assess different aspects (amount versus stability) of atherosclerotic disease.[56]

Several explanations for the strong association of CRP with atherosclerotic disease exist. CRP has been shown to bind and activate complement,[57,58] induce expression of several cytokines and cell adhesion molecules as well as tissue factor,[59-61] mediate low density lipoprotein (LDL)-uptake by endothelial macrophages,[62] induce monocyte recruitment into the arterial wall,[63] and suppress nitric oxide synthesis.[64] However, although these CRP-specific mechanisms may all play a role in atherosclerosis, levels of CRP likely reflect general inflammatory activity, thus representing an even wider range of mechanisms mediated by multiple inflammatory cells and mediators that are involved in the progression of atherosclerotic disease.

Interleukin-6, cell adhesion molecules, and atherosclerosis

Several other inflammation-related molecules have been the focus of epidemiological studies. Of the interleukins, most research has involved interleukin-6 (IL-6), the principal determinant of the hepatic synthesis of CRP, which has been shown to be strongly predictive of future CVD.[65-67] Other molecules of interest are the cellular adhesion molecules, which are expressed under the influence of inflammatory stimuli and can facilitate the emigration of leukocytes into the vessel wall, which is an important feature of atherosclerotic plaque formation.[68,69] Soluble forms of intracellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) can be measured in blood and may result from shedding or the proteolytic cleavage of these molecules from endothelial or other cells.[70,71] sICAM-1 has been reported to predict future CHD,[72,73] but in a recent meta-analysis, it was concluded that cell adhesion molecules are unlikely to add prognostic information to the information provided by traditional cardiovascular risk factors.[74]

Within the Rotterdam Study, associations were investigated of IL-6, sICAM-1, and sVCAM-1 with extracoronary atherosclerosis measured at several sites in the arterial tree (chapter 3.1). Previous reports on this issue have not been conclusive.[75-80] Although the results presented in chapter 3.1 suggest a possible relationship of IL-6 and sICAM-1 with the severity of atherosclerosis, the associations are less consistent than for CRP. More research, at the molecular level and at the population level, is necessary to elucidate the role of IL-6 and CAMs in atherosclerosis.

C-reactive protein in clinical practice

In a recent meta-analysis, it was shown that the risk ratio (95% confidence interval) for subjects in the top third of the population distribution of CRP compared with subjects in the lowest third was 2.0 (1.6 to 2.5).[43] Moreover, in a large, population-based study among nearly 28,000 women, Ridker et al. showed that the level of CRP is a stronger predictor of CVD than the LDL-cholesterol level, and that CRP adds prognostic information to that conveyed by the Framingham risk score.[81] Because of the compelling evidence in favor of CRP, and because of the relatively easy and accurate way in which especially minor elevations in levels of CRP can be measured,[82] it has repeatedly been suggested that CRP be routinely measured as a risk factor for CVD in clinical practice.[83] However, others have objected to this proposal, arguing that it is not yet clear which cut-off points should be used to indicate elevated risk, that the within-subject variability in CRP levels is large, that the positive predictive value of CRP is extremely low, and that data showing beneficial effects of treatment with HMG Co A reductase inhibitors ('statins') among those with elevated levels of CRP come from post hoc analyses and need to be confirmed in randomized clinical trials.[84-86] Despite these objections, the Centers for Disease Control and Prevention and the American Heart Association stated in January 2003 that the measurement of CRP in clinical practice in addition to the major risk factors to further assess absolute CVD risk was reasonable (but still at the discretion of the physician), especially in those with a 10-year CVD risk of 10-20%. However, it was also stated that the benefit of any treatment based on this strategy remains uncertain.[87]

The strong predictive value of CRP for CVD as reported by Ridker et al. needs to be confirmed in different populations of varying backgrounds and ages. Chapter 3.3 presents a study showing that CRP predicts future myocardial infarction in an elderly population, but not independently of more traditional cardiovascular risk factors that are routinely assessed in a clinical setting. Although this study was a relatively small study, which needs to be extended, others have also suggested that the predictive value of CRP decreases with increasing age.[88,89] Therefore, the role of CRP as a new cardiovascular risk factor in elderly people should be critically evaluated.

Genetic variation in Fcγ receptor IIa and peripheral atherosclerosis

A genetic association study is a type of study in which the relationship is studied between a given disease and a gene coding for a protein thought to be important in that particular disease.[90] It provides the opportunity to get more insight in the mechanisms underlying a disease, because genotype is invariable and cannot be influenced by external factors. Fcγ receptor IIa (FcγRIIa) is the low-affinity receptor for immunoglobulin G and possibly also the major leukocyte receptor for CRP.[91] For the FcγRIIa gene, a single amino acid substitution has been described at position 131, which influences the receptor's functional response and its IgG subclass specificity, but also the binding of CRP to the receptor.[92,93] In chapter 3.4, a genetic association study is presented investigating the role of FcγRIIa in peripheral atherosclerosis. It shows that the presence of the H allele of the R/H 131 polymorphism in the FcγRIIa

gene protects against lower extremity atherosclerosis, and that the effect is most pronounced in study participants with modestly elevated levels of inflammation as indicated by the leukocyte count. The results of this study support a role for FcγRIIa in atherosclerotic processes, but, because initial positive reports from genetic association studies can rather frequently not be replicated (see below),[94] they need to be confirmed, both in population-based and in functional in vitro studies.

Programmed cell death in atherosclerosis

The process of programmed cell death is called apoptosis. Although it is an essential physiologic phenomenon, it is also involved in a wide range of pathologic conditions, including CVD.[95,96] In atherosclerotic lesions, large numbers of apoptotic cells are present, as well as apoptosis-related proteins.[97-99] Findings from laboratory studies supporting an active role for apoptosis in CVD include the ability of oxidized LDL-cholesterol to induce apoptosis of endothelial and smooth muscle cells;[100] the induction of pro-inflammatory mediators by apoptotic cells that have not been cleared by macrophages;[101] the pro-thrombogenicity of phosphatidylserine, which is expressed on the surface of apoptotic cells;[102] and the potentially negative influence of apoptosis on the stability of the atherosclerotic plaque, which is suggested by the fact that apoptotic cells are predominantly found in areas of plaque rupture.[103,104]

Binding of Fas-ligand (also known as CD178)) to Fas (Apo-1 or CD95), one of the so-called 'death receptors' on the cell membrane, rapidly leads to the induction of the apoptosis cascade. Soluble (s) forms of these molecules are relatively easy to measure in human blood, and recently, some small studies have reported associations between sFas, sFas-ligand, and CVD. [105-107] However, these results have not yet been confirmed in a population-based study. In chapter 4.1, a study is presented in which associations of sFas and sFas-ligand with coronary and extracoronary atherosclerosis are investigated within the Rotterdam Coronary Calcification Study, a population-based study embedded in the Rotterdam Study. The results from this study do not confirm the previously reported associations between sFas and coronary and extracoronary atherosclerosis. However, although associations of sFas-ligand are not consistent across the various measures of atherosclerosis that are investigated, levels of sFas-ligand show a positive association with the presence of advanced coronary and abdominal calcification. Therefore, further research into the role of sFas-ligand in CVD is warranted.

METHODOLOGICAL CONSIDERATIONS

Study design

In epidemiological research, several study designs can be adopted. In this thesis, three chapters (chapters 3.1, 3.4, and 4.1), describe studies in which the determinant(s) and the outcome of interest were measured at the same point in time. Although these cross-sectional studies provide a relatively quick and easy way to study associations,

their main drawback is that no conclusions can be drawn about cause and effect. For example, from the association between CRP and the severity of atherosclerosis, which is described in chapter 3.1, we cannot infer whether CRP has an active role in the etiology of atherosclerosis or whether levels of CRP should merely be seen as a marker of the disease. Because genotype is an invariable determinant, these considerations do not apply to the genetic association study described in chapter 3.4. Methodological issues that nevertheless remain include the fact that those who are most susceptible to the effects of a given gene may already have died and will therefore not have been included in the study population.

In a prospective study, the measurement of the determinant(s) has taken place before the outcome of interest occurs. Therefore, this type of study may provide insight in the etiology of the disease under study (provided that possible biases have been adequately dealt with). Prospective studies are described in chapters 2.2 (a cohort study) and 3.3 (a nested case-control study). Potential problems with prospective studies are the duration of follow-up needed for a sufficient number of new disease cases to occur, and loss-to-follow-up. In the Rotterdam Study, the duration of follow-up is now more than 10 years; loss-to-follow-up between phases one (1990 – 1993) and three (1997 – 1999) was only 0.4%.

Studies in which the outcome of interest is change or progression in disease status over time are presented in chapters 2.1 and 3.2 of this thesis. The advantages and disadvantages of such a study design are discussed below.

Progression of disease as the outcome of interest.

Studying changes in disease severity (e.g. atherosclerosis) over time instead of a disease state may give more insight into the biological mechanisms underlying an association than a cross-sectional study, and, especially in a relatively elderly population such as the Rotterdam Study population, may identify those factors that even after a certain age are still important determinants of progression of the disease. However, this type of study is complicated by several methodological issues, such as the fact that all participants must have survived until the end of the follow-up period and that precision will decrease because of larger within-person variance when 'change' is measured instead of a state. Most importantly, however, baseline levels of disease may be associated with the determinant of interest (if exposure to the determinant has contributed to the severity of the disease at baseline), but they may also independently determine disease progression. An intuitive approach would therefore be to adjust in the analyses for baseline levels of disease, but this approach may lead to overadjustment and to biased risk estimates because of error in the measurement of the outcome variable.[108] Recently, two studies reported on the use of statistical methods that can adequately deal with measurement error.[109,110] Provided that data on measurement error are actually available, these methods will facilitate research using 'change' as the outcome of interest.

Added clinical value of 'new' cardiovascular risk factors

'New' factors that are found to prospectively predict CVD independently of the traditional cardiovascular risk factors may be of added value in the clinical assessment of CVD risk. The existing debate about whether or not to include CRP measurements in clinical practice is a good example.[87] The area under the receiver-operating curve (AUC) provides a good measure of the overall diagnostic value of a CVD risk function including the factor of interest, and can be compared to the diagnostic value of the risk function without.[111,112] Alternatively, a likelihood ratio test may be performed to test whether a particular factor adds significant information to a statistical model.[81] However, both types of comparison are done over the entire range of values of the CVD risk function, whereas in clinical practice, threshold values are defined to indicate whether or not a patient is at increased risk. In the study presented in chapter 3.3, different thresholds were selected to estimate the sensitivity and specificity of several CVD risk functions in- or excluding CRP. However, threshold values in this study were still chosen arbitrarily, while ideally, they should be based on the prevalence of the disease and the risks and benefits of the proportion of false-positive and false-negative results that is associated with a given threshold.[113] Of note, the conclusions drawn in chapter 3.3 (CRP is not a strong predictor of CVD risk in an elderly population) remained the same when the AUC or likelihood ratio methods were applied to the data.

Lack of reproducibility in genetic association studies

Recently, several suggestions have been done to increase the validity of results obtained from genetic association studies, including the selection of meaningful candidate genes, the most optimal selection of the control population, independent replication of data (even as part of the initial report), and careful attention to the effects of multiple testing and the choice of an appropriate significance threshold.[90,94] However, these 'guidelines' are often neglected, and especially the testing of multiple genetic markers, or the testing of associations in various subgroups of the study population without a clearly defined *a priori* hypothesis, leads to a large number of initially positive publications that cannot be replicated in other studies.[94] In chapter 3.4, a significant association is described between the FcγRIIa gene and peripheral atherosclerosis, which has not been reported before. Because the genetic polymorphism that was studied results in a functional amino acid change in a receptor which has been implicated in the pathogenesis of athersclerosis,[62,63,114] the results of this study are certainly interesting and hypothesis-generating. However, the involvement of FcγRIIa in atherosclerotic disease needs to be confirmed in other studies.

FUTURE RESEARCH

Opportunities for future CVD research in the fields of atherosclerosis imaging, inflammation, and apoptosis are manifold. Research concerning non-invasive methods to measure atherosclerosis is already shifting from the measures of extracoronary atherosclerosis described in chapter 2 to newer techniques, such as electron-beam or multidetector-row computed tomography or high-resolution magnetic resonance imaging, which will allow us to quantify the severity of coronary atherosclerosis and to estimate the vulnerability of the atherosclerotic plaque to rupture.[115] However, use of these techniques in population-based studies, or in a primary prevention setting, is still limited because of the technological knowledge required, the costs involved, and/or the use of contrast media. In general, for (new) methods used to measure atherosclerosis, research should focus on the reliability in the assessment of progression of atherosclerosis over time, and on the predictive value for CVD in populations of varying ages and background. If the predictive value is high, the added value and feasibility of integrating a specific method in clinical practice should be investigated. Of note, atherosclerosis imaging may be especially important for the identification of persons at moderate risk (based on the presence of traditional cardiovascular risk factors) for whom additional testing may resolve whether or not they are at high risk and deserving of aggressive intervention.[116] Future research in the field of atherosclerosis imaging should also focus on the predictive value for clinical CVD of progression of atherosclerosis, which is as yet largely unknown.[117]

In the field of inflammation, many of the mechanisms by which inflammatory mediators are involved in the pathogenesis of atherosclerosis and its progression to clinical disease have not yet been elucidated.[39,118-120] Moreover, it needs to be clarified which factors are responsible for the enhanced inflammatory response within the atherosclerotic lesion. Importantly, endothelial injury and subsequent endothelial dysfunction, which have been proposed to represent the first step in atherosclerosis (the 'response-to-injury' hypothesis), result in a series of changes in the properties of the endothelium that include the production of inflammatory mediators.[39,121] Intriguingly, oxidized LDL-cholesterol is thought to be both a key component in endothelial injury and a potent inducer of the inflammatory response.[121,122]

With respect to epidemiological research, the search for new inflammatory mediators that represent atherosclerotic plaque burden or vulnerability and can predict future CVD will continue in the coming years.[123-126] Part of this search will certainly be conducted by way of genetic association studies.[127-129] The study presented in chapter 3.4 suggests that the FcγRIIa gene is among the potentially interesting candidate genes for further studies in this direction.

Basic research aimed at elucidating the role of apoptotic processes within atherosclerotic plaques will contribute importantly to our understanding of the etiology of CVD. For example, it is not yet known whether the presence of apoptotic cells at sites of plaque rupture is actually a cause of plaque vulnerability or merely a

consequence of the plaque rupture itself.[104] In CVD epidemiology, however, a prominent role for mediators of apoptosis as 'new' CVD risk factors is as yet unlikely because of the limited availability of laboratory assays suitable for the determination of mediators of apoptosis in population-based studies and the negative results reported in chapter 4.1 for sFas in relation to atherosclerosis. The role of sFas-ligand in CVD merits further investigation.

It remains to be established in which individuals measurement of inflammatory status as indicated by levels of CRP is a meaningful and cost-effective addition to CVD risk assessment.[84-86] In the elderly, the clinical value of CRP measurement may be limited, as CRP may lose its predictive value with increasing age.[88,89] On the other hand, CRP measurement may be especially important in individuals with relatively low lipid levels who may still benefit from treatment with HMG Co A reductase inhibitors (statins) if they have high levels of CRP.[81,130] However, although the PRINCE (Pravastatin Inflammation/CRP Evaluation) trial showed that statins can reduce levels of CRP in a largely LDL-independent manner,[131] a randomized clinical trial is needed to investigate the putative beneficial effect of CRP-lowering therapy in subjects with high CRP levels on the occurrence of clinical CVD.[132,133] The upcoming JUPITER (Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin) trial, in which 15,000 men and women with low levels of LDL-cholesterol (< 3.4 mmol/l) and high levels of CRP (> 2.0 mg/l) will be randomized to statin or placebo and followed for the occurrence of major cardiovascular events, may provide some of the answers needed to determine more precisely who will benefit from drug treatment in primary CVD prevention.

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Chapter 6

Summary
Samenvatting

Chapter 6.1

Summary

The discouragement of smoking and the detection and treatment of hypertension, hypercholesterolemia, diabetes mellitus, and obesity take a prominent place in the primary and secondary prevention of cardiovascular disease (CVD). However, half of all patients with CVD do not have any of these traditional cardiovascular risk factors. The aim of the studies described in this thesis was to search for and provide more insight into factors that 1) are involved in the etiology of atherosclerosis and its clinical consequences, and 2) can improve our ability to identify subjects who are at an increased risk of future CVD. All studies in this thesis are based on data from the Rotterdam Study, a population-based cohort study composed of 7,983 men and women aged 55 years and over who live in a well-defined suburb of the city of Rotterdam, the Netherlands.

Chapter 2 focuses on non-invasive measures of (subclinical) extracoronary atherosclerosis. Such measures are valuable tools in epidemiological CVD research. Within the Rotterdam Study, carotid plaques and intima-media thickness (IMT) were measured by ultrasound, abdominal aortic atherosclerosis was measured by X-ray, and lower extremity atherosclerosis was assessed by computing the ankle-arm index (AAI). Although each of these measures has its own advantages and limitations, they are all strongly associated with the presence and amount of coronary calcification and with traditional cardiovascular risk factors.

Because in the Rotterdam Study, atherosclerosis was measured at different points in time, it was possible to compute progression of atherosclerosis over time. The study presented in chapter 2.1 focuses on risk factors for atherslerotic disease progression. The results from this study show that age, smoking, total cholesterol, and systolic blood pressure and/or hypertension strongly predict progression of extracoronary atherosclerosis. However, gender remarkably does not, which emphasizes the need for prevention of progression of extracoronary atherosclerosis in men and women alike.

All of the measures above have been shown to be strongly predictive of future CVD. However, differences between these measures with regard to their predictive value for CVD may exist, either due to factors related to the site at which atherosclerosis was measured, or to factors associated with the measurement method. The study

presented in chapter 2.2 shows that all measures of extracoronary atherosclerosis are good predictors of coronary heart disease. For carotid plaques and aortic atherosclerosis, the predictive value is independent of the other atherosclerosis measures, which shows the value of direct assessment of atherosclerotic plaques in CVD risk prediction.

The importance of inflammatory processes in CVD has only relatively recently been recognized. In **chapter 3**, four studies are described which focus on inflammatory markers in relation to atherosclerosis and clinical CVD. In chapter 3.1, the results are presented from a study investigating the association of inflammatory markers (C-reactive protein (CRP) and interleukin-6 (IL-6)) and soluble forms of cell adhesion molecules (intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1)) with atherosclerosis measured at multiple sites in the arterial tree. This study shows that, whereas associations of IL-6 and sCAMs with atherosclerosis are inconsistent, CRP is associated with carotid plaques and IMT and with the AAI. Moreover, CRP also predicts *progression* of extracoronary atherosclerosis measured at various sites (chapter 3.2), and the risk estimates associated with CRP are as high as those associated with more traditional cardiovascular risk factors.

Because there is strong evidence from large population-based studies that levels of CRP can predict future myocardial infarction and stroke, CRP has been suggested to be a 'new' cardiovascular risk factor, which, along with the traditional cardiovascular risk factors, should be measured in clinical practice to establish a patient's cardiovascular risk profile. However, the study presented in chapter 3.3 shows that CRP predicts future myocardial infarction, but not independently of the traditional cardiovascular risk factors. These results strongly suggest that in men and women above the age of 55, measurement of CRP in clinical practice has no added value when traditional cardiovascular risk factors are known.

The last study presented in chapter 3 investigates the role of Fcγ receptor IIa (FcγRIIa), a receptor for immunoglobulin G and for CRP, in atherosclerotic disease. Because this study shows that variation in the FcγRIIa gene is associated with peripheral atherosclerosis as indicated by the AAI, it supports an active role for FcγRIIa in atherosclerosis, potentially by mediating immunoglobulin G- or CRP-dependent processes.

Chapter 4 concerns the role of programmed cell death, a process called apoptosis, in atherosclerosis. Although laboratory studies have provided evidence for such a role, only a few small epidemiological studies have addressed this issue. A study is presented in which associations with coronary and extracoronary atherosclerosis are investigated for soluble forms of two mediators of apoptosis, Fas and Fas-ligand. The results of this study do not support a role for sFas in the identification of subjects with atherosclerotic disease. However, because sFas-ligand is positively associated with some, but not all of the measures of atherosclerosis that are investigated, further studies into the relationship between sFas-ligand and CVD are warranted.

Finally, **chapter 5** provides a more general discussion of the studies presented in this thesis put in the context of current knowledge and ongoing research in the field of CVD. The main findings are placed in a broader context and some methodological aspects are commented on. These include the design of the various studies, the use of progression of disease as the outcome of interest, the assessment of the added clinical value of new cardiovascular risk factors, and the lack of reproducibility in genetic association studies. In addition, suggestions are made for future research in CVD epidemiology. For (new) methods used to measure atherosclerosis, research should focus on their reliability in the assessment of progression of atherosclerosis over time, and on their predictive value for future CVD. Furthermore, epidemiological studies should continue their search for new factors that represent atherosclerotic plaque burden or vulnerability and can predict future CVD, especially in the field of inflammation, but perhaps also in the field of apoptosis. Finally, the role of measurement of CRP in the clinic as part of the assessment of a patient's cardiovascular risk profile needs to be carefully established in populations of varying ages and backgrounds.

Chapter 6.2

Samenvatting

Een prominente rol bij de primaire en secundaire preventie van hart- en vaatziekten is weggelegd voor het ontmoedigen van roken en het opsporen en behandelen van hypertensie, hypercholesterolemie, diabetes mellitus en obesitas. Toch komen bij de helft van de patiënten met hart- en vaatziekten deze traditionele risicofactoren niet voor. Het doel van de studies die in dit proefschrift beschreven worden is te zoeken naar en meer inzicht te verkrijgen in factoren die 1) betrokken zijn bij het ontstaan van atherosclerose (slagaderverkalking) en de klinische gevolgen daarvan en 2) de identificatie van mensen met een verhoogd risico op hart- en vaatziekten kunnen verbeteren. Alle studies in dit proefschrift zijn gebaseerd op data afkomstig uit het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek ofwel 'the Rotterdam Study'. Dit is een onderzoek onder de algemene bevolking, waaraan 7.983 mannen en vrouwen van 55 jaar en ouder, wonend in de Rotterdamse wijk Ommoord, deelnemen.

Hoofdstuk 2 is gericht op verschillende niet-invasieve maten van (subklinische) extracoronaire atherosclerose. Deze maten zijn waardevolle meetinstrumenten voor epidemiologisch hart- en vaatziekten onderzoek. In het ERGO-onderzoek zijn atherosclerotische plaques in de arteria carotis (de halsslagader) en de intima-media dikte daarvan gemeten met behulp van echoscopie. Atherosclerose van de abdominale aorta werd gemeten aan de hand van een röntgenfoto en atherosclerose in de onderste lichaamshelft werd vastgesteld door de enkel-arm index te berekenen. Hoewel elk van deze maten van atherosclerose voor- en nadelen heeft, tonen ze alle een sterke associatie met de aanwezigheid van en met de hoeveelheid verkalking in de kransslagaderen en met traditionele cardiovasculaire risicofactoren.

Omdat atherosclerose in het ERGO-onderzoek op verschillende tijdstippen gemeten is, kan de progressie van atherosclerose met de tijd worden berekend. De studie die gepresenteerd wordt in hoofdstuk 2.1 richt zich op risicofactoren voor progressie van atherosclerose. De resultaten van deze studie laten zien dat leeftijd, roken, totaal cholesterol en systolische bloeddruk en/of hypertensie sterke voorspellers zijn van progressie van extracoronaire atherosclerose. Opmerkelijk genoeg is geslacht dat niet. Dit benadrukt het belang van preventie van progressie van extracoronaire atherosclerose bij zowel mannen als vrouwen.

Alle bovengenoemde maten van atherosclerose zijn sterke voorspellers van het optreden van hart- en vaatziekten in de toekomst. Toch zijn er mogelijk verschillen in de voorspellende waarde van deze maten, gerelateerd aan de plaats in het lichaam waar atherosclerose gemeten wordt of aan de methode die daarvoor gebruikt wordt. De studie in hoofdstuk 2.2 laat zien dat alle maten van extracoronaire atherosclerose goed het optreden van hart- en vaatziekten kunnen voorspellen. De voorspellende waarde voor hart- en vaatziekten van plaques in de arteria carotis en van atherosclerose in de abdominale aorta is onafhankelijk van de andere maten van atherosclerose. De waarde van methoden die atherosclerotische plaques direct kunnen meten wordt hiermee aangetoond.

Relatief recent is pas het belang van ontstekingsprocessen in het ontstaan van hart- en vaatziekten onderkend. In **hoofdstuk 3** worden vier studies beschreven die ingaan op de relatie tussen ontstekingsfactoren en atherosclerose en zich klinisch presenterende hart- en vaatziekten. In hoofdstuk 3.1 worden de resultaten van een studie gepresenteerd die de associatie onderzoekt van ontstekingsmarkers (C-reactief proteïne (CRP) en interleukine-6 (IL-6)) en in het bloed voorkomende vormen van cel adhesiemoleculen (intercellulair adhesiemolecuul-1 (sICAM-1) en vasculaire cel adhesiemolecuul-1 (sVCAM-1)) met atherosclerose gemeten op meerdere plaatsen in het arteriële vaatstelsel. De associaties van IL-6 en sCAMs met atherosclerose zijn inconsistent, maar CRP is geassocieerd met plaques in en de intima-media dikte van de arteria carotis en met de enkel-arm index. Daarnaast voorspelt CRP ook de *progressie* van extracoronaire atherosclerose gemeten op verschillende plaatsen (hoofdstuk 3.2). De risicoschatters voor CRP zijn even hoog als die voor de meer traditionele cardiovasculaire risicofactoren.

Grote onderzoeken onder de algemene bevolking hebben overtuigend bewijs geleverd dat bloedwaarden van CRP het optreden van een myocardinfarct of cerebrovasculair accident kunnen voorspellen. Daarom is er voorgesteld dat CRP beschouwd moet worden als een 'nieuwe' cardiovasculaire risicofactor, die samen met de traditionele risicofactoren in de klinische praktijk gemeten moet worden om het cardiovasculaire risicoprofiel van een patiënt te bepalen. De studie die gepresenteerd wordt in hoofdstuk 3.3 laat echter zien dat CRP weliswaar het optreden van een myocardinfarct kan voorspellen, maar niet onafhankelijk van de traditionele risicofactoren. Deze resultaten suggereren dat het meten van CRP in de klinische praktijk bij mannen en vrouwen ouder dan 55 jaar geen toegevoegde waarde heeft als informatie over de traditionele risicofactoren bekend is.

De laatste studie die in hoofdstuk 3 gepresenteerd wordt onderzoekt de rol die Fcγ receptor IIa (FcγRIIa), een receptor voor immunoglobuline G en CRP, speelt bij atherosclerose. Deze studie toont aan dat variatie in het FcγRIIa gen geassocieerd is met perifere atherosclerose, gemeten aan de hand van de enkel-arm index. Dit zou kunnen wijzen op een actieve deelname van FcγRIIa aan atherosclerose, mogelijk via immunoglobuline G- of CRP-afhankelijke processen.

Hoofdstuk 4 behandelt de rol bij atherosclerose van geprogrammeerde celdood, een proces dat apoptose genoemd wordt. Hoewel laboratoriumstudies bewijs hebben

aangedragen voor een dergelijke rol, hebben slechts een paar kleine epidemiologische studies dit onderwerp bestudeerd. In dit hoofdstuk wordt een studie gepresenteerd waarin de associaties met coronaire en extracoronaire atherosclerose worden onderzocht van in het bloed voorkomende vormen van de apoptosefactoren Fas en Fas-ligand. De resultaten van deze studie kunnen een rol voor sFas bij het identificeren van mensen met hart- en vaatziekten niet bevestigen. Omdat sFas-ligand echter een positief verband toont met sommige, maar niet alle maten van atherosclerose die onderzocht zijn, is verder onderzoek naar de relatie tussen sFas-ligand en hart- en vaatziekten gerechtvaardigd.

Hoofdstuk 5 tenslotte bevat een meer algemene discussie van de studies beschreven in dit proefschrift in de context van de huidige kennis en onderzoekingen op het gebied van hart- en vaatziekten. De belangrijkste bevindingen worden in een breder daglicht gesteld en enkele methodologische aspecten komen aan de orde, te weten de opzet van de verschillende studies, het gebruik van progressie van een ziekte als de uitkomstmaat in een studie, het beoordelen van de toegevoegde klinische waarde van nieuwe cardiovasculaire risicofactoren en de slechte reproduceerbaarheid van genetische associatiestudies. Daarnaast worden suggesties gedaan voor toekomstig epidemiologisch onderzoek op het gebied van hart- en vaatziekten. Onderzoek op het gebied van (nieuwe) methoden om atherosclerose te meten zal zich moeten richten op de betrouwbaarheid van een methode bij het meten van progressie van atherosclerose met de tijd en op de voorspellende waarde van een methode voor het optreden van hart- en vaatziekten. Eveneens zal men moeten blijven zoeken naar nieuwe, meetbare factoren, met name op het gebied van ontsteking, maar mogelijk ook op het gebied van apoptose, die de hoeveelheid of de kwetsbaarheid van atherosclerotische plaques weergeven en die mensen met een verhoogd risico op het optreden van hart- en vaatziekten kunnen identificeren. Tenslotte zal in bevolkingsgroepen van verschillende leeftijden en met verschillende (etnische) achtergronden zorgvuldig onderzoek moeten worden verricht naar het belang van het meten van CRP in de klinische praktijk voor de bepaling van het cardiovasculaire risicoprofiel van de individuele patiënt.

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Irene Marleen van der Meer was born on March 23rd 1976 in Rotterdam, the Netherlands. In June 1993, she graduated from the Regionale Scholengemeenschap in Brielle (Gymnasium B). She went for nine months to a language school in Cambridge, UK, after which she started her medical studies at the Erasmus University in Rotterdam. In January 1999, she obtained her doctoral degree in medicine and started the work described in this thesis at the department of Epidemiology & Biostatistics of the Erasmus MC, University Medical Center, in Rotterdam (head: Prof. dr. A. Hofman), in close collaboration with the Gaubius Laboratory, TNO-Prevention and Health, in Leiden. In 2001, she obtained a Master of Science degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences. She expects to obtain her medical degree in October 2003, after which she will start her training in internal medicine at the Havenziekenhuis in Rotterdam.

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