Risk Factors, Coronary Calcification and Risk of Coronary Heart Disease

Results from the Rotterdam Study

Hok-Hay S. Oei

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Results from the Rotterdam Study

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Resultaten van het ERGO onderzoek

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Chapter 1

Introduction

In developed countries, cardiovascular disease is and will remain one of the leading causes of death, accounting for almost half of the deaths in the year 2000.[1] Cardiovascular risk factor assessment, which is the first step in primary prevention, has relied on traditional cardiovascular risk factors like elevated blood pressure, elevated cholesterol level, diabetes mellitus and cigarette smoking for many years. However, at least 50% of the coronary heart disease events are not caused by these traditional risk factors.[2] Measurement of (subclinical) atherosclerosis with non-invasive techniques and gaining insight in new risk factors that play a role in the development of cardiovascular disease will improve cardiovascular risk assessment.

Since atherosclerosis is a generalized process, measures of extracoronary atherosclerosis have long been used to predict the risk of coronary heart disease. Non-invasive techniques like ultrasound of the carotid arteries and ankle-arm index have already been used for more than a decade to improve cardiovascular risk prediction. Carotid intima media thickness, the number of plaques in the carotid artery and the ankle-arm index have been shown to predict risk of coronary heart disease. Electron-beam computed tomography (EBT) offers the opportunity to detect calcification in the coronary arteries in a non-invasive way. Since the amount of coronary calcification is strongly associated with the amount of coronary atherosclerotic plaque,[3,4] coronary calcification can be used as a measure of coronary atherosclerosis.

Evidence is accumulating that inflammation plays a role in the development of cardiovascular disease. Markers of inflammation like C-reactive protein and fibrinogen are found to be independent risk factors for cardiovascular disease.[5-10] Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a potential new cardiovascular risk factor with pro-inflammatory properties.[11] The enzyme hydrolyses oxidized phospholids, releasing lysophosphatidylcholine and free fatty acids.[12] The enzyme circulates in blood bound to low-density lipoprotein (LDL) cholesterol. Whether Lp-PLA2 is an independent predictor of cardiovascular disease has still to be established.

The first part of this thesis (chapters 2 to 4) mainly focuses on determinants and predictive value of coronary calcification. Chapter 2 presents studies on risk factors for coronary calcification. Chapter 2.1 gives an overview of the literature on risk factors for coronary calcification. Chapter 2.2 focuses on known cardiovascular risk factors and coronary calcification in the Rotterdam Coronary Calcification Study, while chapter 2.3 describes the association between the angiotensin-converting enzyme insertion/deletion polymorphism and coronary calcification. In chapter 3 the association of coronary calcification with chest pain and the association of coronary calcification with other measures of extracoronary atherosclerosis is described. Chapter 4 describes the association between coronary calcification and risk of coronary heart disease. The second part of this thesis (chapter 5) focuses on the role of Lp-PLA2 in predicting cardiovascular disease. Chapter 5.1 describes its association with risk

of coronary heart disease and stroke. In chapter 5.2 the association between Lp-PLA2 and measures of atherosclerosis is presented. The general discussion (chapter 6) describes the main findings of the studies, discusses its limitations and provides suggestions for future research.

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Chapter 2

Risk factors for coronary calcification

Chapter 2.1

Epidemiology of coronary calcification

The role of sex and age

The amount of coronary calcification depends on sex and age. Studies among self-referred, asymptomatic subjects have shown that men generally have higher calcium scores than women and that calcium scores increase with age.[1-5] Table 1 shows sex- and age-stratified calcium scores of the largest study, which comprises 35246 self-referred subjects. More than 50 percent of the men already have detectable coronary calcification at the age of 40. Median calcium scores increase from 0.5 in men <40 years to 473 in men >74 years. Calcium scores in women are comparable to calcium scores in men who are 15 years younger. Until the age of 54 the median calcium score in women is 0. Median calcium scores in women increase to 75 in women >74. Although the amount of coronary calcification increases with age, age itself is not a risk factor for coronary calcification. Rather, age is a cumulative measure of exposure to cardiovascular risk factors.

Cardiovascular risk factors

It has been known for decades that cardiovascular risk factors like obesity, hypertension, hypercholesterolemia, smoking and diabetes increase the risk of coronary heart disease. These risk factors are also called traditional risk factors. More recently, studies have identified new markers for cardiovascular disease like C-reactive protein, fibrinogen and homocysteine. In the following paragraphs, we will discuss the effect of traditional risk factors and newer risk factors on coronary calcification.

Subjects with obesity have a relative risk of 2-2.5 for coronary heart disease as compared to subjects without obesity.[6] Population-based studies have shown that measures of obesity are strongly associated with coronary calcification. Studies in adults found that body mass index, waist to hip ratio, abdominal height and intra-abdominal fat were associated with coronary calcification.[7-9] In contrast, in older adults body mass index was no risk factor for coronary calcification.[10] Whether the lack of an association between obesity and coronary calcification in elderly is caused by selection due to survival, by frailty due to underlying disease (eg cancer) or by another underlying mechanism is unclear.

Blood pressure is positively and linearly related to cardiovascular disease.[11,12] A net reduction of 5-6 mm Hg in diastolic blood pressure is associated with a 38% reduction in stroke risk and a 16% reduction in coronary heart disease risk.[12] Furthermore, hypertension is an important risk factor for atherosclerosis at extracoronary sites.[13,14] Population-based studies have shown that systolic and diastolic blood pressure are important risk factors for coronary calcification.[7-9,15] Adults with coronary calcification have a higher systolic blood pressure (123 mm Hg versus 117 mm Hg, $p<0.01$) and a higher diastolic blood pressure (82 mm Hg versus 77 mm Hg, $p<0.001$) than adults without any coronary calcification. Adults above the 90th percentile of systolic

Table 1. Electron Beam Tomographic Coronary Artery Calcium Score Percentiles for Men and Women Within Each Age Strata

	Age (years)								
	<40	40-44	45-49	50-54	55-59	60-64	65-69	70-74	>74
Men (25251)	3504	4238	4940	4825	3472	2288	1209	540	235
25th percentile	0	0	0	1	4	13	32	64	166
50th percentile	1	1	3	15	48	113	180	310	473
75th percentile	3	9	36	103	215	410	566	892	1071
90th percentile	14	59	154	332	554	994	1299	1774	1982
Women (9995)	641	1024	1634	2184	1835	1334	731	438	174
25th percentile	0	0	0	0	0	0	1	3	9
50th percentile	0	0	0	0	1	3	24	52	75
75th percentile	1	1	2	5	23	57	145	210	241
90th percentile	3	4	22	55	121	193	410	631	709

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blood pressure were 6.4 times more likely to have coronary calcification. The relative risk for a diastolic blood pressure above the 90th percentile was 4.2 for men and 3.2 for women.[9] Analogous to the attenuation of the predictive value of hypertension for coronary heart disease in the very elderly, no association of hypertension and coronary calcification was found in subjects with a mean age of 80 years.[10]

Compared to subjects who have cholesterol levels below 5.0 mmol/l, the risk of coronary heart disease is 3-fold greater among subjects who have cholesterol levels between 6.5 mmol/l and 7.9 mmol/l and 5-fold greater among subjects who have cholesterol levels >8.0 mmol/l.[16] Population-based studies with an age range of 20 to 70 years have shown that total cholesterol, triglycerides and cholesterol/HDL-cholesterol ratio are positively associated with coronary calcification while HDL-cholesterol is inversely associated with coronary calcification in both men and women.[7-9] However, in the very elderly an association of cholesterol and coronary calcification is lacking.[10]

Pathologic studies in the 1970s already showed that smoking increases the amount of coronary and aortic atherosclerosis.[17] The development of the electron-beam tomography (EBT) offered the opportunity to study the association of smoking and coronary atherosclerosis in vivo. In a population-based study among 740 adults between 20 and 59 years of age, Maher showed that subjects with a history of smoking have higher calcium scores than subjects who never smoked. In a multivariate model a history of smoking was only in men associated with coronary calcification.[7] Even in elderly aged 80 years the number of packyears smoked is strongly associated with coronary calcification.[10] In a self-referred high-risk population (72% of the subjects had >= 1 and 42% had >= 2 cardiovascular risk factors) a history of smoking was associated with a higher prevalence of detectable coronary calcification.[4] On the other hand, studies using intravascular ultrasound to detect coronary calcification in patients who underwent coronary angiography found a similar or even lower amount of calcification in smokers than in non-smokers.[18-20] This apparent contradiction is likely due to selection bias. Therefore, the results of the latter studies cannot be extrapolated to the general population.

Studies on diabetes and coronary calcification consistently showed that diabetes and markers of insulin resistance increase the amount of coronary artery calcification.[8,21-23] Table 2 shows calcium scores for men with diabetes and for men without diabetes in different age-categories. Men with diabetes have higher calcium scores than men without diabetes. Similarly, women with diabetes have higher calcium scores than women without diabetes (table 3).[22] A study in 139 diabetes patients (mean age 58) showed that subjects with diabetes had a mean calcium score of 344 while the control group, which was matched for age, sex and cardiovascular risk factors, had a mean calcium score of 242. Moreover, this study showed that a calcium score >= 400

Table 2. Distribution of coronary calcium scores among males: the visits of patients with diabetes versus those without by age category[a]

Age	N	Mean	Median	SD
Without diabetes				
0-39	219	9.9	0	55
40-49	626	46	0	144
50-59	906	160	15	392
60-69	629	332	126	562
>69	211	635	330	799
With diabetes				
0-39	3	0	0	0
40-49	21	168	1	591
50-59	39	415	164	589
60-69	48	690	320	738
>69	13	787	569	775

[a]P=0.001 via the Mann-Whitney, for those with versus those without diabetes.

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was present in 26% of the diabetes patients and only in 7% of the subjects without diabetes.[21] On the other hand, a population-based study in elderly (mean age 80 years) showed no association between diabetes mellitus and coronary calcification.[10] This is considered to be due to the older age of the subjects. In conclusion it can be stated that diabetes mellitus is strongly associated with coronary calcification.

Population-based follow-up studies have shown that moderate alcohol consumption diminishes the risk of coronary heart disease.[24-28] Although it has been postulated that alcohol increases HDL-cholesterol levels, the mechanism by which alcohol intake exerts this effect is not well understood. So far, only 1 study investigated whether alcohol consumption was associated with coronary calcification. In 1196 high-risk subjects no association was found between alcohol consumption and coronary calcification.[24]

C-reactive protein is a sensitive marker of inflammation that increases the risk of coronary heart disease in healthy subjects,[29-31] in patients with stable and unstable angina pectoris,[32-34] and in high-risk patients.[35] In addition, C-reactive protein has been related both cross-sectionally and prospectively to peripheral arterial disease.[36,37] However, C-reactive protein is not associated with the amount of coronary calcification in most studies.[10,38-40] As a possible explanation for the lack of the association, it has been suggested that hsCRP, in addition to being a marker of atherosclerotic

Table 3. Distribution of coronary calcium scores among females: the visits of patients with diabetes versus those without by age category[a]

Age	N	Mean	Median	SD
Without diabetes				
0-39	86	3.7	0	17
40-49	319	13	0	67
50-59	572	38	0	132
60-69	436	119	8	305
>69	174	197	65	313
With diabetes				
0-39	3	1.3	2	1.15
40-49	21	8.3	0	22
50-59	39	56	1	107
60-69	48	221	28	341
>69	13	300	193	314

[a]P=0.001 via the Mann-Whitney, for those with versus those without diabetes.

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burden, may reflect an underlying propensity to plaque instability whereas coronary calcification may be a marker for mature and hence stable atherosclerotic plaque.[38]

Increased plasma fibrinogen concentration is an independent risk factor for cardiovascular disease.[41,42] There are several mechanisms by which fibrinogen may increase the risk of cardiovascular disease. Fibrinogen is the main coagulation protein in plasma, is an important determinant of blood viscosity and can act as a cofactor for platelet aggregation.[41,43] Fibrinogen may also contribute to cardiovascular disease by other direct effects: it is a component of atherosclerotic plaques and stimulates smooth muscle cell migration and proliferation.[43] Furthermore, the correlation with C-reactive protein suggests that fibrinogen reflects the inflammatory activity of progressing atherosclerosis.[44] Studies on the association of fibrinogen and coronary calcification have found conflicting results. A population-based study in 114 men and 114 women found that subjects who were selected on the basis of their high calcium score had higher fibrinogen than the control group.[40] Furthermore a study in hypercholesterolemia patients found that fibrinogen was positively associated with coronary calcification.[45] However, other studies were not able to confirm these findings.[10,39] In conclusion, studies have suggested that fibrinogen may play a role in the process of atherosclerosis. However, larger population-based studies have to be awaited before conclusions can be drawn on the association of fibrinogen and

coronary calcification.

Although elevated serum homocysteine levels have been shown to correlate with coronary heart disease risk in cross-sectional studies, results from prospective studies are conflicting.[46] A recent meta-analysis of 57 studies showed that homocysteine is only weakly related to coronary heart disease and somewhat stronger related to cerebrovascular disease.[47] Although recent experimental studies have shown that hyperhomocysteinemia is atherogenic, at least at early stages and in the presence of another potent risk factor,[48,49] epidemiologic studies found no effect of serum homocysteine levels on coronary calcification.[9,50,51] Whether this is due to the lack of power or due to the measurement of calcification, which is a late process in the atherosclerosis proces, remains to be established.

Racial differences

There is still uncertainty regarding differences between black and white subjects in the prevalence, progression, and risk of coronary artery disease. Pathological studies have found more extensive fatty streaks in the coronary arteries of blacks than of whites [52-54] but similar amount of raised lesions,[53] which are likely to contain calcium. In accordance with pathological studies a population-based study in young adults showed no racial difference in the presence of coronary calcification.[55] On the other hand population-based studies in elderly found that calcium scores were lower in black than in white subjects.[56,57] It has been suggested that this difference at older ages is due to a higher survival rate in white subjects as compared to black subjects with similar coronary calcium scores. Another explanation could be the racial differences in the calcification process of atherosclerosis.[56]

Coronary calcification and measures of extracoronary atherosclerosis

Pathology studies in the 1960s already revealed that atherosclerosis is a generalized process that is not limited to the coronary arteries but is present in different vessel beds. Measures of extracoronary atherosclerosis have been found to predict the risk of coronary heart disease.[58-61] Recently, the development of the EBT scanner offered the opportunity to study the association of extracoronary atherosclerosis and coronary atherosclerosis in the living. Since then several studies have examined to what extent atherosclerosis in the extracoronary arteries reflects coronary atherosclerosis. Carotid atherosclerosis, as measured by intima media thickness and the number of plaques, is strongly associated with the amount of coronary calcification.[62-64] Similar associations with coronary atherosclerosis are present for aortic atherosclerosis[62,64,65] and peripheral atherosclerosis.[62,64]

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Chapter 2.2

Risk factors for coronary calcification

Abstract

Aims. We examined associations between cardiovascular risk factors and coronary calcification assessed by electron-beam tomography (EBT) in an unselected population of older subjects.

Methods and results. The Rotterdam Coronary Calcification Study is a population-based study in subjects >=55 years. Participants underwent EBT scanning. Coronary calcification was quantified according to the Agatston score. Cardiovascular risk factors were assessed 7 years before and concurrently to scanning. We used the first 2013 participants for the present analyses. Risk factors assessed 7 years before scanning were strongly associated with calcium score. Associations with blood pressure and cholesterol attenuated when measured concurrently to scanning. Although the number of risk factors was strongly associated with a high calcium score in asymptomatic subjects, 29% of the men and 15% of the women without risk factors had a high calcium score.

Conclusions. This population-based study in older subjects shows that cardiovascular risk factors are associated with coronary calcification. Associations were stronger for risk factors measured at earlier age. Almost 30% of the men and 15% of the women without risk factors had extensive coronary calcification.

Introduction

Cardiovascular risk factors like obesity, hypertension, hypercholesterolemia, diabetes and smoking are associated with atherosclerosis at different sites[1-7] and with an increased risk of coronary heart disease.[8-12] Several population-based studies have investigated the association between cardiovascular risk factors and coronary calcification. In asymptomatic adults, these cardiovascular risk factors were strongly associated with the amount of coronary calcification.[13-24] However, only one population-based study has been performed in older subjects (mean age 80 years).[25] In the latter study, only smoking and triglycerides were associated with coronary calcification.

We investigated the associations of cardiovascular risk factors and coronary calcification in a population of older subjects. Risk factors were measured on average 7 years before and at the time of electron-beam tomography (EBT) scanning.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study is embedded in the Rotterdam Study. The Rotterdam Study is a population-based study, which started with a baseline visit between 1990 and 1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited (response 78%). The rationale and design of the Rotterdam Study have been described elsewhere.[26] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1999 onwards the study population is extended with a second cohort comprising inhabitants who reached the age of 55 years after the baseline examination in 1990 to 1993 and subjects aged 55 years and over who migrated into the research area.

From 1997 onwards, participants through 85 years of age completing the third phase of the first cohort or the baseline examination of the second cohort of the Rotterdam Study were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an EBT scan. We restricted the present analyses to participants recruited from the first cohort, who were scanned from 1997 to 2000. Of the 3371 eligibles, scans were obtained for 2063 subjects (response: 61%). Due to several causes, i.e. metal clips from cardiac surgery, severe artifacts and registration errors (ECG, acquisition), image acquisition data could not be reconstructed or analyzed in 50 subjects. Thus, scores were available for 2013 participants. The median duration between risk factor assessment and EBT scanning was 7 years (1990-1993) and 50 days (1997-2000). The Medical Ethics Committee of the Erasmus MC approved the

study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcification in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California, U.S.A.). Before the subjects were scanned, they exercised breath holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. The scanner was calibrated on a daily basis using a water phantom. Quantification of coronary calcification was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California, U.S.A.) displaying all pixels with a density of over 130 Hounsfield Units. Trained scan readers were blinded to the clinical data of the participants. The presence of calcification was defined as a minimum of two adjacent pixels (area =0.52 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[27] We added the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system.

Cardiovascular risk factors

The Rotterdam Coronary Calcification Study is embedded in the ongoing Rotterdam Study. Therefore, information was available on risk factors assessed 7 years before EBT scanning (1990-1993) and concurrent to scanning (1997-2000). Apart from blood sampling methods, protocols for the interview and clinical examination were identical at both examinations. Information on smoking and medication was obtained during the home interview of the Rotterdam Study and the number of packyears of smoking was computed. Clinical measures were obtained during a visit at the Rotterdam Study research center. Height and weight were measured and body mass index (BMI) was calculated (weight (kg)/height $(m)^2$). We defined obesity as a BMI >=30. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses. We defined hypertension as a systolic blood pressure >=160 and/or a diastolic blood pressure >=100 and/or the use of blood pressure lowering medication with the indication hypertension.

In 1990-1993 non-fasting blood samples were obtained while in 1997-2000 blood

samples were obtained after an overnight of fasting. Between 1990 and 1993, serum total cholesterol was determined by an enzymatic procedure and high-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[28] Between 1997 and 2000, serum total cholesterol was determined by an automated enzymatic procedure using Roche CHOD-PAP reagent kit and HDL was measured with the Roche direct HDL-cholesterol assay using PEG-modified enzymes and dextran sulfate. We defined hypercholesterolemia as a total cholesterol level >= 6.2 mmol/l and/or the use of cholesterol lowering medication. Glucose was determined enzymatically by the Hexokinase method. Diabetes was defined as the use of anti-diabetic medication and/or non-fasting glucose levels ≥11.1 mmol/l (1990-1993) and/or fasting glucose levels ≥7.0 mmol/l (1997-2000). Impaired glucose tolerance was defined as no use of antidiabetic medication and/or non-fasting glucose levels of 7.8 - 11.0 mmol/l (1990-1993) and/or fasting glucose levels of 6.1 - 7.0 mmol/l (1997-2000).[29]

Statistical analysis

Median calcium scores were computed for age categories. The distribution of the residuals was highly skewed when we used the total calcium score for linear regression analysis. After log-transformation of the calcium score, the residuals were normally distributed with a constant variance. Therefore, log (total calcium score +1) was used for linear regression analysis. We used age and the cardiovascular risk factors as independent variables and the log calcium score as dependent variable. We computed the increase in log calcium score per standard deviation increase of the independent continuous variables. Since calcium scores were much higher in men than in women, all analyses were performed in men and women separately. In a multivariate model we entered age and all cardiovascular risk factors except diastolic blood pressure and computed regression coefficients for age and all entered cardiovascular risk factors. Regression coefficients for diastolic blood pressure were computed by entering age and all cardiovascular risk factors except systolic blood pressure. Regression analyses were repeated after exclusion of subjects with a history of coronary artery disease (myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass graft (CABG) and angina pectoris on the Rose questionnaire.[30]

Analysis of covariance was used to compute age-adjusted geometric mean calcium scores for categories of risk factors. For this, we categorized BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol into quartiles and divided the number of packyears smoked into 4 categories (0, 1-10, 11-20, ≥21).

We added the number of risk factors (0, 1, 2, >=3). After exclusion of subjects with coronary artery disease, logistic regression analysis with age and dummy variables

Table 1. Characteristics of 2013 men and women at the time of EBT scanning.

Variable	Risk factors measured concurrently to scanning		Risk factors measured 7 years before scanning	
	Men (n=933)	Women (n=1080)	Men (n=933)	Women (n=1080)
Age (years)	71.2±5.6	71.3±5.8	64.3±5.5	64.2±5.6
Body mass index (kg/m^2)	26.5±3.2	27.4±4.4	26.0±2.8	26.5±3.8
Systolic blood pressure (mm Hg)	144±21	142±21	136±20	134±21
Diastolic blood pressure (mm Hg)	77±11	75±11	75±11	73±11
Total cholesterol (mmol/l)	5.6±0.9	6.0±0.9	6.4±1.2	6.9±1.2
HDL-cholesterol (mmol/l)	1.2±0.3	1.5±0.4	1.2±0.3	1.5±0.4
Serum glucose (mmol/l)†	6.1±1.7	5.8±1.3	6.9±2.3	6.4±2.0
Smokers (%)				
Current	18	15	27	18
Past	72	39	65	33
Blood pressure lowering medication (%)	38.3	38.9	21.9	24.1
Cholesterol lowering medication (%)	15.9	15.3	3.5	2.9
History of MI (%)	18.0	6.0	14.0	5.0
History of PTCA and/or CABG (%)	10.3	2.3	5.1	0.6
Angina pectoris (%)	6.0	5.5	4.4	5.8
Calcium score‡	312 (62-969)	56 (5-261)		
Log calcium score	5.3±2.1	3.7±2.3		

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.

* EBT = electron-beam computed tomography, MI = myocardial infarction, PTCA = percutaneous transluminal coronary angioplasty, CABG = coronary artery bypass graft.

† Glucose measured concurrently to scanning is fasting, glucose measured 7 years before scanning is non-fasting.

‡ Value of the calcium score is expressed as median (interquartile range) because of its skewed distribution.

for 1, 2 and >=3 risk factors in the model was used to compute the percentage of subjects with a calcium score >400 (high calcium score according to Rumberger[31]) for the number of risk factors. Risk factors were defined as follows: (1) obesity, (2) hypertension, (3) hypercholesterolemia, (4) diabetes and (5) current smoking. Median calcium scores were computed for subjects with and without a history of coronary artery disease. SPSS 11.0 for Windows (SPSS, Inc., Chicago, Illinois) was used for data analysis.

Results

Table 1 shows the baseline characteristics of the study population. EBT scans were obtained in 2063 subjects. Subjects undergoing an EBT scan had approximately the same levels of cardiovascular risk factors as the non-responders. There were slight differences between responders and non-responders in age (70.6 versus 72.4 years), sex (46% versus 38% male), BMI (27.0 versus 26.7 kg/m^2) and ever smoking (90%

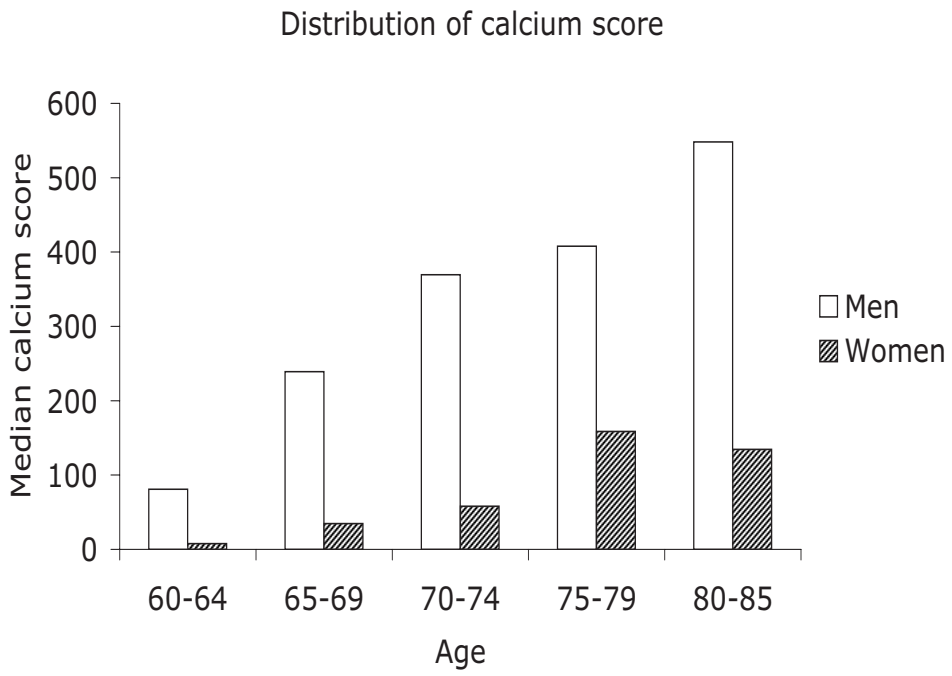


Figure 1. Median calcium scores for age categories in men and women separately.

versus 86% for men, 53% versus 49% for women). Median calcium scores increased from 81 in men aged 60-64 to 548 in men aged 80-85. In women, median calcium scores increased from 8 to 135. Men had much higher calcium scores than women in all age categories: median calcium scores in men were on average 5 times higher than in women (figure 1).

Table 2 shows that BMI, systolic blood pressure, diastolic blood pressure (not significant), total cholesterol, diabetes and smoking measured 7 years before scanning were positively associated with the calcium score while HDL-cholesterol was inversely associated with the calcium score. When we measured risk factors concurrently to EBT scanning, the strength of the association did not change for BMI. However, systolic and diastolic blood pressure were not associated with the calcium score when measured concurrently. After exclusion of subjects with blood pressure lowering medication, systolic blood pressure was positively associated with the calcium score although this did not reach significance in men while no association was present between diastolic blood pressure and the calcium score (data not shown). The association of total cholesterol and the calcium score disappeared when we measured cholesterol concurrently to coronary calcification. After exclusion of subjects with lipid lowering medication, total cholesterol was strongly associated with the calcium score in women while no association was present in men (data not shown). HDL-cholesterol measured at the time of scanning was only in women associated with the calcium score; in men no association was present. In women, the association between diabetes and coronary calcification was stronger when measured at the time of scanning. In men, the strength of the association between diabetes and coronary calcification slightly decreased. Associations for smoking were slightly stronger when measured at the time of scanning. Results slightly changed after exclusion of subjects with a history of coronary artery disease (table 3). In men, BMI measured 7 years before EBT scanning was not significantly associated with the calcium score and systolic blood pressure measured at the time of scanning was positively associated with the calcium score. In women, cholesterol measured at the time of scanning was positively associated with the calcium score.

Figure 2 shows geometric mean calcium scores for categories of risk factors measured 7 years before scanning. While the calcium score is only gradually increasing in the higher categories of BMI and smoking, a gradual increase in calcium score was seen for systolic blood pressure, diastolic blood pressure and total cholesterol. HDL-cholesterol was inversely associated with the calcium score. Compared to subjects with normal glucose tolerance, calcium scores were elevated in subjects with diabetes but not in subjects with an impaired glucose tolerance.

Figure 3 shows the percentage of subjects with a high calcium score according to the number of risk factors in subjects without a history of coronary artery disease.

Table 2. Multivariate-adjusted regression coefficients for risk factors, describing the increase in log calcium score per standard deviation increase of the cardiovascular risk factors.

	Risk factors measured 7 years before scanning		Risk factors measured concurrently to scanning	
	SD	regression coefficients (95% CI)	SD	regression coefficients (95% CI)
Men				
Age	5,5	0.47 (0.34, 0.60)‡	5,6	0.50 (0.36, 0.64)‡
Body mass index	2,8	0.16 (0.02, 0.29)*	3,2	0.20 (0.06, 0.34)†
Systolic blood pressure	20	0.29 (0.16, 0.42)‡	21	0.09 (-0.05, 0.22)
Diastolic blood pressure	11	0.08 (-0.06, 0.21)	11	-0.03 (-0.17, 0.11)
Total cholesterol	1,2	0.29 (0.16, 0.42)‡	0,9	-0.09 (-0.23, 0.05)
HDL-cholesterol	0,3	-0.12 (-0.25, 0.02)	0,3	0.06 (–0.08, 0.21)
Diabetes (yes/no)		0.47 (0.00, 0.94)*		0.34 (-0.06, 0.74)
Smoking	24	0.15 (0.02, 0.27)*	25	0.23 (0.09, 0.37)†
Women				
Age	5,6	0.63 (0.49, 0.77)‡	5,8	0.73 (0.59, 0.88)‡
Body mass index	3,8	0.15 (0.02, 0.29)*	4,4	0.13 (-0.02, 0.28)
Systolic blood pressure	21	0.17 (0.06, 0.35)*	21	0.08 (-0.07, 0.22)
Diastolic blood pressure	11	0.07 (-0.08, 0.22)	11	0.00 (-0.15, 0.14)
Total cholesterol	1,2	0.34 (0.21, 0.47)‡	0,9	0.09 (-0.06, 0.23)
HDL-cholesterol	0,4	-0.17 (–0.34, 0.00)*	0,4	-0.16 (-0.31, -0.02)*
Diabetes (yes/no)		0.42 (-0.23, 1.07)		0.68 (0.07, 0.37)†
Smoking	17	0.26 (0.13, 0.39)‡	18	0.35 (0.21, 0.49)‡

CI = confidence interval, SD = standard deviation
* $0.01<p<0.05$, † $0.001<p<0.01$, ‡ $p<0.001$.

Table 3. Multivariate-adjusted regression coefficients for risk factors, describing the increase in log calcium score per standard deviation increase of the cardiovascular risk factors in subjects without a history of coronary artery disease.

	Risk factors measured 7 years before scanning		Risk factors measured concurrently to scanning	
	SD	regression coefficients (95% CI)	SD	regression coefficients (95% CI)
Men				
Age	5.6	0.43 (0.28, 0.57)‡	5.6	0.46 (0.30, 0.62)‡
Body mass index	2.8	0.10 (-0.05, 0.25)	3.2	0.26 (0.10, 0.43)†
Systolic blood pressure	20	0.34 (0.19, 0.49)‡	21	0.21 (0.05, 0.36)†
Diastolic blood pressure	11	0.09 (-0.06, 0.24)	11	0,06 (–0.10, 0.21)
Total cholesterol	1.2	0.32 (0.17, 0.47)‡	0.9	0.01 (-0.15, 0.17)
HDL-cholesterol	0.3	-0.08 (-0.23, 0.06)	0.3	0.14 (-0.02, 0.30)
Diabetes (yes/no)		0.57 (0.03, 1.11)*		0.38 (-0.10, 0.86)
Smoking	24	0.16 (0.01, 0.30)*	25	0.23 (0.07, 0.38)†
Women				
Age	5.8	0.63 (0.49, 0.78)‡	5.8	0.71 (0.55, 0.86)‡
Body mass index	3.8	0.19 (0.04, 0.34)*	4.4	0.11 (-0.05, 0.27)
Systolic blood pressure	21	0.21 (0.06, 0.36)†	21	0.14 (-0.02, 0.29)
Diastolic blood pressure	10	0.10 (-0.05, 0.25)	11	0.07 (-0.09, 0.22)
Total cholesterol	1,2	0.34 (0.20, 0.49)‡	0.9	0.15 (0.00, 0.30)*
HDL-cholesterol	0,3	-0.16 (-0.30, -0.01)*	0.4	-0.14 (-0.29, 0.02)
Diabetes (yes/no)		0.42 (-0.27, 1.10)		0.59 (0.11, 1.07)*
Smoking	17	0.25 (0.11, 0.40)†	18	0.31 (0.16, 0.46)‡

CI = confidence interval, SD = standard deviation.
* $0.01<p<0.05$, † $0.001<p<0.01$, ‡ $p<0.001$

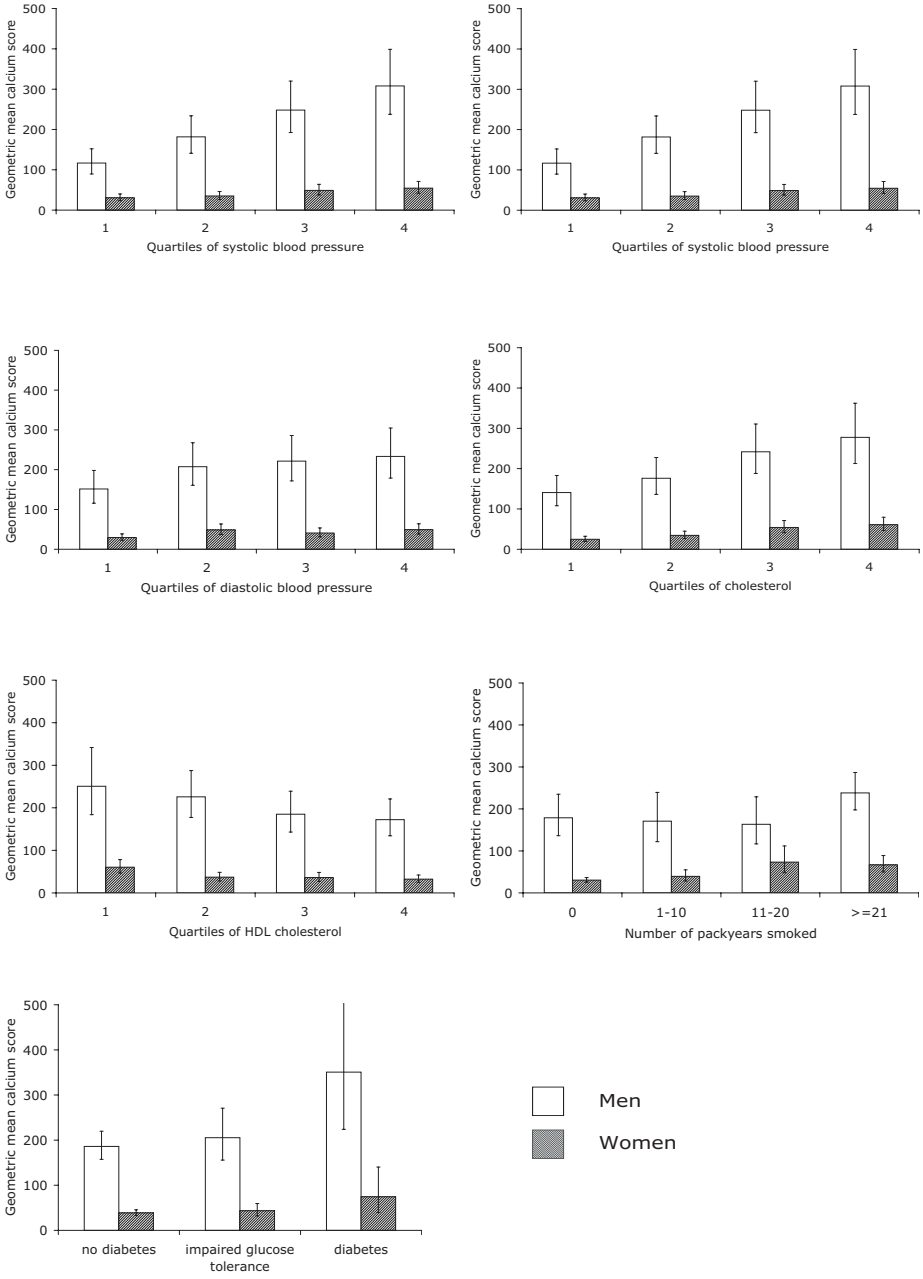
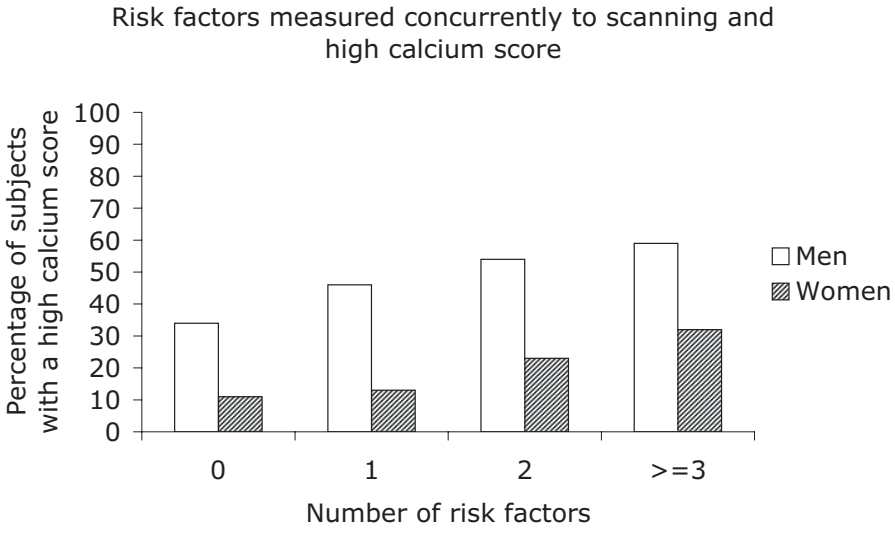


Figure 2. Age-adjusted geometric mean calcium scores for categories of cardiovascular risk factors assessed 7 years before electron-beam CT scanning in 2013 men and women.



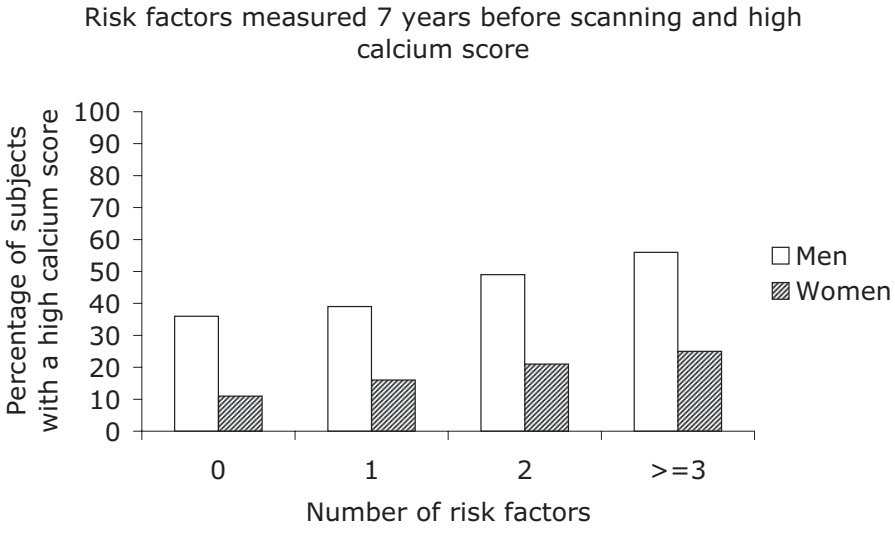


Figure 3. Age-adjusted percentages of subjects with a high calcium score for the number of cardiovascular risk factors assessed 7 years before and concurrently to electron-beam CT scanning in subjects without history of coronary artery disease.

The number of risk factors measured concurrently to scanning was strongly associated with a high calcium score in both men and women: while 29% of the men without risk factor had a high calcium score, 52% of the men with 3 or more risk factors had a high calcium score. The corresponding percentages in women were 15% and 26%. When we measured risk factors 7 years before scanning, strength of the association slightly attenuated in both men and women.

At the time of scanning, 24% of the men and 12% of the women had a history of coronary artery disease. Corresponding percentages for 7 years before scanning were 18% and 10%. Figure 4 shows that subjects with a history of coronary artery disease had a 5 times higher calcium score than subjects without coronary artery disease.

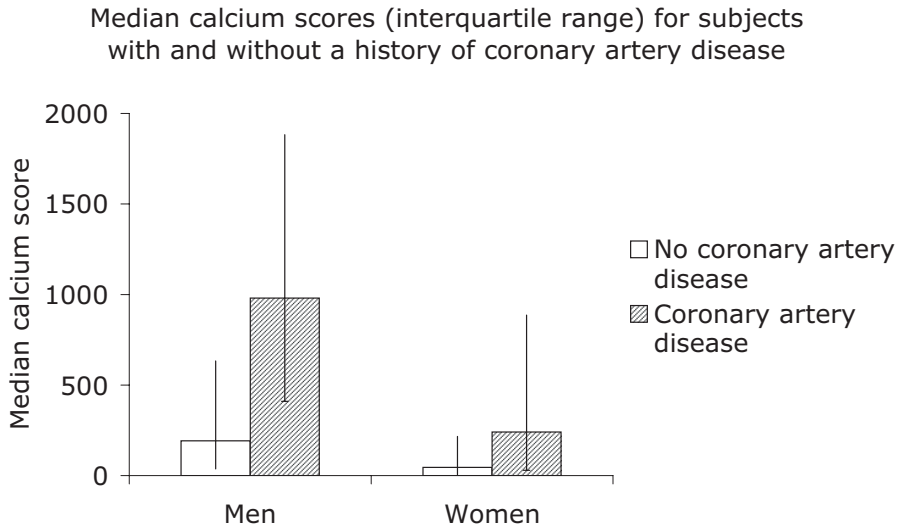


Figure 4. Median calcium scores (interquartile range) for subjects with and without a history of coronary artery disease.

Discussion

The present population-based study shows that age and male sex are the most important risk factors for coronary calcification. Cardiovascular risk factors measured 7 years before EBT scanning were strongly associated with the amount of coronary calcification. Associations for blood pressure and cholesterol attenuated or even disappeared when measured concurrently to EBT scanning. Although the number of

cardiovascular risk factors was strongly associated with a high calcium score, 29% of the men and 15% of the women without risk factors had a high calcium score.

Age and sex are strongly associated with the amount of coronary calcification. Age had a strong and graded association with the calcium score in this population of older subjects: a 6-fold increase in calcium score was seen in men and a 10-fold increase in women. Men had calcium scores that were 5 times higher than in women.

The present study shows that cardiovascular risk factors measured 7 years before EBT scanning were strongly associated with the amount of coronary calcification while associations were weaker for blood pressure and cholesterol when measured at the time of EBT scanning. Several causes should be considered. Firstly, the observation of weaker associations for blood pressure and cholesterol in 7 year older subjects is in line with the observation that the predictive value of cardiovascular risk factors attenuates with increasing age.[32-34] This hypothesis is supported by a recent study in older adults with a mean age of 80 years finding no association of BMI, hypertension, total cholesterol, HDL-cholesterol, diabetes with coronary calcification. In the latter study, only the number of packyears smoked and triglycerides were associated with coronary calcification.[25] Conversely, studies in young and middle-aged adults found that BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, diabetes and smoking were positively associated with the amount of coronary calcification while an inverse association with HDL-cholesterol was observed.[13-19,23] Secondly, at the time of EBT scanning more subjects were treated with blood pressure lowering medication (39% versus 23%) and with cholesterol lowering medication (16% versus 3%) than 7 years before EBT scanning. Misclassification of risk factors due to treatment will lead to an underestimation of the strength of the associations. The stronger associations for systolic blood pressure and cholesterol (women) after exclusion of subjects with medication use support this hypothesis.

It has been suggested that the decision to perform EBT scanning in asymptomatic subjects should be based on risk factor assessment.[35] In asymptomatic subjects, 29% of the men and 15% of the women without risk factors had extensive coronary calcification. This should be taken into account when performing EBT scanning based on cardiovascular risk factor assessment.

In conclusion, this population-based study shows that age and male sex are the most important risk factors for coronary calcification. Cardiovascular risk factors assessed 7 years before EBT scanning are strongly associated with coronary calcification. Associations of blood pressure and cholesterol with the calcium score attenuated when risk factors were measured concurrently to EBT scanning. Although cardiovascular risk factors are strongly associated with the amount of coronary calcification in asymptomatic subjects, almost 30% of the men and 15% of the women without cardiovascular risk factors have extensive coronary calcification.

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Chapter 2.3
The angiotensin-converting enzyme insertion/deletion polymorphism and coronary calcification

Abstract

Background. An insertion/deletion (I/D) polymorphism in the gene encoding angiotensin-converting enzyme (ACE) has been associated with serum ACE levels. The association between the ACE I/D polymorphism and coronary heart disease is unclear. Electron-beam computed tomography (EBT) is a technique to non-invasively quantify the amount of coronary calcification. We investigated the association between the ACE I/D polymorphism and coronary calcification.

Methods and results. The Rotterdam Coronary Calcification Study is a population-based study in subjects aged 55 years and over. EBT scanning was performed in 2013 participants. Coronary calcification was quantified according to the Agatston score. The ACE I/D polymorphism was available for 1976 subjects. Geometric mean calcium scores in men with the II, ID and DD genotype were 167, 207 and 219, respectively. However the difference in calcium score ($p=0.19$ for ID versus II, $p=0.15$ for DD versus II) and the trend ($ptrend=0.17$) were not significant. Calcium scores in women with the II, ID and DD genotype were 44, 42 and 36, respectively. There were no significant differences in calcium score ($p=0.78$ for ID versus II, $p=0.29$ for DD versus II), neither was the trend ($ptrend=0.27$). After we stratified on cardiovascular risk factors, no associations were present.

Conclusion. The present study failed to show an association between the ACE I/D polymorphism and coronary calcification in the general population. Also, no significant associations were present between the ACE I/D polymorphism and coronary calcification in strata of cardiovascular risk factors.

Introduction

The angiotensin-converting enzyme (ACE) is an important enzyme in the renin-angiotensin system. ACE converts angiotensin I into angiotensin II and inactivates bradykinin. An insertion/deletion (I/D) polymorphism in the gene encoding ACE has been associated with serum ACE levels: subjects with the D allele have higher ACE levels than subjects with the I allele.[1,2]

The association between the ACE I/D polymorphism and coronary heart disease is unclear. Several studies have investigated the association between the ACE I/D polymorphism and myocardial infarction with conflicting results: some studies found that the DD genotype was associated with an increased risk of a myocardial infarction[3-6] whereas others did not find an association.[7-11] Furthermore, angiography studies on the ACE I/D polymorphism and the extent of coronary artery disease have been inconsistent.[4-6,8,10,12]

Electron-beam tomography (EBT) is a technique to non-invasively quantify the amount of coronary calcification. Since coronary calcification is strongly correlated with the amount of coronary atherosclerotic plaque,[13,14] the amount of coronary calcification can be used as a measure of coronary atherosclerosis. In a population-based study we investigated the association between the ACE I/D polymorphism and coronary calcification. We hypothesized that the presence of the D allele is associated with more extensive coronary calcification.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study is embedded in the Rotterdam Study. The Rotterdam Study is a population-based study, which started with a baseline visit between 1990 and 1993. All inhabitants of a suburb of Rotterdam aged 55 years and over were invited (response 78%). The rationale and design of the Rotterdam Study have been described elsewhere.[15] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1999 onwards the study population is extended with a second cohort comprising inhabitants who reached the age of 55 years after the baseline examination in 1990 to 1993 and subjects aged 55 years and over who migrated into the research area.

From 1997 onwards, participants through 85 years of age completing the third phase of the first cohort or the baseline examination of the second cohort of the Rotterdam Study were invited to participate in the Rotterdam Coronary Calcification Study and

to undergo an EBT scan. We restricted the present analyses to participants recruited from the first cohort, who were scanned from 1997 to 2000. Of the 3371 eligibles, scans were obtained for 2063 subjects (response: 61%). Due to several causes, i.e. metal clips from cardiac surgery, severe artifacts and registration errors (ECG, acquisition), image acquisition data could not be reconstructed or analyzed in 50 subjects. Thus, scores were available for 2013 participants. The Medical Ethics Committee of the Erasmus MC approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcification in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California, U.S.A.). Before the subjects were scanned, they exercised breath holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. The scanner was calibrated on a daily basis using a water phantom. Quantification of coronary calcification was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California, U.S.A.) displaying all pixels with a density of over 130 Hounsfield Units. Trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area =0.52 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[16] We added the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system.

Genotyping

The II, ID and DD genotypes were detected using the polymerase chain reaction technique (PCR) according to the method of Lindpaintner et al. with some modifications.[17] The insertion and deletion alleles of the ACE gene were identified using a set of oligonucleotide primers flanking the polymorphic site in intron 16 (sense primer, 5'GCC CTG CAG GTG TCT GCA GCA TGT3' and antisense primer, 5'GGA TGG CTC TCC CCG CCT TGT CTC3'). The final volume of the PCR mix was 20 μl, containing 50 ng DNA, PCR buffer (Invitrogen), 1.3 mM MgCl2, 200 μmol dNTPS, 20 pmol primer mix and 0.35 units Taq polymerase. The thermo cycling procedure was identical to the method of Lindpaintner et al. The result of amplification was a 319-bp amplicon for the D-allele and a 597-bp amplicon for the I-allele. Because the

D allele in heterozygous subjects is preferentially amplified, there is a tendency of misclassification of ID genotypes into DD genotypes (4-5%). In order to avoid this misclassification, a second independent PCR was performed with a primer pair that recognizes an insertion specific sequence (5'TGG GAC CAC AGC GCC CGC CAC TAC3' and 5'TCG CCA GCC CTC CCA TGC CCA TAA3'). To optimize the second PCR, 10% DMSO, 0.35 units AmpliTaq Gold DNA polymerase and GeneAmp PCR Gold buffer (Applied Biosystems) were used in the PCR mix. This reaction yielded a 335-bp amplicon only if the I-allele was present. All reactions were performed in 96-well plates with the help of a robot (Beckman Biomek® 2000). Fragments were separated and visualized using 3% Agarose gels, Ethidium Bromide staining and UV trans-illumination. Two independent investigators read pictures from each gel. All ambiguous samples were analyzed a second time.

Statistical analysis

Hardy-Weinberg equilibrium proportions of the ACE I/D polymorphism were tested with a chi square test. In men and women separately, we computed age-adjusted geometric mean calcium scores for the II, ID and DD genotype. A student t-test was used to compare the geometric mean calcium scores in the different categories. Linear regression analysis using the ACE I/D polymorphism as a continuous variable was used as test for trend. To investigate interaction between the ACE I/D polymorphism and cardiovascular risk factors on coronary calcification, we computed interaction terms between the ACE I/D polymorphism and each of the cardiovascular risk factors. Risk factors were defined as follows: (1) obesity (body mass index $\geq$30 kg/m^2), (2) hypertension (systolic blood pressure $\geq$160 mm Hg and/or diastolic blood pressure $\geq$100 mm Hg and/or the use of blood pressure lowering medication), (3) hypercholesterolemia (total cholesterol $\geq$6.2 mmol/l and/or the use of lipid lowering medication), (4) diabetes (fasting glucose >7.0 and/or the use of glucose lowering medication) and (5) current smoking. We added interaction terms in the model including age, sex, the ACE I/D polymorphism and cardiovascular risk factors. In addition, we repeated the analyses in strata of cardiovascular risk factors. To increase statistical power, we pooled men and women for these analyses.

Results

EBT scans were obtained in 2063 subjects. Subjects undergoing an EBT scan had approximately the same levels of risk factors and social class as the non-responders. There were slight differences between responders and non-responders in age (70.6 versus 72.4 years), gender (46 percent versus 38 percent male), body mass index (27.0

versus 26.7 kg/m^2) and history of smoking (72 percent versus 68 percent for men, 39 percent versus 34 percent for women). The percentage current smokers was similar in the responders and non-responders (18% for men and 15% for women).

Genotype frequencies were in Hardy-Weinberg equilibrium (p=0.78 for men and p=0.96 for women). Table 1 shows characteristics of the study population. Figure 1 shows geometric mean calcium scores for the different genotypes in men. Calcium scores in men with the II, ID and DD genotype were 167, 207 and 219 respectively. However, the difference in calcium score over genotypes (p=0.19 for ID versus II, p=0.15 for DD versus II) and the trend (Ptrend=0.17) were not significant. Calcium scores in women with the II, ID and DD genotype were 44, 42 and 36 respectively. There were no significant differences in calcium score between the genotypes (p=0.78 for ID, p=0.29 for DD versus II), neither was the trend (Ptrend=0.27) (figure 2). After we stratified on cardiovascular risk factors, no significant associations were found between the ACE I/D polymorphism and the calcium score in different strata (table 2). Interaction terms between the ACE I/D polymorphism and cardiovascular risk factors entered into the model were not significant. Although we observed no significant association between the ACE I/D polymorphism and coronary calcification in both non-smokers and smokers, we found a non-significant difference in

Table 1. Characteristics of 2013 men and women at the time of electron-beam CT scanning.

Variable	Men (n = 933)	Women (n = 1080)
Age (years)	71.2±5.6	71.3±5.8
Body mass index (kg/m^2)	26.5±3.2	27.4±4.4
Systolic blood pressure (mm Hg)	144±21	142±21
Diastolic blood pressure (mm Hg)	77±11	75±11
Total cholesterol (mmol/l)	5.6±0.9	6.0±0.9
HDL-cholesterol (mmol/l)	1.2±0.3	1.5±0.4
Serum glucose (mmol/l)	6.1±1.7	5.8±1.3
Smokers (%)		
Current	18	15
Past	72	39
History of MI (%)*	18	6
Calcium score†	312 (62-969)	56 (5-261)
Log calcium score	5.3±2.1	3.7±2.3

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation
* MI = myocardial infarction
† Value of the calcium score is expressed as median (interquartile range) because of its skewed distribution.

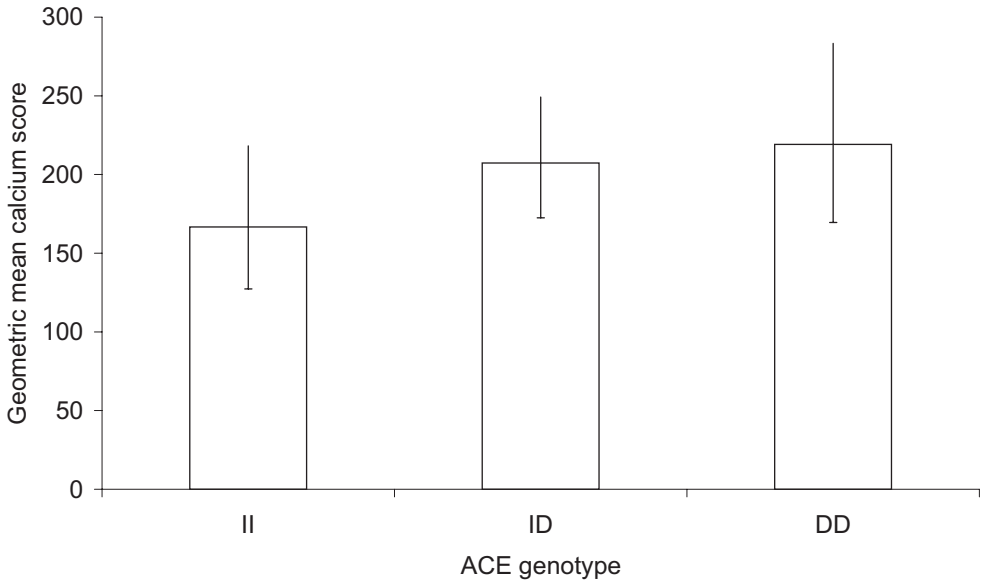

Figure 1. Geometric mean calcium scores adjusted for age in men with the II, ID and DD genotype.

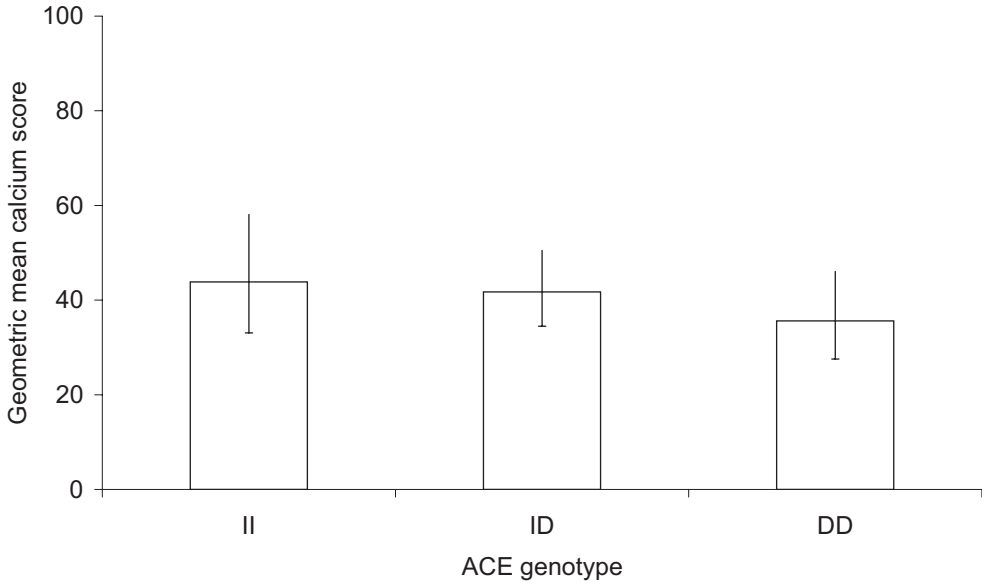

Figure 2. Geometric mean calcium scores adjusted for age in women with the II, ID and DD genotype.

Table 2. Age- and sex-adjusted geometric mean calcium scores (95% confidence interval) for the ACE I/D polymorphism in strata of cardiovascular risk factors.

	N	II	ID	DD	p-value for ID vs II	p-value for DD vs II	ptrend
Obesity							
No	1574	79 (63-98)	83 (71-96)	85 (69-105)	0.73	0.61	0.16
Yes	395	86 (57-131)	115 (86-153)	75 (51-110)	0.27	0.61	0.65
Hypertension							
No	1039	47 (37-61)	56 (47-68)	54 (42-69)	0.30	0.48	0.17
Yes	934	160 (120-213)	141 (117-170)	135 (104-174)	0.48	0.38	0.62
Hypercholesterolemia							
No	1120	70 (54-90)	72 (60-86)	76 (60-96)	0.85	0.63	0.15
Yes	856	101 (75-136)	110 (91-135)	97 (73-129)	0.62	0.85	0.59
Diabetes							
No	1585	66 (53-83)	77 (67-90)	78 (63-96)	0.27	0.31	0.27
Yes	237	200 (118-338)	202 (143-284)	175 (111-275)	0.98	0.71	0.78
Current smoker							
No	1654	75 (60-93)	81 (70-94)	75 (62-92)	0.55	0.97	0.46
Yes	315	107 (69-166)	142 (103-194)	139 (90-215)	0.32	0.41	0.33

N=number of subjects, I=insertion, D=deletion

calcium score between the genotypes in smokers (139 versus 107 for DD versus II, p=0.41 and 142 versus 107 for ID versus II, p=0.32).

Discussion

In the present population-based study, the ACE I/D polymorphism was not significantly associated with coronary calcification. In strata of cardiovascular risk factors, we found no significant association between the ACE I/D polymorphism and coronary calcification.

It is unclear whether the ACE I/D polymorphism is associated with coronary heart disease: studies on the ACE I/D polymorphism and myocardial infarction show conflicting results.[3-11] Similarly, studies on the ACE I/D polymorphism and angiographically determined coronary artery disease are inconsistent.[4-6,8,10,12] To our knowledge, this is the first population-based study examining the association of the ACE I/D polymorphism and coronary calcification. In the present study we did not find a significant association between the ACE I/D polymorphism and coronary calcification.

Cambien and coworkers found a stronger association between the ACE I/D polymorphism and myocardial infarction in subgroups of male patients who were at low risk for coronary heart disease according to cardiovascular risk factors.[3] Ever since, studies on the ACE I/D polymorphism and coronary heart disease have stratified on cardiovascular risk factors with inconsistent findings in low risk patients[4,5,7,8,11,12,18] In the present study, no associations between the ACE I/D polymorphism and coronary calcification were present in low risk subjects.

Studies on the association between the ACE I/D polymorphism and atherosclerosis so far mainly focussed on the association between the polymorphism and carotid intima media thickness. However, the results have been controversial: while some studies found an association between the ACE I/D polymorphism and carotid intima media thickness,[19-21] others failed to show an association.[22-32] A recent meta-analysis showed evidence of a positive association between the D-allele and carotid intima media thickness.[33]

It has been suggested that smoking-dependent effects may play a role in the association between the ACE I/D polymorphism and atherosclerosis.[34-36] A Japanese study in patients with coronary heart disease showed that smokers with the DD genotype had more diseased coronary arteries, more stenotic lesions and more extensive coronary atherosclerosis than smokers with the II genotype. These associations were absent in non-smokers.[34] Recently, two studies showed an association of the ACE I/D polymorphism with hypertension[36] and carotid IMT[35] in smokers but not in non-

smokers. While no association between the ACE I/D polymorphism and coronary calcification was seen in non-smokers, a non-significant difference in calcium score between the genotypes was observed in smokers. Whether the lack of significance is due to low power or whether smoking-dependent effects play no role in the association between the ACE I/D polymorphism and coronary atherosclerosis remains to be established in future studies.

In conclusion, the present study failed to show an association between the ACE I/D polymorphism and coronary calcification in the general population. In strata of risk factors, we found no association between the ACE I/D polymorphism and coronary calcification. Larger studies and studies in subgroups of cardiovascular risk factors are needed to further elucidate the association between the ACE I/D polymorphism and coronary calcification.

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Chapter 3

The association of coronary calcification with measures of extracoronary atherosclerosis and symptoms of chest pain

Chapter 3.1
Measures of extracoronary atherosclerosis and coronary calcification

Abstract

Objectives. This study was designed to examine the associations of coronary calcification assessed by electron-beam computed tomography with measures of extracoronary atherosclerosis.

Background. Although measures of extracoronary atherosclerosis have been used to predict coronary events it is not yet known to what extent those measures reflect coronary atherosclerosis.

Methods. The Rotterdam Coronary Calcification Study is a population-based study in subjects aged 55 years and over. Participants of the study underwent an electron-beam computed tomography scan. Coronary calcification was quantified according to the Agatston calcium score. Measures of extracoronary atherosclerosis included common carotid intima media thickness, carotid plaques, ankle-arm index and aortic calcification. We used the first 2013 participants for the present analyses. Age-adjusted geometric mean calcium scores were computed for categories of extracoronary measures using analyses of variance.

Results. Graded associations with coronary calcification were found for the carotid and aortic measures. Associations were strongest for carotid plaques and aortic calcification; coronary calcification increased from the lowest category (no plaques) to the highest category 9- and 11-fold in men and 10- and 20-fold in women, respectively. A non-linear association was found for ankle-arm index with an increase in coronary calcification only at lower levels of ankle-arm index.

Conclusions. In this population-based study graded associations were found between coronary calcification and common carotid intima media thickness, carotid plaques and aortic calcification. A non-linear association was found between coronary calcification and the ankle-arm index.

Introduction

Non-invasive measures of extracoronary atherosclerosis have been used to predict the risk of coronary heart disease.[1-5] Although clear associations exist between measures of extracoronary atherosclerosis and coronary events, it is not yet known to what extent those extracoronary measures reflect coronary atherosclerosis. Electron-beam computed tomography (CT) is a relatively new technique to measure coronary calcification. Since coronary calcification assessed by electron-beam CT is strongly correlated with the amount of coronary atherosclerotic plaque[6,7] the amount of coronary calcification can be used as a measure of coronary atherosclerosis.

Only few studies have examined the association between measures of extracoronary atherosclerosis and coronary calcification.[8-11] However, these studies performed measurements only at one extracoronary site[8,9] or included only subjects at high risk for cardiovascular disease.[10,11] Therefore, in a large population-based study we examined the associations between coronary calcification and common carotid artery (CCA) intima media thickness (IMT), carotid plaques, ankle-arm index (AAI) and aortic calcification.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by electron-beam CT. The study is embedded in the Rotterdam Study. The Rotterdam Study is a population-based study, which started with a baseline visit between 1990 and 1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited (response 78%). The rationale and design of the Rotterdam Study have been described elsewhere.[12] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1999 onwards the study population is extended with a second cohort comprising inhabitants who reached the age of 55 years after the baseline examination in 1990 to 1993 and subjects aged 55 years and over who migrated into the research area. Baseline and follow-up visit examinations included non-invasive measurements of atherosclerosis. Measurement protocols for the first and second cohort were identical.

From 1997 onwards, participants through 85 years of age completing the third phase of the first cohort or the baseline examination of the second cohort of the Rotterdam Study were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an electron-beam CT scan. We restricted the present analyses to participants recruited from the first cohort, who were scanned from 1997 to 2000. Of the

3371 eligibles, scans were obtained for 2063 subjects (response: 61%). Due to several causes, i.e. metal clips from cardiac surgery, severe artifacts and registration errors (ECG, acquisition), image acquisition data could not be reconstructed or analyzed in 50 subjects. Thus, scores were available for 2013 participants. The median duration between the examination of non-invasive measures of extracoronary atherosclerosis and electron-beam CT scanning was 50 days. The Medical Ethics Committee of the Erasmus MC approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcification in the epicardial coronary arteries detected on electron-beam CT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California, U.S.A.). Before the subjects were scanned, they exercised breath holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. The scanner was calibrated on a daily basis using a water phantom. Quantification of coronary calcification was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California, U.S.A.) displaying all pixels with a density of over 130 Hounsfield Units. Trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area =0.52 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[13] We added the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system.

Measures of extracoronary atherosclerosis

Ultrasonography of both left and right carotid artery was performed according to the protocol of the Rotterdam Study.[14] Off-line, the mean and maximum IMT of the CCA were measured.[14] For the current analyses the average of the mean anterior and posterior IMT of both the left and right CCA was used. When one or more of the IMT measurements could not be obtained the average of the remaining measurements was used. On line, the left and right CCA, bifurcation and internal carotid artery were evaluated for the presence (yes/no) of atherosclerotic lesions (plaques). A plaque was defined as a focal widening (of at least 1.5 times the average IMT) relative to adjacent segments, with protrusion into the lumen. The anterior and posterior wall

were evaluated and the number of affected locations counted. If one of the affected locations could not be visualized the subject was excluded from the analyses. This resulted in a plaque score between 0 and 6.

The AAI is the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm. According to the protocol of the Rotterdam Study the AAI was measured at both legs.[15] For the current analyses we used the lowest measurement. Because of possible measurement artifacts reflecting the presence of rigid or calcified walls, 7 participants with an AAI >1.5 were excluded. Subjects with an unmeasurable ankle pressure, which was considered to be due to atherosclerosis, were classified as having an AAI of 0.

Aortic calcification was diagnosed by radiographic detection of calcified deposits in the abdominal aorta.[16] Lateral abdominal films were made from a fixed distance with the subject seated. Calcifications in the abdominal aorta were classified as present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). Calcification of the anterior and posterior wall was scored separately. The extent of atherosclerosis was classified as absent, dubious, mild, moderate and severe, according to the length of the involved area of the posterior wall (0, <=1.0 cm, 1.1-2.4 cm, 2.5-4.9 cm, and >=5 cm respectively).

Cardiovascular risk factors

Information on smoking was obtained during the home interview of the Rotterdam Study and the number of packyears of smoking was computed. Clinical measures were obtained during a visit at the Rotterdam study center. Height and weight were measured and the body mass index was calculated (weight (kg)/height $(m)^2$). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses. After an overnight of fasting, blood samples were obtained at the research center. Serum total cholesterol was determined by an enzymatic procedure. High-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[17] Fasting glucose was determined enzymatically by the Hexokinase method. A history of myocardial infarction was based on self-report checked with records from general practitioner or cardiologist and/or on electrocardiographic evidence. Two research physicians independently coded events, according to the ICD-10 classification;[18] final decisions were made by a medical expert in the field who reviewed the coded events.

Statistical analysis

The distribution of the calcium score was highly skewed and therefore, log (total calcium score +1) was used for linear regression analysis. Age-adjusted regression

coefficients were computed using extracoronary measures as independent variables and log calcium score as dependent variable. Aortic calcification and carotid plaques were considered as ordinal variables (carotid plaques 0-6, aortic calcifications 0-4). In subsequent models, we additionally adjusted for cardiovascular risk factors and excluded subjects with a history of myocardial infarction. Age-adjusted standardized regression coefficients were computed to compare the strength of the associations.

We performed analyses of variance to compute age-adjusted geometric means of the calcium score for categories of extracoronary measures. For this endeavor, CCA IMT was categorized into quintiles (cut-off values were 0.78, 0.86, 0.93 and 1.03 for men and 0.73, 0.80, 0.87 and 0.96 for women) and carotid plaques according to the number of carotid plaques present (0, 1, 2, 3, and ≥4). Similarly, AAI was divided into 5 categories (>=1.20, 1.10-1.19, 1.00-1.09, 0.90-0.99, and <0.90). For all measures, the category reflecting the lowest amount of atherosclerosis was used as the reference category. A student t-test was performed to compare the geometric mean calcium score of each category with the reference category. We used linear regression analysis with continuous variables (IMT, AAI) or ordinal variables (carotid plaques 0-6, aortic calcifications 0-4) as test for trend. The number of subjects with measurements of CCA IMT, carotid plaques, AAI and aortic calcification were 1857, 1734, 1949, and 1751, respectively. All analyses were performed in men and women separately.

Results

Table 1 shows characteristics of the Rotterdam Coronary Calcification Study population. Men had a median calcium score of 312 (interquartile range 62-969) whereas women had a median calcium score of 56 (5-261). CCA IMT, carotid plaques and aortic calcification were positively and AAI was inversely associated with coronary calcification (table 2). Additional adjustment for cardiovascular risk factors (table 2) and exclusion of subjects with a history of myocardial infarction (data not shown) slightly attenuated the strength of the associations. Standardized regression coefficients (table 3) showed that associations with coronary calcification were stronger for carotid plaques and aortic calcification than for CCA IMT and AAI.

Geometric mean calcium scores for categories of the measures of extracoronary atherosclerosis for men and women are shown in figure 1 and 2 respectively. A graded increase in coronary calcification was seen across quintiles of CCA IMT. Further subdivision of the fifth quintile showed that the highest average calcium score was observed in the upper decile of CCA IMT (373 for men and 100 for women). A strong and graded increase in coronary calcification was seen with increasing number of carotid plaques present. A further increase in calcium score was found when only

5 or 6 carotid plaques were classified in the highest category (854 for men, 212 for women).

AAI was inversely associated with coronary calcification. While only a slight increase (men) or no increase (women) in coronary calcification was seen in the upper three categories of AAI, increased levels of coronary calcification were found in subjects with an AAI of 0.90-0.99 ($p<0.05$ for men and $p=0.26$ for women, compared to the reference category) and those with an AAI <0.90 ($p<0.001$ for men and $p<0.001$ for women). Further subdivision of the lowest category of AAI showed that subjects with an AAI <0.70 had the highest calcium score (407 in men, 128 in women).

Aortic calcification was strongly associated with coronary calcification. In men, there was an 11-fold increase in coronary calcification from the lowest category of aortic calcification (calcification absent) to the category of severe calcification. In women, this increase was 20-fold.

Table 1. Characteristics of the study population

Variable	Men (n=933)	Women (n=1080)
Age (years)	71.2±5.6	71.3±5.8
Body mass index (kg/m^2)	26.5±3.2	27.4±4.4
Systolic blood pressure (mm Hg)	144±21	142±21
Diastolic blood pressure (mm Hg)	77±11	75±11
Total cholesterol (mmol/l)	5.6±0.9	6.0±0.9
HDL-cholesterol (mmol/l)	1.2±0.3	1.5±0.4
Serum glucose (mmol/l)	6.1±1.7	5.8±1.3
Smokers (%)		
Current	18	15
Past	72	39
History of myocardial infarction (%)	18	6
Calcium score*	312 (62-969)	56 (5-261)
Log calcium score	5.3±2.1	3.7±2.3
Common carotid intima media thickness (mm)	0.90±0.17	0.84±0.13
Carotid plaque (%) †	78	59
Ankle-arm index	1.03±0.21	1.04±0.16
Aortic calcification (%) ‡	47	35

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.
* Value of the calcium score is expressed as median (interquartile range) because of its skewed distribution.
† Percentage of subjects with one or more carotid plaques.
‡ Percentage of subjects with aortic calcification over a length of at least 2.5 cm.

Table 2. Regression coefficients for men and women separately, describing the increase in log calcium score per unit increase of the extracoronary variables.

	Model 1		Model 2	
	beta coefficients (95% CI)	p-value	beta coefficients (95% CI)	p-value
Men				
CCA IMT	2.13 (1.31, 2.96)	<0.001	1.83 (0.75, 2.91)	0.001
Carotid plaques	0.47 (0.39, 0.54)	<0.001	0.45 (0.36, 0.54)	<0.001
AAI	-1.28 (-1.92, -0.65)	<0.001	-1.02 (-0.28 -1.77)	0.007
Aortic calcification	0.59 (0.49, 0.69)	<0.001	0.59 (0.48, 0.70)	<0.001
Women				
CCA IMT	2.16 (1.08, 3.24)	<0.001	1.77 (0.47, 3.06)	0.008
Carotid plaques	0.55 (0.45, 0.64)	<0.001	0.53 (0.42, 0.64)	<0.001
AAI	-1.63 (-2.41, -0.84)	<0.001	-1.04 (-0.06, -2.02)	0.04
Aortic calcification	0.74 (0.65, 0.84)	<0.001	0.70 (0.59, 0.81)	<0.001

Model 1 is adjusted for age, model 2 is adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL-cholesterol, serum glucose and number of packyears smoked. Regression coefficients describe the increase in log calcium score per unit increase (IMT: per mm, plaques: per plaque, AAI: per unit, aortic calcification: per unit (0-4)). CCA = common carotid artery, IMT = intima media thickness, AAI = ankle-arm index.

Table 3. Age-adjusted standardized regression coefficients for men and women separately, describing the increase in log calcium score per standardized unit increase of the extracoronary variables.

	age-adjusted standardized beta coefficients, p-value			
Variable	Men		Women	
CCA IMT	0.17	<0.001	0.12	<0.001
Carotid plaques	0.39	<0.001	0.35	<0.001
AAI	-0.13	<0.001	-0.12	<0.001
Aortic calcification	0.38	<0.001	0.46	<0.001

CCA = common carotid artery, IMT = intima media thickness, AAI = ankle-arm index.

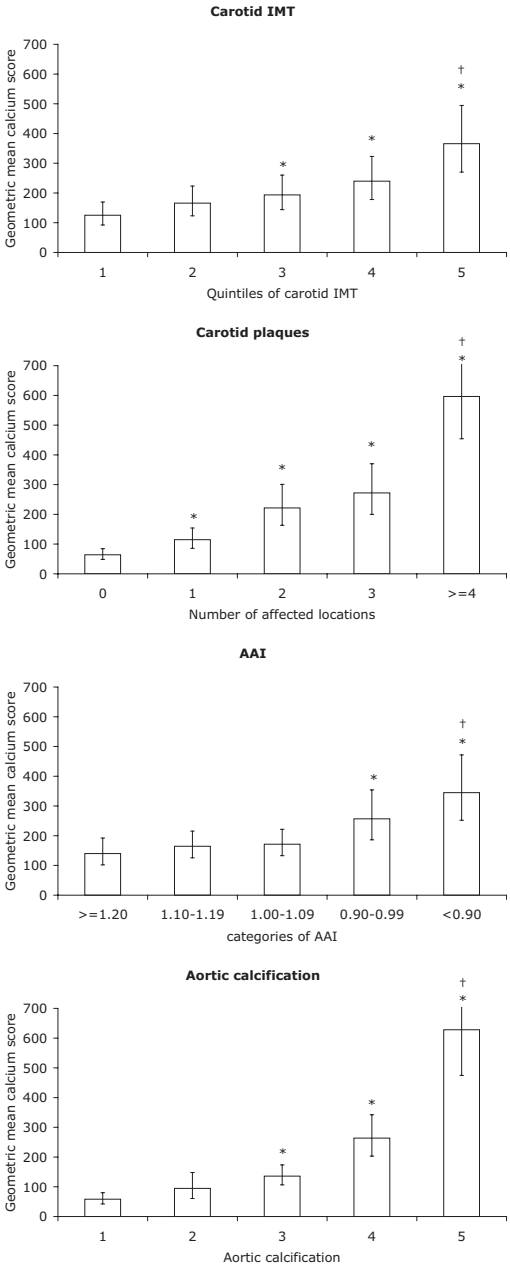

Figure 1. Age-adjusted geometric mean calcium scores for categories of carotid intima media thickness (IMT), carotid plaques, ankle-arm index (AAI) and aortic calcification, for men.
*Significant higher calcium score compared to the reference category ($p<0.05$)
†P for trend < 0.001

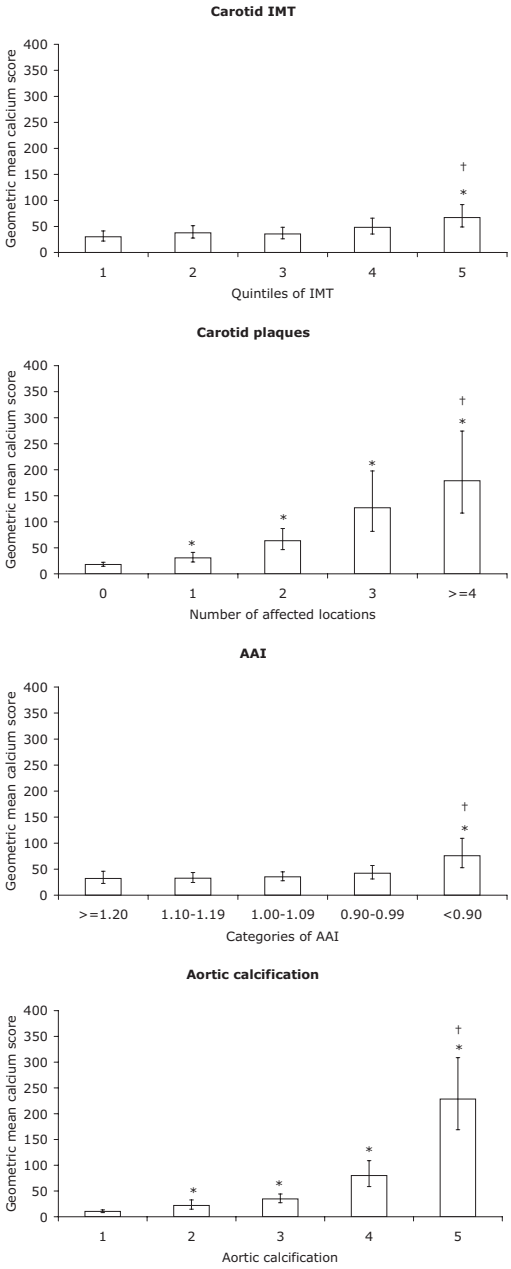

Figure 2. Age-adjusted geometric mean calcium scores for categories of carotid intima media thickness (IMT), carotid plaques, ankle-arm index (AAI) and aortic calcification, for women.
*Significant higher calcium score compared to the reference category ($p<0.05$)
†P for trend < 0.001

Discussion

Our population-based study shows clear associations between coronary calcification assessed by electron-beam CT and CCA IMT, carotid plaques, AAI, and aortic calcification. While graded associations with coronary calcification were found for carotid and aortic measures, a non-linear association was found for AAI. The associations were strongest for carotid plaques and aortic calcification. Associations were present in both men and women and were only slightly attenuated after correction for cardiovascular risk factors and exclusion of subjects with a history of myocardial infarction.

Study limitations

Electron-beam CT scans were obtained in 2063 subjects. Subjects undergoing an electron-beam CT scanning had approximately the same levels of risk factors and social class as the non-responders. There were slight differences between responders and non-responders in age (70.6 versus 72.4 years), gender (46% versus 38% male), body mass index (27.0 versus 26.7 kg/m^2) and ever smoking (90% versus 86% for men, 53% versus 49% for women). Measurements of extracoronary atherosclerosis and electron-beam CT scanning were not performed during the same session. However, since the median duration between the measurements was only 50 days, it is not likely that this has affected our results. All measurements of extracoronary atherosclerosis included missing values. Since these missing values were largely due to logistic reasons, we can assume them to be random.

Previous studies

Associations between coronary and extracoronary atherosclerosis have been known for decades. Necropsy studies in the 1960s already found a close association between atherosclerosis in the coronary arteries and atherosclerosis in the aorta,[19] the iliac artery and the carotid artery.[20] Studies in the living had to be awaited until non-invasive measures of atherosclerosis became available. So far, only a limited number of studies examined associations between measures of coronary and extracoronary atherosclerosis. In a population-based study among young subjects (33 to 42 years), an association was found between carotid IMT and coronary calcification.[8] Similarly, aortic and coronary calcium scores both assessed by electron-beam CT were found to be associated in postmenopausal women.[9] In hypercholesterolemic asymptomatic patients, the presence of ultrasonographically detected plaques in the femoral artery and the aorta were found to be associated with coronary calcification assessed by electron-beam CT.[10] In the latter study, the association between carotid plaques and

coronary calcification did not reach statistical significance. In men with at least one cardiovascular risk factor, ultrasound was used to detect plaques in the carotid and femoral artery and the aorta. The number of affected extracoronary sites was found to be associated with coronary calcification assessed by electron-beam CT.[11] The major limitations of the previous studies are the measurement of atherosclerosis only at one extracoronary site[8,9] and the use of a selected population.[10,11] Furthermore, only one study included both men and women.[8]

Coronary calcification and measures of carotid atherosclerosis

It is still a matter of debate whether increased carotid IMT indicates atherosclerosis or merely reflects an adaptive response of the vessel wall to changes in shear and tensile stress.[21] The present study showed a graded association between coronary calcification and CCA IMT, which supports the existing evidence that carotid IMT may be regarded as a continuous measure of generalized atherosclerosis. We made a simple quantification of carotid plaques by counting the number of affected locations. The plaque score, however, showed a strong and graded association with coronary calcification.

Coronary calcification and AAI

Our results showed the association between AAI and coronary calcification to be non-linear. Levels of AAI above 1.00 were only weakly associated with the calcium score. This may suggest that in the higher range, AAI may not reflect the severity of atherosclerosis and is consistent with the view that an AAI >1.00 rules out significant peripheral arterial narrowing. If so, this implies that the AAI should not be considered as a continuous measure of generalized atherosclerosis. On the other hand, the results show that not only subjects with an index below 0.90, which is generally used as a cut-off value for the presence of peripheral arterial disease, but also subjects with an index between 0.90 and 0.99 have increased levels of coronary calcification compared to subjects with higher values.

Coronary and aortic calcification

In the present study, strong and graded associations were shown for coronary and aortic calcification. The 10-fold increase in calcium score in men and the 20-fold increase in calcium score in women implies that aortic calcification can be seen as a continuous measure of generalized atherosclerosis.

Measurement techniques

By using different measurement techniques we measured different stages of atherosclerosis. Intima media thickening is considered to reflect a less advanced stage

of atherosclerosis than the presence of plaques.[22] In addition, AAI is considered to be a marker of atherosclerosis that is not only influenced by the presence of plaques but also by hemodynamic factors and vascular stiffness. Furthermore, calcified plaques are generally thought to reflect a more advanced stage of atherosclerosis than non-calcified plaques.[23] The use of coronary calcification as a measure of coronary atherosclerosis and the use of aortic calcification as a measure of aortic atherosclerosis may have favored the association between those measures in our study. Due to the measurement of different stages of atherosclerosis, no definite conclusions can be drawn concerning the strength of associations between coronary atherosclerosis and atherosclerosis in the carotid artery, peripheral arteries and aorta.

Calcium and plaques

Despite the observation that calcium is frequently present in complicated plaques, histopathologic studies are not conclusive on the role of coronary calcium; calcium is a marker for neither unstable nor stable plaques.[24] However, the close association of coronary calcium with the total amount of coronary atherosclerotic plaques[6,7] offers the opportunity to categorize subjects with respect to the extent of atherosclerosis.

Conclusions

Our population-based study shows that graded associations are present between coronary calcification as measured by electron-beam CT and CCA IMT, carotid plaques and aortic calcification. A non-linear association is present between coronary calcification and AAI. This large population-based study supports the concept of generalized atherosclerosis for a variety of vessels of the vascular tree.

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Chapter 3.2
Rose questionnaire angina pectoris and coronary calcification

Abstract

Purpose. The Rose questionnaire is a standardized method of measuring angina pectoris in general populations. Electron-beam computed tomography (CT) is a non-invasive technique to quantify the amount of coronary calcification. In a population-based study, we investigated the association between Rose questionnaire angina pectoris and coronary calcification.

Methods. The Rotterdam Coronary Calcification Study is embedded in the Rotterdam Study, a population-based study in subjects >=55 years. Participants of the Rotterdam Coronary Calcification Study underwent an electron-beam CT scan between 1997 and 2000. Coronary calcification was quantified in a calcium score according to Agatston's method. Rose questionnaire angina pectoris was assessed during a home interview. The first 2013 participants were included in the present analyses.

Results. In men, the presence of Rose questionnaire angina pectoris was associated with a 12.9-fold (95% confidence interval: 3.8-43.7) increased risk of a calcium score >1000 (reference: calcium score 0-100). The corresponding relative risk in women was 4.8 (2.0-11.3). Similar results were found when we computed sex-specific quartiles of the calcium score.

Conclusions. Rose questionnaire angina pectoris is strongly associated with the amount of coronary calcification. Rose questionnaire angina pectoris corresponds better with the amount of coronary calcification in men than in women.

Introduction

Chest pain is a complaint which is frequently caused by ischemia of the heart. In order to study angina pectoris in a general population, several questionnaires have been developed to discriminate between cardiac and non-cardiac causes of chest pain. The Rose questionnaire has been introduced in 1962 as a standardized method of measuring angina pectoris in general populations.[1] Several studies have shown that the diagnosis of angina pectoris based on the Rose questionnaire predicts coronary heart disease morbidity and mortality.[2-6] Although subjects with Rose questionnaire angina pectoris have an increased risk of coronary heart disease morbidity and mortality, to our knowledge only one population-based study has investigated to what extent subjects with Rose questionnaire angina pectoris indeed have more extensive coronary atherosclerosis.[7] The use of coronary angiography to assess coronary atherosclerosis in the latter study is a major disadvantage since only subjects suspected for coronary heart disease underwent an angiography.

Electron-beam computed tomography (CT) is a non-invasive technique to adequately quantify the amount of coronary calcification. Since coronary calcification assessed by electron-beam CT is strongly correlated with the amount of coronary atherosclerotic plaque burden[8,9] the amount of coronary calcification can be used as a measure of coronary atherosclerosis. Therefore, in a large population-based study we investigated whether and to what extent subjects with Rose questionnaire angina pectoris have more coronary calcification than subjects without Rose questionnaire angina pectoris.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by electron-beam CT. The study is embedded in the Rotterdam Study. The Rotterdam Study is a population-based study, which started with a baseline visit between 1990 and 1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited (response 78%). The rationale and design of the Rotterdam Study have been described elsewhere.[10] Follow-up visits took place in 1993 to 1994 and 1997 to 1999. From 1999 onwards the study population is extended with a second cohort comprising inhabitants who reached the age of 55 years after the baseline examination in 1990 to 1993 and subjects aged 55 years and over who migrated into the research area. Baseline and follow-up visit examinations included assessment of angina pectoris by the Rose questionnaire.

Measurement protocols for the first and second cohort were identical.

From 1997 onwards, participants through 85 years of age completing the third phase of the first cohort or the baseline examination of the second cohort of the Rotterdam Study were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an electron-beam CT scan. We restricted the present analyses to participants recruited from the first cohort, who were scanned from 1997 to 2000. Of the 3371 eligibles, scans were obtained for 2063 subjects (response: 61%). Due to several causes, i.e. metal clips from cardiac surgery, severe artifacts and registration errors (electrocardiogram, acquisition), image acquisition data could not be reconstructed or analyzed in 50 subjects. Thus, scores were available for 2013 participants. The median duration between assessment of angina pectoris by the Rose questionnaire and electron-beam CT scanning was 50 days. The Medical Ethics Committee of Erasmus MC approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcification in the epicardial coronary arteries detected on electron-beam CT scans. Imaging was performed with a C-150 Imatron scanner (Imatron, South San Francisco, California, U.S.A.). Before the subjects were scanned, they exercised breath holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. The scanner was calibrated on a daily basis using a water phantom. Quantification of coronary calcification was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, California, U.S.A.) displaying all pixels with a density of over 130 Hounsfield Units. Trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area =0.52 mm^2) with a density over 130 Hounsfield Units. We placed a region of interest around each high-density lesion in the epicardial coronary arteries. The peak density in Hounsfield Units and the area in mm^2 of the individual coronary calcifications were calculated. A calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the individual area, as proposed by Agatston et al.[11] We added the scores for individual calcifications, resulting in a calcium score for the entire epicardial coronary system. Participants were not informed about their calcium score, in accordance with the research protocol, agreed by the Medical Ethics Committee.

Rose questionnaire

Questions concerning chest pain were based on the World Health Organization (WHO) angina pectoris questionnaire.[1] The presence or absence of angina pectoris

was assessed during a home interview. Angina pectoris was defined according to standard criteria as chest pain or discomfort with the following characteristics: (1) the site must include either the sternum (any level) or the left anterior chest and the left arm, (2) it must be provoked by either hurrying or walking uphill or walking on the level, (3) when it occurs on walking it must make the subject either stop or slacken pace, unless nitrates are taken, (4) it must disappear within 10 minutes from the time the subject stands still. If one of the criteria was not met the subject was classified as absence of angina pectoris.

Cardiovascular risk factors

Information on smoking and medication was obtained during the home interview of the Rotterdam Study and the number of packyears of smoking was computed. Clinical measures were obtained during a visit at the Rotterdam study center. Height and weight were measured and the body mass index was calculated (weight (kg)/height $(m)^2$). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses. After an overnight of fasting, blood samples were obtained at the research center. Serum total cholesterol was determined by an enzymatic procedure. High-density lipoprotein (HDL) was measured similarly after precipitation of the non-HDL fraction.[12] Fasting glucose was determined enzymatically by the Hexokinase method. Diabetes was defined as the use of anti-diabetic medication and/or a fasting glucose level >=7.0.

Statistical analyses

Geometric mean calcium scores adjusted for age and cardiovascular risk factors were computed for subjects with and without Rose questionnaire angina pectoris. We divided calcium scores into 4 categories (0-100, 101-500, 501-1000 and >1000) and computed the percentage of subjects in each category for subjects with and without Rose questionnaire angina pectoris. Multinomial regression adjusted for age (model 1) was performed to compute the association between Rose questionnaire angina pectoris and a calcium score of 101-500, 501-1000 and >1000 (reference: calcium score 0-100). In a subsequent model (model 2) we additionally adjusted for cardiovascular risk factors.

In order to adjust for the large sex-difference in calcium score, we additionally divided calcium scores into sex-specific quartiles (cut-off values were 63, 312 and 969 for men and 5, 55 and 261 for women). Multinomial regression adjusted for age (model 1) and additionally cardiovascular risk factors (model 2) was performed using the first (=lowest) quartile of the calcium score as the reference. All analyses were performed in men and women separately.

Additionally, multivariate adjusted analyses were performed after exclusion of subjects with a history of myocardial infarction. Since the use of beta-blockers and nitrates may reduce the occurrence of chest pain, which leads to misclassification of the diagnosis Rose questionnaire angina pectoris, we repeated multivariate adjusted analyses after exclusion of beta-blocker and nitrate users.

In an additional analysis, we dichotomised the calcium score (0-100, >=101) and performed multivariate adjusted logistic regression using the categories of calcium score as dependent and Rose questionnaire angina pectoris as independent variable.

Results

Table 1 shows the characteristics of the participants. Rose questionnaire angina pectoris was present in 6.0% of men and in 5.5% of women. Subjects with Rose questionnaire angina pectoris were approximately 2 years older than subjects without angina pectoris (73.8 vs 71.1 years for men and 73.5 vs 71.1 years for women). Figure 1 shows geometric mean calcium scores adjusted for age and cardiovascular risk factors for subjects with and without Rose questionnaire angina pectoris. Geometric mean

Table 1. Characteristics of the study population at the time of electron-beam CT scanning

Variable	Men (n=933)	Women (n=1080)
Age (years)	71.2±5.6	71.3±5.8
Body mass index (kg/m^2)	26.5±3.2	27.4±4.4
Systolic blood pressure (mm Hg)	144±21	142±21
Diastolic blood pressure (mm Hg)	77±11	75±11
Total cholesterol (mmol/l)	5.6±0.9	6.0±0.9
HDL cholesterol (mmol/l)	1.2±0.3	1.5±0.4
Diabetes Mellitus (%)	14	12
Smokers (%) Current Past	1872	1539
History of myocardial infarction (%)	18	6
History of CABG (%)	8	1
History of PTCA (%)	4	1
Calcium score*	312 (62-969)	56 (5-261)
Log calcium score	5.3±2.1	3.7±2.3

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.
* Value of the calcium score is expressed as median (interquartile range) because of its skewed distribution.
PTCA = percutaneous transluminal coronary angioplasty, CABG = coronary artery bypass grafting.

calcium scores were 3-4 times higher in subjects with Rose questionnaire angina pectoris than in subjects without Rose questionnaire angina pectoris (592 vs 174 for men, 126 vs 38 for women). Furthermore, calcium scores in men without Rose questionnaire angina pectoris were higher than calcium scores in women with Rose questionnaire angina pectoris (174 vs 126).

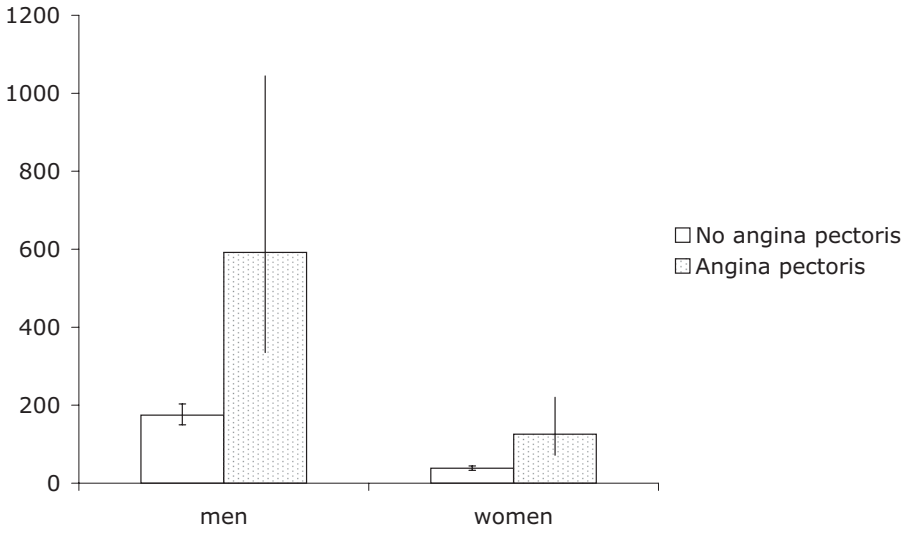

Figure 1. Age and cardiovascular risk factor adjusted geometric mean calcium scores in men and women with and without Rose questionnaire angina pectoris.

Among men with Rose questionnaire angina pectoris, 5.4% had a calcium score of 0-100 and 57.1% had a calcium score >1000 (figure 2). Corresponding percentages for women with Rose questionnaire angina pectoris were 39.0% and 22.0% (figure 3). Table 2 shows the association of presence versus absence of Rose questionnaire angina pectoris on a calcium score of 101-500, 501-1000, >1000 (reference: calcium score 0-100). In men, the presence of Rose questionnaire angina pectoris was associated with a 12.9-fold increased risk of a high calcium score (calcium score >1000). The corresponding relative risk in women was 4.8.

In order to investigate whether the large sex-difference is due to higher absolute calcium scores in men, we computed sex-specific quartiles of the calcium score. In men, the presence of Rose questionnaire angina pectoris was associated with a 14-fold increased risk of a calcium score in the highest quartile (reference: calcium score in

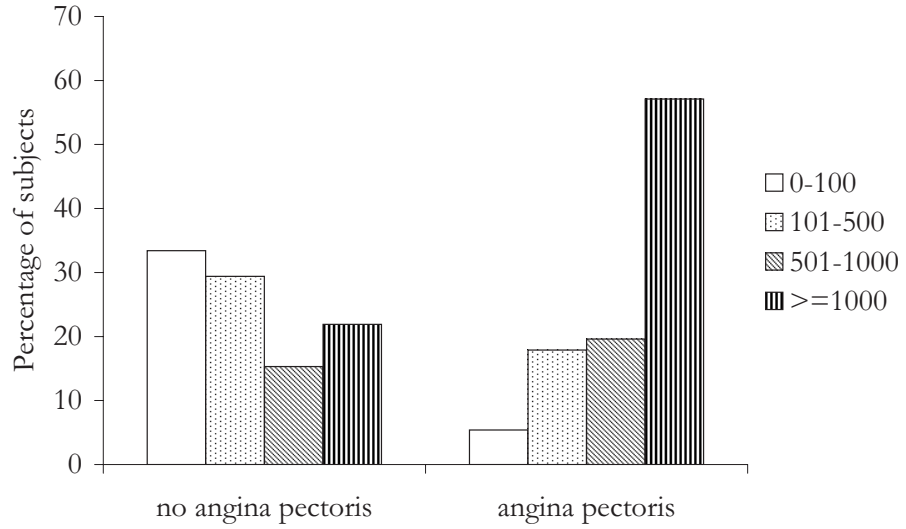

Figure 2. Distribution of calcium score in men with and without Rose questionnaire angina pectoris.

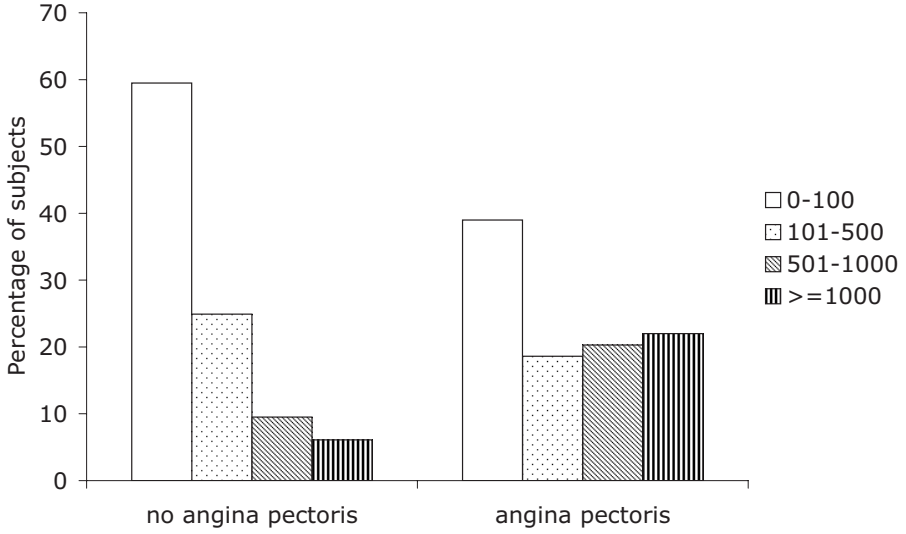

Figure 3. Distribution of calcium score in women with and without Rose questionnaire angina pectoris.

Table 2. Multinomial regression showing the effect of presence versus absence of angina pectoris on a calcium score of 101-500, 501-1000 and >1000 (reference category: calcium score <=100).

				Model 1	Model 2
		N	AP	Odds ratio (95% CI)	Odds ratio (95% CI)
Men	<=100	296	3	1.0 (reference)	1.0 (reference)
	101-500	268	10	3.3 (0.9-12.2)	3.0 (0.8-11.5)
	501-1000	145	11	6.8 (1.9-25.0)	5.8 (1.5-22.0)
	>1000	224	32	13.8 (4.1-45.9)	12.9 (3.8-43.7)
Women	<=100	631	23	1.0 (reference)	1.0 (reference)
	101-500	265	11	1.0 (0.5-2.1)	1.2 (0.5-2.5)
	501-1000	109	12	2.8 (1.3-5.9)	4.2 (1.9-9.3)
	>1000	75	13	4.2 (2.0-9.2)	4.8 (2.0-11.3)

Model 1: adjusted for age.
Model 2: adjusted for age, body mass index, systolic blood pressure, total cholesterol, HDL-cholesterol, diabetes and smoking.
N = number of subjects, AP = Rose questionnaire angina pectoris
CI = confidence interval.

Table 3. Multinomial regression showing the effect of presence versus absence of angina pectoris on a calcium score in the second, third and fourth quartile (reference category: first quartile of calcium score).

				Model 1	Model 2
		N	AP	Odds ratio (95% CI)	Odds ratio (95% CI)
Men	First quartile	233	2	1.0 (reference)	1.0 (reference)
	Second quartile	233	5	2.2 (0.4-11.7)	1.7 (0.3-9.6)
	Third quartile	234	17	7.6 (1.7-33.7)	7.4 (1.6-33.0)
	Fourth quartile	233	32	15.3 (3.6-65.1)	14.1 (3.2-60.8)
Women	First quartile	270	8	1.0 (reference)	1.0 (reference)
	Second quartile	270	12	1.3 (0.5-3.2)	1.4 (0.5-3.9)
	Third quartile	269	10	1.0 (0.4-2.6)	1.1 (0.4-3.0)
	Fourth quartile	271	29	2.9 (1.3-6.6)	3.6 (2.0-11.1)

Model 1: adjusted for age.
Model 2: adjusted for age, body mass index, systolic blood pressure, total cholesterol, HDL-cholesterol, diabetes and smoking.
N = number of subjects, AP = Rose questionnaire angina pectoris
CI = confidence interval.

lowest quartile) (table 3). In women, the relative risk was 3.6 (table 3).

After exclusion of subjects with a history of myocardial infarction the strength of the associations slightly decreased in men while in women the strength of the association remained unaltered (table 4). Exclusion of beta-blocker users and nitrate users the strength of the associations led to an attenuation of the strength of the associations, in particular in men (table 4).

Multivariate adjusted odds ratios for a calcium score >100 were 6.9 (2.1-22.6) and 2.3 (1.2-4.1) for men and women, respectively. After exclusion of subjects with a history of myocardial infarction, odds ratios for a calcium score >100 were 4.8 (1.4-16.7) and 2.5 (1.3-4.6) for men and women, respectively.

Table 4. Multivariate adjusted multinomial regression showing the effect of presence versus absence of angina pectoris on a calcium score of 101-500, 501-1000 and >1000 (reference category: calcium score <=100) after exclusion of subjects with a myocardial infarction and additional exclusion of subjects with anti-anginal medication.

		Subjects without history of MI		Subjects without history of MI and without anti-anginal medication	
		N/AP	Odds ratio (95% CI)	N/AP	Odds ratio (95% CI)
Men	<=100	277/3	1.0 (reference)	255/2	1.0 (reference)
	101-500	235/8	2.7 (0.7-10.8)	210/7	3.9 (0.8-19.8)
	501-1000	112/8	5.1 (1.2-20.9)	93/3	2.4 (0.4-17.8)
	>1000	142/13	8.9 (2.4-33.1)	107/3	3.2 (0.5-20.5)
Women	<=100	606/21	1.0 (reference)	531/13	1.0 (reference)
	101-500	251/11	1.4 (0.6-3.0)	198/6	1.4 (0.5-4.1)
	501-1000	97/10	4.4 (1.9-10.5)	76/3	2.3 (0.6-9.0)
	>1000	62/12	5.6 (2.3-14.1)	46/6	5.0 (1.3-18.7)

All analyses are adjusted for age, body mass index, systolic blood pressure, total cholesterol, HDL-cholesterol, diabetes and smoking.
N = number of subjects, AP = Rose questionnaire angina pectoris
CI = confidence interval.

Discussion

The present study shows that Rose questionnaire angina pectoris is strongly associated with the calcium score. This association is stronger in men than in women: men with Rose questionnaire angina pectoris had a relative risk of 12.9 for having a high calcium score while women had a relative risk of 4.8. Furthermore, only 6% of the men but almost 40% of the women with Rose questionnaire angina pectoris had a calcium score <100.

Electron-beam CT scans were obtained in 2063 subjects. Subjects undergoing electron-beam CT scanning had approximately the same levels of risk factors and social class as the non-responders. There were slight differences between responders and non-responders in age (70.6 versus 72.4 years, $p<0.001$), gender (46% versus 38% male, $p<0.001$), body mass index (27.0 versus 26.7 kg/m^2, $p=0.01$) and ever smoking (90% versus 86% for men, $p=0.01$; 53% versus 49% for women, $p=0.04$). Assessment of angina pectoris by the Rose questionnaire was performed before electron-beam CT scanning and participants of the study were unaware of their calcium scores. Therefore, differential misclassification of the Rose questionnaire angina pectoris on the basis of the calcium score was absent.

Population-based studies have shown that subjects with Rose questionnaire angina pectoris have an increased risk of incident coronary heart disease.[2-6] However, only one population-based study investigated whether subjects with Rose questionnaire angina pectoris have more extensive coronary atherosclerosis than subjects without Rose questionnaire angina pectoris. In working, presumably healthy, middle aged men, Rose questionnaire angina pectoris was moderately associated with a significant obstruction on coronary angiography.[7] In the latter study, coronary angiography was only performed in subjects with angina pectoris. Therefore, no conclusions can be drawn on the coronary atherosclerotic burden in subjects without angina pectoris. The development of the electron-beam CT scan offers the opportunity to non-invasively study the association of Rose questionnaire angina pectoris and coronary atherosclerosis in a general population. In the present study, angina pectoris assessed by the Rose questionnaire was strongly associated with the amount of coronary calcification: men with Rose questionnaire angina pectoris had a relative risk of 12.9 of having a high calcium score, while women had a relative risk of 4.8.

The present study shows that men with Rose questionnaire angina pectoris have calcium scores that are much higher than women with Rose questionnaire angina pectoris. Calcium scores in men with Rose questionnaire angina pectoris were 5 times higher than in women (592 versus 126). While only 6% of the men with Rose questionnaire angina pectoris had a calcium score <=100, almost 40% of the women had a calcium score <=100. Furthermore, the association of Rose questionnaire angina pectoris and coronary calcification is stronger in men than in women. The stronger association of Rose questionnaire angina pectoris and coronary calcification in men remained after dividing calcium scores into quartiles: men with Rose questionnaire angina pectoris had a relative risk of 14.1 for a calcium score in the highest quartile. Women with Rose questionnaire angina pectoris only had a relative risk of 3.6 for a calcium score in the highest quartile. The results of the present study suggest that the Rose questionnaire misclassifies more often women than men with chest pain. This hypothesis is supported by previous studies: reports have shown that chest pain is

more common in women than men and that the prevalence is higher than the prevalence of other coronary heart disease events would suggest.[13-15] Studies showing a better prognosis of women with chest pain than men with chest pain further support this hypothesis.[16-18]

In conclusion, Rose questionnaire angina pectoris is strongly associated with the amount of coronary calcification. Rose questionnaire angina pectoris better reflects the amount of coronary calcification in men than in women.

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Chapter 4

Predictive value of coronary calcification

Chapter 4.1

Coronary calcification and risk of coronary heart disease

Abstract

Background. Whether coronary calcification detected by electron beam tomography (EBT) improves cardiovascular risk prediction is unresolved. The technique is particularly promising in elderly because the predictive power of cardiovascular risk factors weakens with age. We investigated the prognostic value of coronary calcification for cardiovascular events, and mortality in a general population of older adults.

Methods and Results. From 1997 to 2000, EBT scanning for assessment of coronary calcification was performed in subjects of the population-based Rotterdam Study. Risk factors were measured by standardized procedures. Coronary calcium scores were available for 2013 participants (range, 62 to 85 years). During a mean follow-up period of 3.3 years, 116 cardiovascular events occurred, including 73 coronary events. The risk of coronary events gradually increased with increasing calcium score. Subjects with a calcium score above 1000 had an eight times increased risk of coronary heart disease compared to those with a calcium score of 0 to 100 (age- and sex adjusted relative risk 8.0 (95% confidence interval 3.6-18.2)). The corresponding relative risk in asymptomatic subjects was 8.2 (3.3-20.4). The relative risks only slightly changed after adjustment for cardiovascular risk factors (7.1, 3.0-16.7 and 8.7, 3.4-22.1, respectively). Cardiovascular risk prediction improved when the calcium score was added to risk factors.

Conclusions. Coronary calcification is a strong and independent predictor of coronary heart disease in older adults. Risk stratification by assessment of coronary calcification can have an important role in guiding decisions on drug therapy or life style changes for the primary prevention of coronary heart disease in elderly.

Introduction

Coronary calcification, assessed by electron beam tomography (EBT) may be a useful tool for the identification of subjects at high risk of coronary heart disease. Several studies have shown that the amount of coronary calcification is associated with the risk of coronary heart disease.[1-6] However, whether the measurement of coronary calcification is additive in conventional risk prediction, based on risk factor assessment, is unresolved. Most published studies have used unmeasured, self-reported risk factors, which is an underestimation of the true prevalence of risk factors.[1-5] Since these data did not allow computation of the Framingham risk score, the widely used office-based assessment, no conclusion can be drawn on the incremental value of coronary calcification over the Framingham risk score. The only study with measured risk factors, carried out in a high-risk population, did not find an additive effect on risk prediction.[6]

Furthermore, published studies covered wide age ranges, and therefore, information in specific groups like older adults, is lacking. The predictive power of cardiovascular risk factors decreases with age, partly due to selective survival and the influence of co-morbidity on risk factor levels.[7-9] Since calcification of the coronary arteries can be seen as a cumulative measure of life-time exposure to cardiovascular risk factors, coronary calcification may be especially important for the assessment of cardiovascular risk in older adults.

The Rotterdam Coronary Calcification Study is a prospective population-based study with standardized measurement of cardiovascular risk factors among 2013 older adults. We studied the predictive value of coronary calcification for coronary heart disease, cardiovascular disease, and total mortality.

Methods

Study population

The Rotterdam Coronary Calcification Study was designed to study determinants and consequences of coronary calcification, detected by EBT. The study was embedded in the Rotterdam Study, a prospective, population-based study among subjects aged 55 years and older, which started in 1990. The rationale and design of the Rotterdam Study have been described elsewhere.[10] The third research round took place from April 1997 to December 1999. From December 1997 onward, participants through 85 years of age were invited to participate in the Rotterdam Coronary Calcification Study and to undergo an EBT scan. Subjects in nursing homes did not visit the research center and thus were not invited for the study. Of the 3370 eligibles, scans

were obtained for 2063 subjects (61%). Due to several causes, e.g. metal clips from cardiac surgery, severe artifacts, and registration errors (electrocardiography, acquisition), image acquisition data could not be reconstructed or analysed in 50 subjects, and therefore data were available for 2013 participants. All other information was obtained from the examinations of the Rotterdam Study.

Cardiovascular risk factors

Information on smoking was obtained during the home interview of the Rotterdam Study. We categorised subjects as current, past or never smokers. Anthropometric measures were obtained during the visit at the research center. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used. Hypertension was defined as a systolic blood pressure of at least 160 mm Hg and/or a diastolic blood pressure of at least 100 mm Hg and/or use of blood pressure lowering medication for the indication hypertension. After an overnight of fasting, blood samples were obtained at the research center. Serum total cholesterol was determined by an automated enzymatic procedure using Roche CHOD-PAP reagent agent and high-density lipoproteins (HDL) were with the Roche HDL-cholesterol assay using PEG-modified enzymes and dextran sulfate. We defined hypercholesterolemia as a total cholesterol level of at least 6.5 mmol/l and/or use of cholesterol lowering medication. Glucose was determined enzymatically by the Hexokinase method. Diabetes mellitus was considered present with current use of antidiabetic medication and/or when fasting glucose levels exceeded 7.0 mmol/l. The median duration between risk factor assessment and EBT scanning was 50 days. The Medical Ethics Committee of Erasmus University Rotterdam approved the study, and all participants gave informed consent.

Coronary calcification

We assessed coronary calcifications in the epicardial coronary arteries detected on EBT scans. Imaging was performed with a C-150 Imatron scanner (GE-Imatron). Before the subjects were scanned, they exercised adequate breath-holding. From the level of the root of the aorta through the heart, 38 images were obtained with 100 ms scan time and 3 mm slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of coronary calcifications was performed with AccuImage software (AccuImage Diagnostics Corporation) displaying all pixels with a density of over 130 Hounsfield units. A calcification was defined as a minimum of two adjacent pixels (area = 0.52 mm^2) with a density over 130 Hounsfield Units. Calcium scores were calculated according to Agatston's method.[11] The trained scan readers were blinded to the clinical

data of the participants. To conform with the protocol outlines as approved by the Medical Ethics Committee, participants were not informed about the calcium score.

Clinical outcomes

Two participants were lost to follow-up, in which case the last date of contact was used as census date. Information concerning the vital status of the participants was obtained from the municipal health service of Rotterdam. Subjects in the Rotterdam Study were continuously monitored for the occurrence of cardiovascular events through automated linkage with the files from general practitioners in the research area of the Rotterdam Study (85% of the cohort). For 15% of the cohort of which the general practitioners had practices outside the research area, information was obtained through checking the participant's file and by interviewing the general practitioner regularly. When myocardial infarction, coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), stroke or death was reported, the research assistants collected additional information from medical records of the general practitioner and in addition, obtained information from hospital discharge records or nursing home records, including letters from medical specialists. Two research physicians independently coded the possible cardiovascular events, according to the International Classification of Diseases, 10th version.[12] In case the research physicians disagreed on the diagnosis of a coronary event or stroke, a cardiologist or neurologist, respectively, reviewed the coded event and performed the definitive coding. The research physicians, cardiologist and neurologist were not aware of the calcium score outcome. In the analyses, we used the following outcome measures: coronary heart disease (incident myocardial infarction, CABG, PTCA and coronary heart disease mortality), cardiovascular disease (incident myocardial infarction, CABG, PTCA, stroke and cardiovascular mortality), coronary heart disease mortality, cardiovascular mortality, and total mortality. A history of coronary heart disease included a history of myocardial infarction, CABG or PTCA, while a history of cardiovascular disease included the aforementioned and a history of stroke.

Statistical analysis

The calcium score was divided into four categories: 0 to 100, 101 to 500, 501 to 1000, and above 1000. Age- and sex-adjusted Cox regression analysis was conducted to compute event-free survival curves for the calcium score categories. Hazard ratios of events in increasing calcium score categories were computed as estimates of relative risk. Subjects with a calcium score of 0 to 100 were used as the reference group. We also computed relative risks in a multivariate model, containing the following cardiovascular risk factors in addition to age and sex: body mass index, hypertension, total cholesterol, HDL-cholesterol, smoking, diabetes mellitus, history of coronary heart

disease (for outcomes coronary heart disease and coronary heart disease mortality) or cardiovascular disease (for the other outcomes), and family history of myocardial infarction. Cox analyses were repeated in asymptomatic subjects. Subjects with a history of coronary heart disease were excluded in the analyses with coronary heart disease or coronary heart disease mortality as outcome, while subjects with a history of cardiovascular disease were excluded for the other outcomes. Aforementioned Cox models were also applied in analyses restricted to asymptomatic subjects over 70 years of age, for the outcomes coronary heart disease, cardiovascular disease, and total mortality (due to limited numbers of events, this analysis was not applied for the outcomes coronary heart disease mortality and cardiovascular mortality).

In the asymptomatic population, two additional analyses were conducted. First, the Framingham risk model as derived by Wilson et al.[13] was applied to calculate 10-year risk probabilities. Cox regression analysis adjusted for age and sex was conducted in categories based on the calcium score and the Framingham risk function (below or above 75th percentile). For this analysis, the third and the fourth calcium score category were combined to increase statistical power. Secondly, we computed probabilities of coronary heart disease and cardiovascular disease for each subject as predicted by the multivariate Cox regression model containing only age, sex and cardiovascular risk factors, and by the multivariate model that also included the calcium score. Probabilities of coronary heart disease and cardiovascular disease were also computed as predicted by age, sex and the calcium score alone. We applied the probability values as thresholds to categorize the results as positive or negative. True- and false-positive rates were determined for each threshold, and used to construct receiver operating characteristic (ROC) curves. Differences in the predicted values were estimated by comparing the areas under the ROC curve, taking correlation between the areas into account.[14]

All measures of association are presented with 95% confidence intervals (CI). SPSS 11.0 for Windows (SPSS, Inc., Chicago, Illinois) was used for data analysis. Of the population, 8% missed information on one cardiovascular risk factor, while 3% missed information on two or more risk factors. Before multivariate Cox regression analyses were performed, missing risk factor values were imputed using the multivariate imputation by chained equations (MICE) approach in S-Plus 2000 (MathSoft, Inc., Cambridge, Massachussetts).

Results

Baseline characteristics of the study population are shown in table 1. The distribution of the calcium score was highly skewed, with a median of 134 and a range of

0 to 12611. The mean follow-up duration was 3.3 years (standard deviation, 0.8 years; maximum, 4.9 years). Of the 2013 participants, 140 had died during the follow-up period. Hundred and sixteen subjects suffered a cardiovascular event, including 52 non-fatal myocardial infarctions and coronary heart disease deaths, 21 revascularizations, 43 strokes, and 50 cardiovascular deaths. The rates of events in the calcium score categories are shown in figure 1.

Figure 2 shows the association between the calcium score categories at the start of follow-up and survival free of new cardiovascular events, adjusted for differences in age and sex. The event-free survival decreased with increase of the calcium score, with a cumulative incidence at four years of 3 percent for a calcium score up to 100, and 12 percent for a calcium score above 1000.

Table 2 shows the relative risks of events for categories of coronary calcification. There was an increasing risk of events with increasing calcium score (test for trend, $p<0.01$ for all outcomes). Compared to a calcium score of 0 to 100, relative risks of events in subjects with a calcium score above 1000 ranged from 2.6 for total mortality and 3.6 for cardiovascular disease to 8.0 for coronary heart disease. Additional adjustment for cardiovascular risk factors resulted in generally unchanged risk estimates for all outcomes (2.7, 3.5, and 7.1, respectively). Eleven percent of the population had a history of coronary heart disease, and 13% had a history of cardiovascular disease. The strength of the associations between cardiovascular risk factors

Table 1. Baseline characteristics of the study population (n=2013)

Variable	Mean or percentage*
Age, y	71.3±5.7
Male	46.3
Body mass index, kg/m^2	27.0±3.9
Hypertension	35.2
Total cholesterol, mmol/L	5.8±1.0
HDL-cholesterol, mmol/L	1.4±0.4
Smokers Current	16.2
Past	54.2
Diabetes mellitus	13.2
History of myocardial infarction	11.4
History of stroke	2.5
History of CABG/PTCA	6.0
Family history of myocardial infarction	19.6
Calcium score†	134 (13-578)

* Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean (standard deviation).
† Median (interquartile range) reported because of skewed distribution of the calcium score.

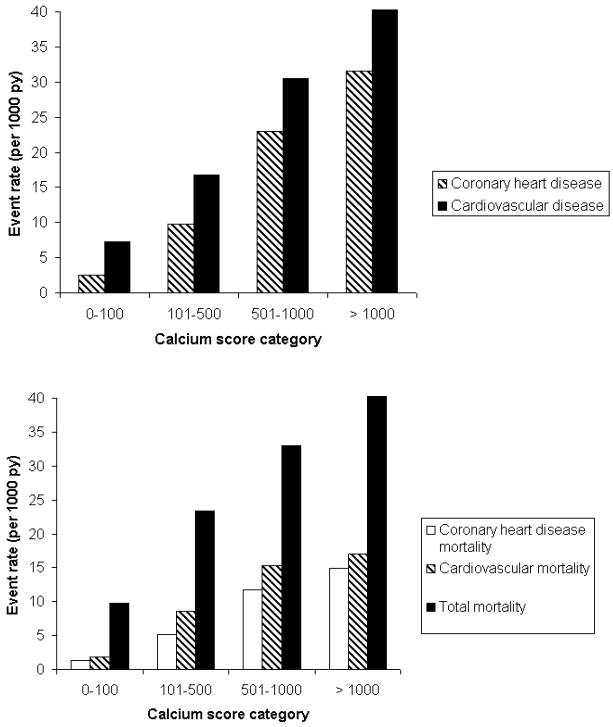

Figure 1. Event rates according to calcium score category

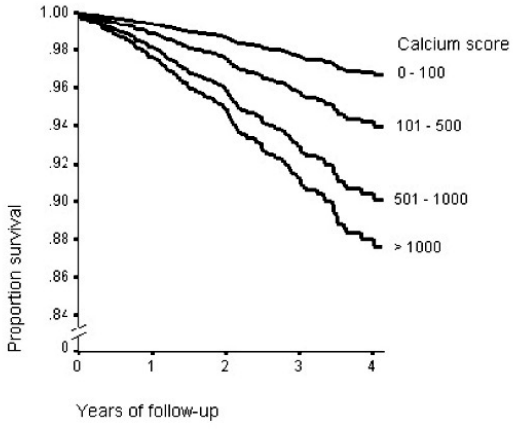

Figure 2. Cardiovascular event-free survival curves according to calcium score category

Table 2. Relative risks of events according to calcium score category in all subjects

	Total/events (n)	Relative risk (95% confidence interval)	
		Age- and sex-adjusted	Multivariate-adjusted
CHD*			
Calcium score:			
0-100	927/8	1.0 (reference)	1.0 (reference)
101-500	533/17	3.1 (1.3-7.2)	2.9 (1.2-6.9)
501-1000	254/19	6.8 (2.9-15.7)	6.3 (2.6-14.8)
>1000	299/29	8.0 (3.6-18.2)	7.1 (3.0-16.7)
CVD*			
Calcium score:			
0-100	927/23	1.0 (reference)	1.0 (reference)
101-500	533/29	1.8 (1.1-3.2)	1.7 (1.0-3.1)
501-1000	254/25	3.1 (1.7-5.6)	2.0 (1.6-5.2)
>1000	299/39	3.9 (2.3-6.8)	3.5 (2.0-6.3)
CHD mortality			
Calcium score:			
0-100	927/4	1.0 (reference)	1.0 (reference)
101-500	533/9	2.8 (0.9-9.2)	2.5 (0.8-8.4)
501-1000	254/10	5.6 (1.7-18.5)	5.7 (1.7-18.9)
>1000	299/14	6.1 (1.9-19.3)	5.7 (1.7-18.8)
CVD mortality			
Calcium score:			
0-100	927/6	1.0 (reference)	1.0 (reference)
101-500	533/15	3.2 (1.2-8.4)	3.0 (1.2-8.0)
501-1000	254/13	5.1 (1.9-13.8)	5.0 (1.8-13.7)
>1000	299/16	5.0 (1.9-13.2)	4.5 (1.6-12.3)
Total mortality			
Calcium score:			
0-100	927/31	1.0 (reference)	1.0 (reference)
101-500	533/41	1.7 (1.0-2.7)	1.7 (1.1-2.8)
501-1000	254/28	2.3 (1.3-3.8)	2.2 (1.3-3.8)
>1000	299/40	2.6 (1.6-4.2)	2.7 (1.6-4.5)

* CHD is coronary heart disease, CVD is cardiovascular disease.

and events ranged from no association to relative risks of 1.7 to 2.3 for hypertension, diabetes mellitus and a history of coronary heart disease or cardiovascular disease. Relative risks among asymptomatic subjects were in general similar to the ones found for the whole population (table 3). However, due to the relatively small number of events in the analyses of coronary heart disease mortality and cardiovascular mortality, the relative risks found for those outcomes had somewhat wider confidence intervals. Additional adjustment for cardiovascular risk factors did not materially change the risk estimates. When the Cox regression analyses were restricted to subjects over

Table 3. Relative risks of events according to calcium score category in asymptomatic subjects

	Total/events (n)	Relative risk (95% confidence interval)	
		Age- and sex-adjusted	Multivariate-adjusted
CHD*			
Calcium score:			
0-100	905/7	1.0 (reference)	1.0 (reference)
101-500	492/16	3.4 (1.4-8.3)	3.3 (1.3-8.1)
501-1000	202/10	4.8 (1.8-12.8)	5.3 (2.0-14.4)
>1000	196/17	8.2 (3.3-20.4)	8.7 (3.4-22.1)
CVD*			
Calcium score:			
0-100	893/21	1.0 (reference)	1.0 (reference)
101-500	477/26	2.0 (1.1-3.6)	1.9 (1.1-3.5)
501-1000	191/14	2.6 (1.3-5.2)	2.5 (1.2-5.0)
>1000	185/22	4.2 (2.2-7.9)	4.0 (2.1-7.5)
CHD mortality			
Calcium score:			
0-100	905/4	1.0 (reference)	1.00 (reference)
101-500	492/9	2.9 (0.9-9.7)	2.6 (0.8-8.8)
501-1000	202/8	5.4 (1.6-18.5)	5.5 (1.6-18.9)
>1000	196/8	5.4 (1.6-18.7)	4.9 (1.4-17.3)
CVD mortality			
Calcium score:			
0-100	893/6	1.0 (reference)	1.0 (reference)
101-500	477/14	3.4 (1.3-8.9)	3.1 (1.2-8.3)
501-1000	191/9	4.9 (1.7-14.1)	4.7 (1.6-13.6)
>1000	185/7	3.8 (1.2-11.7)	3.2 (1.0-10.1)
Total mortality			
Calcium score:			
0-100	893/27	1.0 (reference)	1.0 (reference)
101-500	477/37	2.0 (1.2-3.3)	2.0 (1.2-3.4)
501-1000	191/22	2.8 (1.6-5.1)	2.8 (1.6-5.0)
>1000	185/23	3.0 (1.7-5.3)	3.0 (1.7-5.4)

* CHD is coronary heart disease, CVD is cardiovascular disease.

70 years of age, similar relative risks of coronary and cardiovascular events were found. For example, among asymptomatic subjects over 70 years, the relative risks of coronary heart disease for increasing calcium score categories compared to the reference category were 3.4, 5.9, and 8.2, respectively (table 4).

Figure 3 presents age- and sex-adjusted relative risks of coronary heart disease and cardiovascular disease by categories based on the calcium score and the Framingham risk function. Compared to the reference category (subjects with a calcium score of 0 to 100 and a Framingham risk score below the 75th percentile), there was an increasing

Table 4. Relative risks of events according to calcium score category in asymptomatic subjects over 70 years of age

	Total/events (n)	Relative risk (95% confidence interval)	
		Age- and sex-adjusted	Multivariate-adjusted
CHD*			
Calcium score:			
0-100	398/4	1.0 (reference)	1.0 (reference)
101-500	284/11	3.4 (1.1-10.8)	3.0 (0.9-9.7)
501-1000	128/9	5.9 (1.8-19.5)	6.3 (1.9-20.9)
>1000	127/12	8.2 (2.6-26.0)	7.3 (2.3-23.8)
CVD*			
Calcium score:			
0-100	393/15	1.0 (reference)	1.0 (reference)
101-500	273/17	1.5 (0.7-3.0)	1.3 (0.6-2.7)
501-1000	118/11	2.1 (1.0-4.7)	2.1 (1.0-4.7)
>1000	118/14	2.8 (1.3-5.9)	2.3 (1.1-5.0)
Total mortality			
Calcium score:			
0-100	393/18	1.0 (reference)	1.0 (reference)
101-500	273/28	1.9 (1.0-3.4)	1.8 (1.0-3.3)
501-1000	118/16	2.4 (1.2-4.7)	2.4 (1.2-4.8)
>1000	118/18	2.7 (1.4-5.2)	2.5 (1.3-5.0)

* CHD is coronary heart disease, CVD is cardiovascular disease.

risk with increase in calcium score and Framingham risk score. Relative risks ranged from 1.6 to 9.0 for coronary heart disease, and from 1.1 to 5.4 for cardiovascular disease.

The area under the ROC curve (AUC), which indicates the power to discriminate subjects who will have an event from those who will not, was 0.746 for the model based on age, sex, and calcium score. The multivariate model of age, sex and cardiovascular risk factors, fitted for the current population had an AUC of 0.738.When the amount of coronary calcification was added to the multivariate model, the discriminatory power improved (AUC, 0.760; difference in AUC, 0.022; p for change, 0.06).

Discussion

The results from this population-based study on coronary calcification show that coronary calcification in older adults is a strong and independent predictor of coronary heart disease. The predictive value remained in subjects over 70 years of age. Relative risks for coronary calcification are an order of magnitude higher than those for cardiovascular risk factors.

Strengths of the current study include standardized assessment of risk factor levels, subjects' unawareness of the calcium scoring result, and a population-based sample.

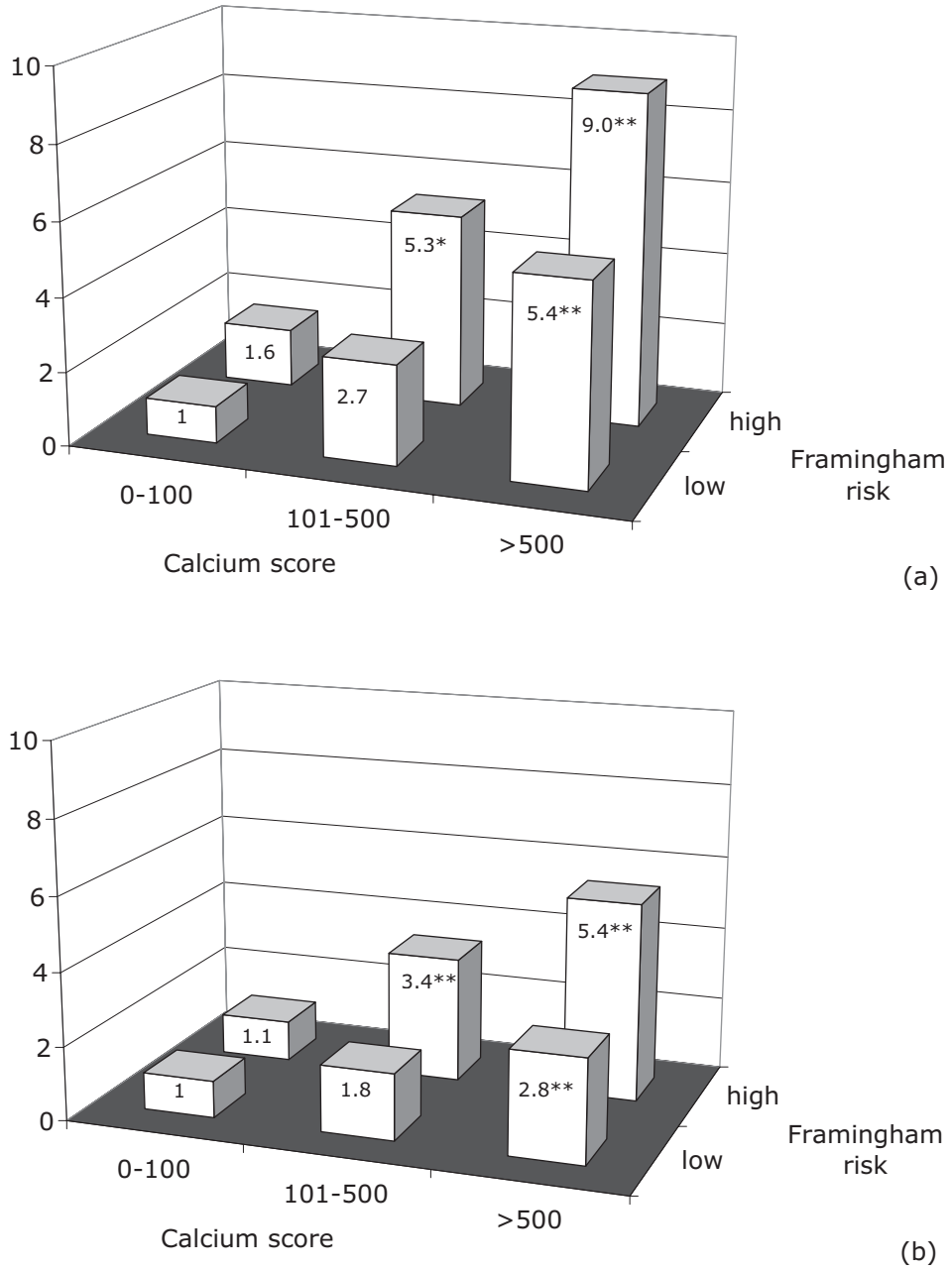

Figure 3. Relative risks of coronary heart disease (a) and cardiovascular disease (b) by low and high Framingham risk score and categories of the calcium score, in asymptomatic subjects. Level of significance: * $p<0.05$, ** $p<0.01$

By using a standardized assessment of levels of risk factors, misclassification of risk factors was reduced. Furthermore, subjects were not informed about the amount of coronary calcification present. In all previously published studies on the predictive value of coronary calcification, participants were notified of their calcium score. Awareness of a high calcium score may have motivated a positive change in health behaviour.[15] Since participants were unaware of the calcium score in our study, change in lifestyle or medication use or further cardiac testing (and possible subsequent revascularization) on the basis of the calcium score was prevented. Therefore, revascularization procedures could be included in our study as coronary outcomes without inducing biased results.

This is the first population-based study on the prognostic value of coronary calcification. Five prospective studies have shown that the amount of coronary calcification increases the risk of coronary heart disease.[1-6] The majority of the studies were conducted in populations of self-referred subjects.[1-3,5] These subjects may be more health-conscious or at high-risk of cardiovascular disease. One study was performed in high-risk subjects.[4,6] In our study, 61% of the invited population participated in the study. Characteristics of the study population were highly similar to those of the non-responders, except that the study population included somewhat more men and smokers. Since the study population reliably reflected the total population, non-response will not have affected the results.

Whether measurement of coronary calcification improves the identification of high-risk subjects in addition to cardiovascular risk factors, is unresolved. Four studies with self-reported data on risk factors showed that coronary calcification was a strong predictor of coronary events, independent of cardiovascular risk factors.[1-3,5] In the only published prospective study in which levels of cardiovascular risk factors were assessed, three-year risk prediction did not significantly improve when coronary calcification was added.[6] In nondiabetic subjects from the same study, coronary calcification contributed independent information to the six-year risk prediction based on traditional risk factors and C-reactive protein.[4] In our study, coronary calcification added independent information to risk prediction based on cardiovascular risk factors.

The predictive power of cardiovascular risk factors decreases with age, partly due to selective survival and the influence of co-morbidity on risk factor levels.[7-9] Calcification of the coronary arteries can be seen as a cumulative measure of lifetime exposure to cardiovascular risk factors, and may therefore improve risk stratification at older age. In our study among older adults, coronary calcification was a strong predictor of coronary heart disease, but also of cardiovascular disease and total mortality. The predictive power remained in subjects over age 70. In older adults, the choice between preventive drug therapy and lifestyle modification for the primary prevention of

coronary heart disease is pressing. Therefore, accurate cardiovascular risk stratification is of utmost importance in the elderly. Our study shows that assessment of coronary calcification can be used as a tool for risk stratification in older adults. The relative risks for coronary calcification are an order of magnitude higher than those found for cardiovascular risk factors.

In summary, this population-based study shows that coronary calcification is a strong and independent predictor of coronary heart disease in older adults. Risk stratification by assessment of coronary calcification can have an important role in guiding decisions on drug therapy or life style changes for the primary prevention of coronary heart disease in elderly people.

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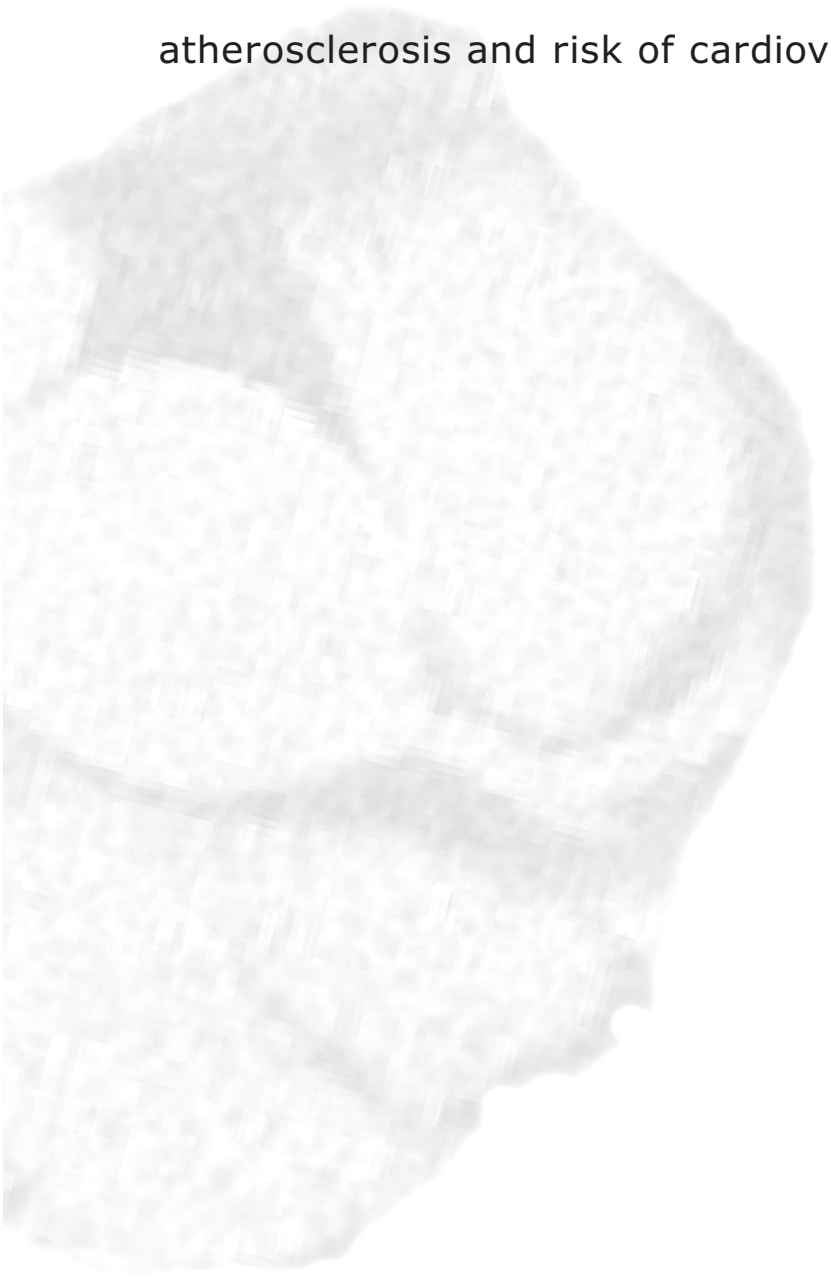
Chapter 5

Lipoprotein-associated phospholipase A2, atherosclerosis and risk of cardiovascular disease



Chapter 5.1

Lipoprotein-associated phospholipase A2 and risk of coronary heart disease and stroke

Abstract

Background. Lipoprotein-associated phospholipase A2 (Lp-PLA2) has been proposed as an inflammatory marker of cardiovascular disease. In the present study we investigated whether Lp-PLA2 is an independent predictor of coronary heart disease and stroke.

Methods and results. The Rotterdam Study is a population-based follow-up study in 7983 subjects aged 55 years and over. We performed a case-cohort study, including 377 coronary heart disease cases, 200 stroke cases and a random sample of 1822 subjects. Cox proportional hazard models were used with modification of the standard errors based on robust variance estimates. Compared to the first quartile of Lp-PLA2 activity, age and sex adjusted hazard ratios for coronary heart disease for the second, third and fourth quartile were 1.40 (95% confidence interval:0.96-2.05), 2.09 (1.45-3.02) and 2.25 (1.57-3.22), respectively (Ptrend<0.0001). After additional adjustment for cardiovascular risk factors, including total cholesterol and HDL-cholesterol, corresponding hazard ratios were 1.26 (0.86-1.86), 1.75 (1.19-2.57) and 1.77 (1.19-2.64), respectively (Ptrend=0.006). For stroke, age and sex adjusted hazard ratios for the second, third and fourth quartile of Lp-PLA2 were 1.08 (0.68-1.71), 1.45 (0.93-2.27) and 1.60 (1.04-2.47), respectively (Ptrend=0.02), compared to the first quartile. Corresponding hazard ratios after additional adjustment for cardiovascular risk factors were 1.17 (0.73-1.88), 1.60 (0.98-2.60) and 1.76 (1.09-2.85), respectively (Ptrend=0.01).

Conclusions. This study shows that Lp-PLA2 activity is a new and strong predictor of stroke in the general population. Furthermore, this study confirms that Lp-PLA2 is associated with risk of coronary heart disease. Both associations are independent of known cardiovascular risk factors.

Introduction

Lipoprotein-associated phospholipase A2 (Lp-PLA2) has been proposed as an inflammatory marker of cardiovascular disease. The enzyme circulates in the blood bound to low-density lipoprotein (LDL) cholesterol. The pro-inflammatory properties of the enzyme have been ascribed to its capacity to hydrolyze oxidized phospholipids leading to the generation of lysophosphatidylcholine and oxidized free fatty acids.[1] On the other hand, Lp-PLA2, sometimes called platelet-activating factor acetylhydrolase (PAF-AH), is also suggested to have anti-inflammatory properties[2] by hydrolyzing platelet-activating factor, which plays a role in the activation of platelets, monocytes and macrophages.[3]

The West of Scotland Coronary Prevention Study (WOSCOPS) suggested that Lp-PLA2 may be a risk factor for coronary heart disease, independent of traditional cardiovascular risk factors and C-reactive protein.[4] The study was conducted among middle-aged men with elevated levels of LDL-cholesterol who were enrolled in a primary prevention trial. In a nested case-control study within the Women's Health Study, Lp-PLA2 was associated with coronary heart disease, but only in univariate analyses. No association was present after adjustment for cardiovascular risk factors.[5] Recently, the Atherosclerosis Risk In Communities (ARIC) study found that Lp-PLA2 was an independent predictor of coronary heart disease in subjects with low LDL-cholesterol.[6] Currently, no data on the association between Lp-PLA2 and risk of stroke are available.

Within the Rotterdam Study, a population-based cohort study among men and women aged 55 years and over, we investigated whether Lp-PLA2 activity is an independent predictor of coronary heart disease and stroke.

Methods

Study population

The Rotterdam Study is a prospective population-based cohort study comprising 7983 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 was 78%. The Medical Ethics Committee of the Erasmus University Rotterdam 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.[7]

Study design

We used a case-cohort design,[8,9] in which a random sample, or a "subcohort," is drawn from the source population. Subjects who develop the disease but who are not included in the subcohort are selected as additional cases.[8,9] Baseline exposure is measured in the cases and controls included in the subcohort and in the additional cases.

Measurement of Lp-PLA2 activity

Plasma aliquots prepared from non-fasted blood samples were collected at baseline and stored at -80°C, and Lp-PLA2 activity was measured with a high throughput radiometric activity assay as described.[10] Briefly, plasma samples were aliquoted into 96-well microtiter plates and mixed with a substrate solution consisting of 0.4 mM [3H]-PAF (Specific Activity 21.5 Ci/mmol, Perkin Elmer Life Sciences) and 99.6 mM C16-PAF (Avanti Polar Lipids Inc) in assay buffer (100mM HEPES, 150mM NaCl, 5mM EDTA, pH7.4). The reactions were allowed to proceed at room temperature for 5 minutes before sequestering of the phospholipid substrates by an ice-cold fatty acid-free bovine serum albumin solution at a final concentration of 16.1 mg/ml. The BSA-lipid complexes were then precipitated with ice-cold trichloroacetic acid at a final concentration of 7.8% and pelleted by centrifugation at ~6,000 g for 15 min at 4°C. Aliquots of the supernatant containing the reaction products were transferred to another microplate (Perkin Elmer) and the radioactivity counted in a Topcount liquid scintillation counter (Perkin Elmer Life Sciences) upon addition of Microscint-20 scintillation cocktail (Perkin Elmer Life Sciences). Lp-PLA2 activity was expressed as nmoles of PAF hydrolysed per minute per ml of plasma samples. The coefficient of variation was 8.4%.

Assessment of covariates

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 behaviour. Additionally, during two visits to the research center clinical measures were obtained. Height and weight were measured and the body mass index was calculated (weight (kg)/length (m^2)). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Non-fasting blood samples were drawn and total cholesterol, high-density lipoprotein (HDL) cholesterol and glucose were measured within 2 weeks, as described previously.[11] In a random sample of 42 subjects, LDL-cholesterol was determined using an enzymatic method (Roche, Mannheim, Germany). Immediately after blood sampling, white cell count

was assessed in citrate plasma using a Coulter Counter T540® (Coulter electronics, Luton, England), which has a coefficient of variation less than 2.0%. Quality of assessments was continuously monitored by Instruchemi® (Hilversum, the Netherlands). Using a nephelometric method (Immage®, Beckman Coulter), C-reactive protein was measured in 267 blood samples which were kept frozen at -20 °C. We defined diabetes mellitus as a random or postload glucose level >=11.1 mmol/l and/or the use of blood glucose lowering medication.

Follow-up procedures

For each subject, follow-up started after the baseline examination. For coronary heart disease the study lasted until January 1st 2000 and for stroke the study lasted until January 1st 1999. 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 general practitioners. Two research physicians independently coded all reported events according to the International Classification of Diseases, 10th edition (ICD-10).[12] Codes on which the research physicians disagreed were discussed in order to reach consensus. Finally, a medical expert in cardiovascular disease, whose judgment was considered final, reviewed all events. Incident coronary heart disease 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), other forms of acute (I24) or chronic ischemic (I25) heart disease, sudden (cardiac) death (I46 and R96), and death due to ventricular fibrillation (I49) and congestive heart failure (I50) during follow-up. Strokes were classified as definite, probable and possible stroke. A stroke was considered definite if the diagnosis was based on typical clinical symptoms and neuro-imaging excluded other diagnoses. A stroke was considered probable if typical clinical symptoms were present but neuro-imaging was not performed. For the diagnosis of a fatal stroke, other causes of death, especially cardiac, must have been excluded. All reported transient ischemic attacks also were reviewed in order to screen and classify all strokes. For the present analyses we used definite and probable strokes. Subclassification in hemorrhagic or ischemic stroke was based on neuro-imaging, which was available for 61% of all cases. For the diagnosis ischemic stroke,

we excluded all definite hemorrhagic strokes. During the follow-up for coronary heart disease, which lasted until January 1st 2000, 47 (2.2%) subjects were lost to follow-up. During the follow-up for stroke, which lasted until January 1st 1999, 49 subjects (2.5%) were lost to follow-up. For these subjects, the follow-up time was computed until the last date of contact.

Statistical analysis

We used a t-test for continuous and a chi-square test for dichotomous variables to test differences between the random cohort and the remainder of the Rotterdam Study. In the random cohort, age- (except for age) and sex-adjusted (except for sex) correlation coefficients were computed for the association of age, sex, cardiovascular risk factors and inflammatory markers with Lp-PLA2.

The association of Lp-PLA2 activity with coronary heart disease and stroke was evaluated in a case-cohort design using standard Cox proportional hazards models with modification of the standard errors based on robust variance estimates.[8,9] We used the method according to Barlow in which the random cohort is weighted by the inverse of the sampling fraction from the source population. In the case-cohort analysis only subjects from the random cohort contribute to follow-up time.

We made quartiles of Lp-PLA2 activity and used the lowest quartile as the reference category. Cox proportional hazard models for both coronary heart disease and stroke were performed entering age, sex and quartiles of Lp-PLA2 activity into the model (model 1). We excluded subjects with a history of myocardial infarction for the analyses on coronary heart disease and we excluded subjects with a history of stroke for the analyses on stroke. In model 2 we added total cholesterol and HDL-cholesterol. In model 3, body mass index, systolic blood pressure, diabetes, smoking status, cholesterol lowering medication and white cell count were added. We did not include C-reactive protein in the model because measurements were only available in a subset of our study population. In the test for trend analysis we replaced the quartiles of Lp-PLA2 activity by continuous values of Lp-PLA2 activity. We had missing values for covariates in less than 5%. Therefore, we imputed the median value for continuous variables and assumed subjects with missing values for diabetes and smoking to be non-diabetic and non-smoker.

To investigate the combined effect of total cholesterol and Lp-PLA2 on coronary heart disease, we divided the population into nine groups according to tertiles of total cholesterol and Lp-PLA2. Cut-off points for tertiles were based on the distribution in the random cohort (39 and 48 nmol/min/ml for Lp-PLA2 activity, 6.1 and 7.1 mmol/l for total cholesterol). We used the group with subjects who were in the lowest tertile for both total cholesterol and Lp-PLA2 as the reference group. Cox proportional hazard analysis for coronary heart disease was performed by entering dummy

variables of the eight groups together with age, sex, body mass index, systolic blood pressure, total cholesterol, HDL-cholesterol, diabetes, smoking status, cholesterol lowering medication and white cell count into the model. Since total cholesterol was not associated with stroke, analysis on the combined effect of total cholesterol and Lp-PLA2 on risk of stroke was not performed.

Results

Table 1 shows baseline characteristics of the random cohort. The characteristics of the random cohort were similar to the baseline characteristics of the total population of the Rotterdam Study with few minor exceptions. Subjects in the random cohort were younger (69.6 years versus 71.7 years) and had a lower systolic blood pressure (138 mm Hg versus 140 mm Hg). Lp-PLA2 activity was higher in men than in women and was positively associated with body mass index, systolic blood pressure, total cholesterol and white cell count (table 2). An inverse association was present with HDL-cholesterol. Lp-PLA2 activity was not significantly associated with age, diastolic blood pressure, diabetes and smoking. Associations with Lp-PLA2 were strongest for

Table 1. Baseline characteristics of the random cohort

Variable	Total (n=1820)
Age (years)	69.6±9.3
Men (%)	38
Body mass index (kg/m^2)	26.2±3.6
Systolic blood pressure (mm Hg)	138±22
Diastolic blood pressure (mm Hg)	73±11
Total cholesterol (mmol/l)	6.6±1.3
HDL-cholesterol (mmol/l)	1.3±0.4
Diabetes (%)	10
Smokers (%)	
Current	21
Past	41
Cholesterol lowering medication (%)	2.1
White cell count (x10^9/l)	2.5
Lp-PLA2 activity (nmol/min/ml plasma)	44±12
History of myocardial infarction (%)	6
History of stroke (%)	3

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.

Table 2. Spearman correlation coefficients showing associations between Lp-PLA2 activity and cardiovascular risk factors in the random cohort.

	Lp-PLA2	p-value
Age	0.025	0.29
Male sex	0.16	<0.001
Body mass index	0.074	0.002
Systolic blood pressure	0.070	0.003
Diastolic blood pressure	0.005	0.83
Total cholesterol	0.42	<0.001
HDL-cholesterol	-0.28	<0.001
Diabetes	0.028	0.24
Smoking	0.021	0.37
White cell count	0.060	0.01
C-reactive protein*	0.024	0.67

All correlation coefficients, except for age (only adjusted for sex) and sex (only adjusted for age), are adjusted for age and sex.

*Available in random sample of 267 subjects.

total and HDL-cholesterol with correlation coefficients of 0.42 and –0.28, respectively. In a subset of the random cohort (n=267), Lp-PLA2 activity was not associated with C-reactive protein (r=0.02, p=0.31). Total cholesterol was strongly associated with LDL-cholesterol in a random sample of 42 subjects (r=0.91, p<0.001).

During a mean follow-up of 6.8 years, incident coronary heart disease occurred in 377 subjects. Lp-PLA2 activity was strongly associated with the risk of coronary heart disease (table 3). Compared to the first quartile of Lp-PLA2 activity, age- and sex-adjusted hazard ratios for the second, third and fourth quartile were 1.40, 2.09 and 2.25, respectively (Ptrend<0.0001). After additional adjustment for cardiovascular risk factors the strength of the association attenuated. Compared to the first quartile of Lp-PLA2 activity, hazard ratios for the second, third and fourth quartile were 1.26, 1.75 and 1.77, respectively (Ptrend=0.006). The attenuation of the association was mainly caused by adjustments for total cholesterol and HDL-cholesterol. When we used myocardial infarction as the outcome, the strength of the association slightly increased (table 3).

During a mean follow-up of 6.2 years, incident stroke occurred in 200 subjects. Lp-PLA2 activity showed a graded association with the risk of stroke. Compared to the first quartile of Lp-PLA2 activity, age- and sex-adjusted hazard ratios for the second, third and fourth quartile were 1.08, 1.45 and 1.60, respectively (Ptrend=0.02)

Table 3. Hazard ratios for events according to quartile of Lp-PLA2 activity.

	Hazard ratio (95% confidence interval)		
	Model 1	Model 2	Model 3
Coronary heart disease (N=377)			
Quartile of Lp-PLA2:	1.00 (reference)	1.00 (reference)	1.00 (reference)
1	1.40 (0.96-2.05)	1.28 (0.87-1.88)	1.26 (0.86-1.86)
2	2.09 (1.45-3.02)	1.78 (1.22-2.62)	1.75 (1.19-2.57)
3	2.25 (1.57-3.22)	1.81 (1.22-2.68)	1.77 (1.19-2.64)
4	<0.0001	0.006	0.006
Ptrend			
Myocardial infarction (N=153)			
Quartile of Lp-PLA2:			
1	1.00 (reference)	1.00 (reference)	1.00 (reference)
2	1.32 (0.70-2.49)	1.14 (0.60-2.16)	1.08 (0.56-2.07)
3	2.76 (1.55-4.92)	1.93 (1.06-3.50)	1.89 (1.03-3.48)
4	3.25 (1.85-5.70)	1.90 (1.02-3.53)	1.88 (1.00-3.55)
Ptrend	<0.0001	0.08	0.14
Stroke (N=200)			
Quartile of Lp-PLA2:			
1	1.00 (reference)	1.00 (reference)	1.00 (reference)
2	1.08 (0.68-1.71)	1.12 (0.70-1.78)	1.17 (0.73-1.88)
3	1.45 (0.93-2.27)	1.55 (0.96-2.51)	1.60 (0.98-2.60)
4	1.60 (1.04-2.47)	1.80 (1.12-2.89)	1.76 (1.09-2.85)
Ptrend	0.02	0.01	0.01
Ischemic stroke (N=178)			
Quartile of Lp-PLA2:			
1	1.00 (reference)	1.00 (reference)	1.00 (reference)
2	1.28 (0.78-2.09)	1.31 (0.80-2.15)	1.37 (0.83-2.27)
3	1.54 (0.95-2.51)	1.62 (0.96-2.72)	1.67 (0.99-2.82)
4	1.85 (1.17-2.94)	2.03 (1.23-3.37)	2.00 (1.20-3.34)
Ptrend	0.01	0.01	0.01

N=number of events, Lp-PLA2=lipoprotein-associated phospholipase A2.
Model 1. Adjusted for age and sex.
Model 2. Adjusted for age, sex, body mass index, systolic blood pressure, total cholesterol level, HDL-cholesterol level, diabetes, smoking, cholesterol lowering medication and white cell count.

(table 3). After additional adjustment for cardiovascular risk factors, hazard ratios for the second, third and fourth quartile of Lp-PLA2 were 1.17, 1.60 and 1.76 respectively, compared to the first quartile (Ptrend=0.01). The absence of a decrease in the hazard ratio's for stroke after adjustment for cardiovascular risk factors is due to the absence of an association of total cholesterol and HDL-cholesterol with risk of stroke (hazard ratio's were 0.91 (0.79-1.04) and 1.02 (0.68-1.54) per mmol/l increase in total cholesterol and HDL-cholesterol, respectively). When we used ischemic stroke as the outcome, the strength of the association slightly increased (table 3).

Figure 1 shows hazard ratios for coronary heart disease in tertiles of total cholesterol and Lp-PLA2. Within each tertile of total cholesterol, a gradual increase in hazard ratio was seen with increasing tertiles of Lp-PLA2. Compared to subjects in

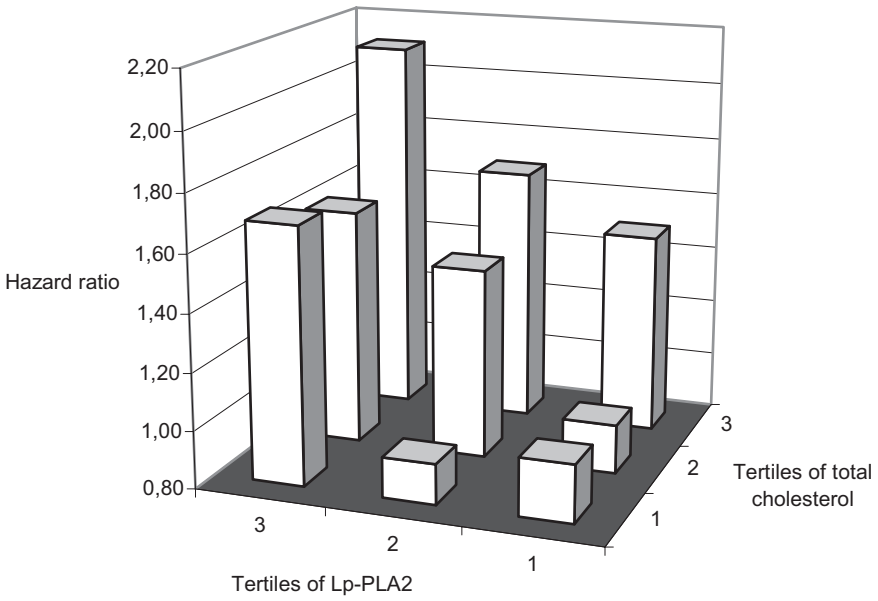


Figure 1. Multivariate-adjusted hazard ratios for coronary heart disease for tertiles of cholesterol and tertiles of Lp-PLA2 activity.

the lowest tertile of both cholesterol and Lp-PLA2, the hazard ratio increased to 2.11 (1.25-3.54) in subjects in the highest tertile of both total cholesterol and Lp-PLA2.

Discussion

The present population-based study shows that Lp-PLA2 is a strong predictor of stroke. Furthermore, the study confirms that Lp-PLA2 is a risk factor for coronary heart disease. The associations are independent of known cardiovascular risk factors.

Several methodological issues should be considered before interpreting the results.

We have to be aware of potential confounding factors. Lp-PLA2 is bound to LDL-cholesterol and therefore highly correlated with LDL-cholesterol levels. In the present study, no LDL-cholesterol levels were available and therefore we adjusted for total cholesterol levels. Because of the high correlation between LDL-cholesterol and total cholesterol in a random sample of our cohort (r=0.91), we believe that residual confounding by LDL-cholesterol cannot explain our results. Furthermore, since cholesterol is not a strong predictor of stroke,[13,14] the observed association between Lp-PLA2 and stroke supports the absence of confounding by LDL-cholesterol in our study. Since Lp-PLA2 is considered to have pro-inflammatory properties, it is important to know whether the effects of Lp-PLA2 on coronary heart disease and stroke are independent of known inflammatory markers. Consequently, we adjusted for white cell count in the analyses. C-reactive protein is probably a better marker of inflammation, but was only available for a subset of the population. In the present study, the association between C-reactive protein and Lp-PLA2 was only weak, which confirms results of previous studies.[4,6] In the WOSCOPS and the ARIC studies, additional adjustment for C-reactive protein did not affect the strength of the association between Lp-PLA2 and coronary heart disease.[4,6] Therefore, we do not expect that the absence of C-reactive protein in our model would have affected our risk estimates. Our results thus suggest that the effect of Lp-PLA2 on coronary heart disease and stroke is independent of other markers of inflammation. Other factors related to Lp-PLA2 in our study were male sex, body mass index and systolic blood pressure. We adjusted for these factors in our multivariate models.

This is the first study that shows an association between Lp-PLA2 and risk of stroke. The importance of this result is twofold. First, the present study shows that Lp-PLA2 is a new and independent predictor of stroke in the general population. Subjects in the highest quartile had a 76% increased risk compared to those in the lowest quartile. The Women's Health Study investigated the association between Lp-PLA2 and cardiovascular disease. However, no separate analyses on Lp-PLA2 and stroke were performed.[5] Second, since total cholesterol is not associated with risk of stroke,[13,14] the association between Lp-PLA2 activity and stroke suggests that Lp-PLA2, although carried by LDL-cholesterol, may convey a different risk. Evidence is accumulating that inflammation plays are role in the pathogenesis of ischemic stroke. A number of markers of inflammation are found to be associated with risk of stroke.[15-19] Inflammatory processes are involved in atherosclerosis and plaque rupture, which in turn contribute to the development of stroke. Lp-PLA2 is an enzyme that hydrolyzes oxidized phospholipids, releasing lysophosphatidylcholine, which has pro-inflammatory properties.[1] Our findings suggest that Lp-PLA2 may be added as an inflammatory marker that predicts risk of ischemic stroke.

Three studies reported on the association of Lp-PLA2 and coronary heart disease.

In the WOSCOPS study, middle-aged men with elevated LDL-cholesterol levels without a history of myocardial infarction were randomly assigned to pravastatin or placebo. In a nested case-control study with 560 cases and 1160 controls, assigned to either pravastatin or placebo, a relative risk of 1.18 for coronary heart disease per standard deviation increase in Lp-PLA2 was found. This association was independent of cardiovascular risk factors.[4] The Women's Health Study is a trial of aspirin and vitamin E in women above 45 years of age without history of cardiovascular disease or cancer. In a nested case-control study with 123 cases and 123 controls, Lp-PLA2 levels were higher in the subjects with cardiovascular disease than in the controls. However, after adjustment for cardiovascular risk factors this association disappeared.[5] In a case-cohort design with 608 coronary heart disease cases, the ARIC study found that subjects in the highest tertile of Lp-PLA2 had a relative risk of 1.78 for coronary heart disease as compared to subjects in the lowest tertile of Lp-PLA2. After adjustment for cardiovascular risk factors, an independent association was still present in subjects with a LDL-cholesterol below the median.[6] In the present study, we found that LP-PLA2 is an independent risk factor for coronary heart disease and the association is present in all strata of total cholesterol. Since total cholesterol is highly correlated with LDL-cholesterol, strata based on total cholesterol will only slightly differ from strata based on LDL-cholesterol. Therefore, our results are not likely to be much different if we would have examined the association in strata of LDL-cholesterol.

How can the difference between our findings and those of the Women's Health Study and ARIC study be explained? The Women's Health Study included middle-aged women with a low event rate and comprised a relatively low number of cardiovascular events.[5] Both the ARIC study and the Rotterdam Study were conducted in population-based cohorts of men and women, with a comparable duration of follow-up, and with large numbers of coronary heart disease events. The Rotterdam Study cohort included subjects aged 55 years and over (mean age 70 years) while the cohort of the ARIC study included subjects aged 45-64 (mean age 58 years).[6] Previous studies controlled for LDL-cholesterol while in our study we adjusted for total cholesterol level. However, the correlation between Lp-PLA2 and total cholesterol in our study was comparable in size to the correlation between Lp-PLA2 and LDL-cholesterol in the WOSCOPS[4] and ARIC[6] study. Therefore we do not think that the absence of LDL-cholesterol in our study can explain the different results. While previous studies measured Lp-PLA2 mass, Lp-PLA2 activity was measured in our study, with reasonable reproducibility. However, a correlation of 0.86 between Lp-PLA2 mass and activity has been reported[20] and a difference in assays is thus not likely to fully explain the difference in findings. More studies are needed to shed light on possible explanations for the diverging results and the characteristics of populations in which

Lp-PLA2 can be used to indicate high risk of cardiovascular disease.

In conclusion, our results suggest that Lp-PLA2 activity is a new and independent predictor of stroke in the general population. Our findings confirm the association between Lp-PLA2 and coronary heart disease. The results suggest that the effect of Lp-PLA2 on cardiovascular disease is independent of a subject's total cholesterol level and markers of inflammation.

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Chapter 5.2

Lipoprotein-associated phospholipase A2 and measures of extracoronary atherosclerosis

Abstract

Background. Lipoprotein-associated phospholipase A2 (Lp-PLA2) may be a new and independent predictor of coronary heart disease. It is unclear whether the effect of Lp-PLA2 on cardiovascular disease is exerted through the development of atherosclerosis. Therefore, we investigated the association between Lp-PLA2 and measures of atherosclerosis.

Methods and results. The Rotterdam Study is a population-based study in 7983 subjects aged 55 years and over. Lp-PLA2 was determined in a random sample of 1822 subjects. Measures of atherosclerosis included common carotid intima media thickness (IMT), carotid plaques, ankle-arm index (AAI) and aortic calcification. We used linear regression to examine associations of Lp-PLA2 with carotid IMT and AAI and used logistic regression to examine associations of Lp-PLA2 with the presence of carotid plaques and aortic calcification. After adjustment for age and sex, Lp-PLA2 was positively associated with carotid IMT (Ptrend=0.015) and inversely associated with AAI (Ptrend=0.004). After additional adjustment for cardiovascular risk factors, Lp-PLA2 was no longer significantly associated with carotid IMT (Ptrend=0.30) nor with AAI (Ptrend=0.44). Subjects in the third tertile of Lp-PLA2 had an age and sex adjusted odds ratio of 2.0 (95% confidence interval: 1.4-2.7) for the presence of aortic calcification and an odds ratio of 1.3 (1.0-1.7) for the presence of carotid plaques. After additional adjustment for cardiovascular risk factors the strength of the association attenuated for aortic calcification and disappeared for carotid plaques.

Conclusion. Lp-PLA2 activity is only univariately associated with measures of atherosclerosis. The lack of an independent association suggests that the effect of Lp-PLA2 on coronary heart disease is at least partly exerted through another mechanism than atherosclerosis.

Introduction

Recently, several studies have suggested that lipoprotein-associated phospholipase A2 (Lp-PLA2) may be a new and independent risk factor for coronary heart disease.[1,2] Lp-PLA2 hydrolyses oxidated low-density lipoprotein (LDL) cholesterol generating lysophosphatidylcholine and oxidized free fatty acids. Lysophosphatidylcholine and oxidized free fatty acids are both chemoattractants for monocytes and may account for a part of the pro-inflammatory capacities of oxidized LDL-cholesterol.[3]

It is unclear whether the effect of Lp-PLA2 on cardiovascular disease is exerted through effects of the enzyme on the development of atherosclerosis. So far, no studies have investigated whether Lp-PLA2 is associated with the extent of atherosclerosis. Therefore, we investigated whether Lp-PLA2 activity is associated with atherosclerosis at different sites of the vascular tree in a large population-based study.

Methods

Study population

The Rotterdam Study is a prospective population-based cohort study comprising 7983 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 was 78%. The Medical Ethics Committee of the Erasmus University Rotterdam 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.[4]

Measurement of Lp-PLA2 activity

Plasma aliquots prepared from non-fasted blood samples were collected at baseline and stored at -80°C, and Lp-PLA2 activity was measured with a high throughput radiometric activity assay as described.[5] Briefly, plasma samples were aliquoted into 96-well microtiter plates and mixed with a substrate solution consisting of 0.4 mM [3H]-PAF (Specific Activity 21.5 Ci/mmol, Perkin Elmer Life Sciences) and 99.6 mM C16-PAF (Avanti Polar Lipids Inc) in assay buffer (100mM HEPES, 150mM NaCl, 5mM EDTA, pH7.4). The reactions were allowed to proceed at room temperature for 5 min before sequestering of the phospholipid substrates by an ice-cold fatty acid-free bovine serum albumin solution at a final concentration of 16.1 mg/ml. The BSA-lipid complexes were then precipitated with ice-cold trichloroacetic acid at a

final concentration of 7.8% and pelleted by centrifugation at ~6,000 g for 15 min at 4°C. Aliquots of the supernatant containing the reaction products were transferred to another microplate (Perkin Elmer) and the radioactivity counted in a Topcount liquid scintillation counter (Perkin Elmer Life Sciences) upon addition of Microscint-20 scintillation cocktail (Perkin Elmer Life Sciences). Lp-PLA2 activity was expressed as nmoles of PAF hydrolysed per minute per ml of plasma samples.

Measures of 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, Bethel, Washington, USA). Common carotid intima media thickness (IMT) was determined as the average of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[6]

The internal carotid artery, carotid bifurcation, and common carotid artery were examined both left and right for the presence of plaques. 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.[6] The anterior and posterior wall were evaluated for the presence of a plaque. This resulted in a carotid plaque score between 0 and 6. For the analyses, we compared no carotid plaques versus 1 or more plaques.

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. Values of the AAI larger than 1.50 were considered invalid.[7]

Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. The extent of abdominal atherosclerosis was scored according to the length of the involved area (with scores 0 to 5 corresponding to 0, <1 cm, 1-2.5 cm, 2.5-4.9, 5.0-9.9, and >=10.0 cm).[8] For the analyses, we compared absence of aortic calcification versus presence of calcification.

Assessment of covariates

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 behaviour. Additionally, during two visits to the research center clinical measures were obtained. Height and weight were measured and the body mass index was calculated (weight (kg)/length (m^2)). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Non-fasting blood samples were drawn and total cholesterol, high-density lipoprotein (HDL) cholesterol and glucose were measured within 2 weeks, as described previously.[7] In a random sample of 42 subjects, LDL-cholesterol was determined using an enzymatic method (Roche, Mannheim, Germany). Immediately after blood sampling, white cell count was assessed in citrate plasma using a Coulter Counter T540® (Coulter electronics, Luton, England), which has a coefficient of variation less than 2.0%. Quality of assessments was continuously monitored by Instruchemi® (Hilversum, the Netherlands). Using a nephelometric method (Immage®, Beckman Coulter), C-reactive protein was measured in 267 blood samples which were kept frozen at -20 °C. We defined diabetes mellitus as a random or postload glucose level >=11.1 mmol/l and/or the use of blood glucose lowering medication.

Statistical analysis

We used a t-test for continuous and a chi-square test for dichotomous variables to test differences between the random cohort and the remainder of the Rotterdam Study. We divided Lp-PLA2 activity into tertiles (cut-offs 39 and 48 nmol/min/ml) and calculated age, sex, total cholesterol and HDL-cholesterol adjusted mean values of carotid IMT and AAI for tertiles of Lp-PLA2 activity. Linear regression using continuous values of Lp-PLA2 activity was used to investigate the association of Lp-PLA2 activity with carotid IMT and AAI. The p-value of the linear regression analysis using continuous values was used as p for trend. Logistic regression was used to examine the association of Lp-PLA2 activity tertiles with the presence of aortic calcification and carotid plaques. In model 1 we adjusted for age and sex. In model 2, total cholesterol and HDL-cholesterol were added. In model 3, body mass index, systolic blood pressure, diabetes and smoking were additionally added. The p-value of the logistic regression analysis using continuous values of Lp-PLA2 was used as p for trend.

Results

Table 1 shows baseline characteristics of the study population. The mean age was 69.6 years and 38% were male. The characteristics of the subcohort were similar to the baseline characteristics of the remaining population of the Rotterdam Study with

few minor exceptions. Subjects in the subcohort were slightly younger (69.6 years versus 71.7 years) and had a slightly lower mean systolic blood pressure (138 mm Hg versus 140 mm Hg). The correlation coefficient between total cholesterol and LDL-cholesterol was high ($r=0.91$, $p<0.001$).

After adjustment for age and sex, Lp-PLA2 was positively associated with carotid

Table 1. Baseline characteristics of the study population (1990-1993)

Variable	Total (n=1820)
Age (years)	69.6±9.3
Men (%)	38
Body mass index (kg/m^2)	26.2±3.6
Systolic blood pressure (mm Hg)	138±22
Diastolic blood pressure (mm Hg)	73±11
Total cholesterol (mmol/l)	6.6±1.3
HDL-cholesterol (mmol/l)	1.3±0.4
Diabetes (%)	10
Smokers (%)	
Current	21
Past	41
Cholesterol lowering medication (%)	2.1
White cell count (x10^9/l)	2.5
Lp-PLA2 activity (nmol/min/ml plasma)	44±12
History of myocardial infarction (%)	6
History of stroke (%)	3

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation.

Table 2. Characteristics of measures of atherosclerosis.

Variable	Total (n=1820)
Carotid intima media thickness (mm)	0.80±0.17
Carotid plaques* (%)	42
Ankle-arm index	1.06±0.22
Aortic calcification† (%)	68

Categorical variables are expressed as a percentage. Values of continuous variables are expressed as mean ± standard deviation.
*Percentage of subjects with one or more carotid plaques.
†Percentage of subjects with aortic calcification.

IMT (Ptrend=0.015) and negatively associated with AAI (Ptrend=0.004). After additional adjustment for cardiovascular risk factors, Lp-PLA2 was no longer significantly associated with carotid IMT nor with AAI (figure 1) (Ptrend=0.30 for carotid IMT and Ptrend=0.44 for AAI). Table 2 shows that Lp-PLA2 is positively associated with the presence of carotid plaques and the presence of abdominal calcification. Compared to subjects in the first tertile of Lp-PLA2, subjects in the third tertile had an age and sex adjusted odds ratio of 2.0 (1.4-2.7) for the presence of aortic calcification and an odds ratio of 1.3 (1.0-1.7) for the presence of carotid plaques. After additional adjustment for cardiovascular risk factors the strength of the association attenuated for aortic calcification and disappeared for carotid plaques.

Table 3. Odds ratios for presence of carotid plaques and abdominal aorta calcification for tertiles of Lp-PLA2 activity.

	Odds ratio (95% confidence interval)		
	Model 1	Model 2	Model 3
Carotid plaques			
Tertile of Lp-PLA2:			
1	1.0 (reference)	1.0 (reference)	1.0 (reference)
2	1.0 (0.8-1.4)	0.9 (0.7-1.2)	0.9 (0.6-1.2)
3	1.3 (1.0-1.7)	1.1 (0.8-1.5)	1.0 (0.7-1.5)
Ptrend	0.003	0.17	0.16
Aortic calcification			
Tertile of Lp-PLA2:			
1	1.0 (reference)	1.0 (reference)	1.0 (reference)
2	1.3 (1.0-1.8)	1.0 (0.7-1.4)	1.1 (0.7-1.5)
3	2.0 (1.4-2.7)	1.2 (0.8-1.7)	1.3 (0.9 -1.9)
Ptrend	<0.001	0.10	0.028

Lp-PLA2=lipoprotein-associated phospholipase A2
Model 1. Adjusted for age and sex
Model 2. Adjusted for age, sex, total cholesterol level and HDL-cholesterol level
Model 3. Adjusted for age, sex, body mass index, systolic blood pressure, total cholesterol level, HDL-cholesterol level, diabetes, smoking, cholesterol lowering medication and white cell count

Discussion

In the present population-based study Lp-PLA2 is only univariately associated with atherosclerosis at different sites of the arterial tree. After adjustment for total cholesterol and HDL-cholesterol the association between Lp-PLA2 and measures of atherosclerosis disappeared.

In this study, we failed to show an independent association between Lp-PLA2

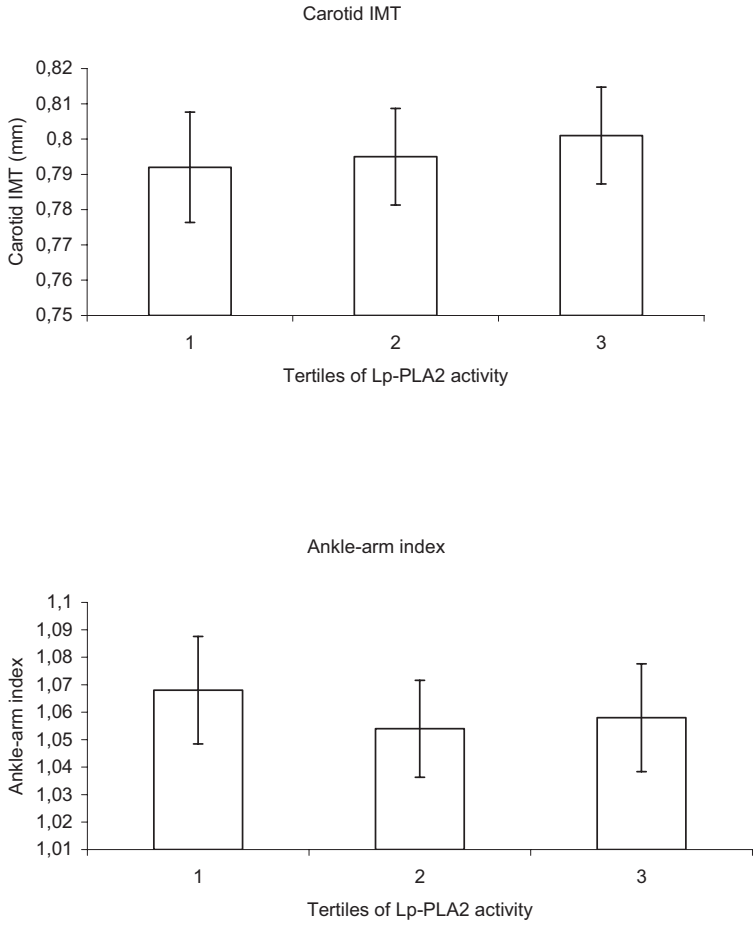

Figure 1. Mean and 95% confidence interval of multivariate-adjusted carotid intima media thickness and ankle-arm index for tertiles of Lp-PLA2 activity.

activity and measures of atherosclerosis at different sites of the vascular tree. The study was performed within the Rotterdam Study, a large population-based study in subjects aged 55 years and older. We used several techniques to measure atherosclerosis. Ultrasound was used to measure the IMT in the common carotid artery and to detect plaques in the common carotid artery, bifurcation and internal carotid artery.

We performed X-ray to assess the amount of aortic calcification and used AAI as a measure of lower extremity atherosclerosis. The measures of carotid, aortic and lower extremity atherosclerosis have all shown to be associated with cardiovascular risk factors and cardiovascular risk and are considered to be measures of generalized atherosclerosis.[6,9-12] In a previous study we found that C-reactive protein is associated with all these measures of atherosclerosis.[13] This suggests that the lack of an association is unlikely to be caused by the use of inappropriate measures of atherosclerosis in our study.

Lp-PLA2 is bound to LDL-cholesterol and therefore highly correlated with LDL-cholesterol levels. In the present study, no LDL-cholesterol levels were available and therefore we adjusted for total cholesterol levels. Because of the high correlation between LDL and total cholesterol in a random sample of the present study and because the correlation between Lp-PLA2 and total cholesterol in our study was even higher than that of Lp-PLA2 with LDL-cholesterol in the WOSCOPS[1] and the ARIC study,[2] we believe that we adjusted sufficiently for LDL-cholesterol. Furthermore, whereas residual confounding would lead to an overestimation of the effect, we did not observe an association between Lp-PLA2 and measures of atherosclerosis in the present study.

Recently, three studies have investigated the association between Lp-PLA2 and cardiovascular disease. The WOSCOPS study, a randomized controlled trial of pravastatin versus placebo in middle-aged men, showed that Lp-PLA2 was an independent risk factor for cardiovascular disease.[1] The Women's Health Study, a randomized controlled trial in women without a history of cardiovascular disease and cancer, found an association between Lp-PLA2 and cardiovascular disease, but only in univariate analyses.[14] In a case-cohort design with 608 coronary heart disease cases, the ARIC study found that subjects in the highest tertile of Lp-PLA2 had a relative risk of 1.78 for coronary heart disease as compared to subjects in the lowest tertile of Lp-PLA2. However, after adjustment for cardiovascular risk factors, the association was only present in subjects with a LDL-cholesterol below the median.[2] In the present study Lp-PLA2 activity was only univariately associated with measures of atherosclerosis. After adjustment for total cholesterol and HDL-cholesterol the association disappeared.

Several mechanisms may account for a role of Lp-PLA2 in cardiovascular disease. Lp-PLA2 is suggested to have pro-inflammatory and pro-atherogenic properties by hydrolyzing oxidized phospholipids releasing lysophospholipids and oxidized fatty acids.[3] Lp-PLA2, sometimes called platelet-activating factor acetylhydrolase (PAF-AH), is also suggested to have anti-inflammatory properties[15] by hydrolyzing platelet-activating factor, which plays a role in the activation of platelets, monocytes

and macrophages.[16] The positive association between Lp-PLA2 and cardiovascular disease suggests that the pro-inflammatory effects outweigh the anti-inflammatory effect of the enzyme. The lack of an association with atherosclerosis as found in the present study suggests that the effect of Lp-PLA2 on coronary heart disease is at least partly exerted through another mechanism than atherosclerosis.

In conclusion, Lp-PLA2 activity is only univariately associated with measures of atherosclerosis. The lack of an independent association with atherosclerosis suggests that the effect of Lp-PLA2 on coronary heart disease is at least partly exerted through another mechanism than atherosclerosis. Future studies are needed to further elucidate the role of Lp-PLA2 in the development of atherosclerosis and cardiovascular disease.

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Chapter 6

General discussion

The aim of the studies described in this thesis is to examine the roles of a new non-invasive measure of atherosclerosis, coronary calcification measured by EBT, and a new marker of inflammation, Lp-PLA2, in cardiovascular risk prediction. The merits and shortcomings of the described studies have been discussed in the previous chapters. The present chapter will provide a more general discussion in the light of current knowledge in the field of cardiovascular risk prediction. It includes the background of the thesis, the main findings and suggestions for future research.

Background

Despite a gradual decline in cardiovascular disease in the past decades, cardiovascular disease is and will remain one of the leading causes of death in developed countries. In 2000, 48.6% of the deaths were caused by cardiovascular disease. By 2020, still more than 45% of all deaths are expected to be attributable to cardiovascular disease. Furthermore, in developing countries, cardiovascular disease will soon become the main cause of death with an expected one third attributable to cardiovascular disease in 2020.[1] Known cardiovascular risk factors like obesity, hypertension, hypercholesterolemia, diabetes and smoking only predict 50% of the cardiovascular events.[2] To improve cardiovascular risk assessment, non-invasive measurement of atherosclerosis like coronary calcification has received increasing attention over the past decades. Coronary calcification can be accurately quantified by electron-beam computed tomography (EBT). The amount of coronary calcification measured by EBT is strongly correlated with the amount of coronary atherosclerotic plaque in histopathological studies.[3,4] Therefore, the amount of coronary calcification can be used as a measure of coronary atherosclerosis.

Also the search for new biochemical risk factors may improve cardiovascular risk assessment. Evidence is accumulating that inflammation is a risk factor for cardiovascular disease. Many studies have shown that C-reactive protein is associated with risk of coronary heart disease and risk of stroke.[5-7] Recently, lipoprotein-associated phospholipase A2 (Lp-PLA2) has been suggested as new inflammatory mediator, which predicts risk of cardiovascular disease. The enzyme circulates in the blood bound to low-density lipoprotein (LDL) cholesterol. Its pro-inflammatory properties have been ascribed to its capacity to hydrolyse oxidized phospholipids into lysophosphatidylcholine and free fatty acids.[8] In contrast, Lp-PLA2 also has anti-inflammatory properties by hydrolysing platelet-activating factor (PAF).[9]

The Rotterdam Coronary Calcification Study is a population-based cohort study which started in 1997. The study is embedded in the Rotterdam Study, an ongoing population-based cohort study in 7983 subjects aged 55 years and over who live in

Ommoord, a suburb of Rotterdam. Its overall aim is to investigate determinants of chronic disabling diseases, such as cardiovascular disease, dementia, osteoporosis and visual impairment.[10] At phase one of the Rotterdam Study (1990-1993), all participants were extensively interviewed at home and visited the research center twice. Amongst others, information on cardiovascular risk factors was collected, blood samples were drawn and non-invasive measurement of atherosclerosis was performed. These procedures were repeated at phase two (1994-1995) and phase three (1997-2000). At the third examination, participants were invited to undergo EBT scanning and to participate in the Rotterdam Coronary Calcification Study. All non-institutionalised subjects under age 85 years were invited. Of the 3371 eligible subjects, 2063 participated (response rate: 61%). Complete data were available for 2013 subjects. The studies described in chapter 2, 3 and 4 of this thesis are based on the Rotterdam Coronary Calcification Study. The studies in chapter 5 are based on the Rotterdam Study.

Main findings

Known cardiovascular risk factors like obesity, hypertension, hypercholesterolemia, diabetes and smoking are associated with the amount of coronary calcification.[11-23] However, studies in older subjects are lacking. So far, only one study has been performed in older subjects, finding only associations of smoking and triglycerides with coronary calcification.[23] In chapter 2 results from studies on risk factors for coronary calcification are described. Chapter 2.2 describes the association between known cardiovascular risk factors and coronary calcification in a population of older subjects. Risk factors were measured 7 years before EBT scanning and concurrently to EBT scanning. Age and gender were the most important risk factors for coronary calcification. Calcium scores in men were 5 times higher than in women. The reasons for this large sex difference in calcium score are currently unknown. All known cardiovascular risk factors measured 7 years before scanning are strongly associated with the amount of coronary calcification. However, systolic blood pressure and total cholesterol measured at the time of EBT scanning were not associated with the amount of coronary calcification. An explanation is the more frequent use of antihypertensive and/or lipid lowering drugs at the time of scanning (39% versus 23% for antihypertensive drug use and 16% versus 3% for lipid lowering drug use). This explanation is supported by the stronger associations between those risk factors and coronary calcification in subjects without drug use. Another explanation is the older age of the cohort at the time of scanning. We know from other studies that the strength of cardiovascular risk factors decreases with increasing age.[24-26]

The angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism

is suggested to be associated with coronary heart disease in the general population. Subjects with the DD and ID genotype are considered to have a higher risk of coronary heart disease than subjects with the II genotype. Chapter 2.3 describes a study on the ACE I/D polymorphism and coronary calcification. The ACE I/D polymorphism was not significantly associated with the amount of coronary calcification. This suggests that the ACE I/D polymorphism doesn't play a role in the development of coronary atherosclerosis. However, genetic association studies on candidate genes need large numbers to find small effects. Although we included 2000 subjects in our analyses, the study may lack power to show a significant but relatively weak association. Another explanation for the absence of a positive finding may be that calcification is not a good measure for assessing the effects of the ACE I/D polymorphism. A recent meta-analysis in almost 10.000 subjects showed that the D-allele was positively associated with carotid intima media thickness.[27] While thickening of the intima occurs early in atherosclerosis development, calcification of plaques occurs at a later stage in atherosclerosis development.[28] However, studies have found that the amount of calcified plaques correlates well with the total amount of plaques, and thus should not be considered solely as a measure of calcification.[3,4]

It has long been known that atherosclerosis is a generalised process, which affects all the vessels of the vascular tree. Therefore, measures of extracoronary atherosclerosis like carotid intima media thickness, carotid plaques, aortic calcification and ankle-arm index have been used to predict coronary heart disease events. The development of the EBT scan in the 1980s offered the opportunity to non-invasively measure the amount of coronary calcification. Chapter 3.1 describes to what extent those measures reflect coronary atherosclerosis. All measures of extracoronary atherosclerosis are strongly associated with the amount of coronary calcification. The stronger associations of carotid plaques and aortic calcification with coronary calcification can be ascribed to the measurement of a similar stage of atherosclerosis rather than to a stronger location specific association.

Chapter 4.1 describes the association between angina pectoris according to the Rose questionnaire and coronary calcification. We used the Rose questionnaire, a standardized questionnaire that has been used for decades to assess angina pectoris in population-based studies, to distinguish between non-cardiac and cardiac causes of chest pain. While men with Rose questionnaire angina pectoris compared to those without angina pectoris had a 13-fold increased risk of a calcium score above 1000, women only had a five-fold increased risk of a calcium score above 1000. After adjustment for the large sex difference in the distribution of the calcium score, by using sex-adjusted percentiles, this sex difference remained. The results suggest that the Rose questionnaire more often misclassifies chest pain in women than in men. This is supported by the higher prevalence of Rose questionnaire angina pectoris in

women[29-31] and by studies finding a better prognosis for women with Rose questionnaire angina pectoris than for men.[32-34] An alternative interpretation of the results is that men with angina pectoris have calcium scores that are 5 times higher than women with angina pectoris.

Coronary calcification assessed by EBT may be a useful tool to identify subjects at high risk of coronary heart disease. Several studies have shown that coronary calcification is associated with risk of coronary heart disease.[35-40] Whether the measurement of coronary calcification is additive in conventional risk prediction in all age groups is unresolved. The study described in chapter 4.2 shows that coronary calcification measured by EBT is a strong predictor of coronary heart disease in a general population of older subjects. Furthermore, it shows that coronary calcification predicts coronary heart disease events in all strata of Framingham risk. This study provides useful data on the role of coronary calcification in cardiovascular risk prediction. Most of the former studies have been performed in self-referred populations[36-38,40] which can considered to be extremes of the population and may therefore not be suitable for evaluation of the use of coronary calcification in primary prevention.

Lp-PLA2, an enzyme which is mainly bound to LDL-cholesterol and HDL-cholesterol, is a potential new risk factor for cardiovascular disease.[41-44] Since one of its properties is hydrolysis of oxidized phospholipids leading to lysophosphatidylcholine and free fatty acids, Lp-PLA2 is considered to have pro-inflammatory properties.[8] Several studies have shown an association between Lp-PLA2 and risk of coronary heart disease, but results are inconsistent about the independent role of the enzyme in risk prediction.[41,42,44] So far, no studies have investigated the role of Lp-PLA2 in atherosclerosis and in risk of stroke in the general population. Chapter 5.1 describes the association between Lp-PLA2 and risk of coronary heart disease and stroke. Lp-PLA2 is associated with coronary heart disease and stroke independent of known cardiovascular risk factors, including total cholesterol. To investigate whether atherosclerosis is an intermediate, we studied the association between Lp-PLA2 and several measures of atherosclerosis. The results described in chapter 5.2 suggest that the association between Lp-PLA2 and measures of extracoronary atherosclerosis is weak or absent. No association between Lp-PLA2 and coronary calcification was present in the Rotterdam Coronary Calcification Study, confirming this hypothesis (data not presented). This suggests that atherosclerosis is not an important pathway through which the effects of Lp-PLA2 on cardiovascular disease are exerted.

Future research

Traditional cardiovascular risk factors like diabetes, cigarette smoking, hyper-

tension and hypercholesterolemia are the basis of cardiovascular risk assessment[45,46] accounting for 50% of the coronary heart disease events.[2,47] Recent studies have questioned whether "only 50%" of the coronary heart disease events are explained by traditional cardiovascular risk factors. In these studies the authors measured the number of cardiovascular risk factors in subjects with coronary heart disease. Those studies showed that more than 80% of the subjects suffering from coronary heart disease have at least one of the traditional cardiovascular risk factors.[48,49] However, the latter does not mean that 80% of the coronary heart disease events can be attributed to the known cardiovascular risk factors. In fact, in the Rotterdam Study the population attributable risk of the traditional risk factors was only 40% (Rogier Nijhuis, personal communication) implying that more than 50% of the coronary heart disease events cannot be explained by cardiovascular risk factors. Therefore, improvement of cardiovascular risk prediction is and will remain a key research topic in cardiovascular research for many years. For this purpose, new non-invasive measures of atherosclerosis and new cardiovascular risk factors are of particular interest.

Carotid intima media thickness can be non-invasively assessed by B-mode ultrasound. B-mode ultrasound is a safe, non-invasive and relatively cheap technique. Studies have shown that carotid intima media thickness has a high reproducibility.[63,64] Carotid intima media thickness is an independent predictor of coronary heart disease but its additive role in cardiovascular risk prediction has been shown to be moderate.[65] Ankle-arm index is the ratio between the systolic blood pressure at the ankle (posterior tibial artery) and the systolic blood pressure in the arm. It can simply be measured with a blood pressure cuff and a Doppler ultrasonic sensor, which makes it suitable for general practitioners to use as a screenings tool. Although it is influenced by hemodynamic factors and vascular stiffness, it is considered to be a marker of peripheral atherosclerosis. Studies have shown that it is an independent risk factor for coronary heart disease.[66-70] Studies on its additive role in cardiovascular risk prediction, however, are not yet published.

EBT offers the opportunity to non-invasively quantify the amount of coronary calcification. Since the amount of coronary calcification is closely associated with the amount of coronary atherosclerotic plaque,[3,4] it can be considered as a measure of coronary atherosclerosis. Its main advantage above the non-invasive measures of extracoronary atherosclerosis is the direct measurement of coronary atherosclerosis. So far, most of the studies have been performed in selected populations. Since these studies are conducted in high-risk subjects, the results cannot be extrapolated to the general population. In order to evaluate the role of coronary calcification scanning in primary prevention, large population-based studies are needed to provide stable relative risk estimates. In the Rotterdam Coronary Calcification Study, subjects with a calcium score >1000 had an eight times increased risk of coronary heart disease as

compared to subjects with a calcium score <100, independent of known cardiovascular risk factors. The strength of the association makes measurement of coronary calcification a candidate for screening in the general population. Before coronary calcification screening can be used in the population for assessment of cardiovascular risk, however, screening for coronary calcification in the general population need to be evaluated for its (cost-) effectiveness. Such cost-effectiveness analyses also should select specific subgroups of subjects that benefit most from screening, but more data are needed before these analyses can be performed.

The disadvantage of the EBT scan is its limited availability. The newest generation multidetector CT (MDCT) is widespread available and has the ability to non-invasively quantify the amount of coronary calcification with similar accuracy as the EBT. Using phantom vessels with known calcium content, the sensitivity, specificity and reproducibility of the calcium score measured by the helical ungated CT was shown to be comparable with the calcium score measured by the EBT.[71] This result is promising for the MDCT for identifying subjects at high risk.

Future studies should evaluate the measurement of progression of coronary calcification by EBT or MDCT. The reliability of the Agatston score to score progression of coronary calcification is moderate. A volumetric-based method with isotropic interpolation[72,73] and a mass-based method[73] are shown to have a lower variability than the Agatston score and may be better candidates. Accurate measurement of progression of coronary calcification allows the use of this measure in etiological studies on risk factors for atherosclerosis and as a surrogate endpoint in clinical trials.

MRI is another promising tool for identifying subjects at high risk of coronary heart disease. Studies have shown that MRI can non-invasively measure atherosclerotic plaque composition in extracoronary arteries rather than only detecting calcium.[74] Another advantage of MRI for primary prevention is the absence of radiation. However, due to cardiac and respiratory motion non-invasive detection of atherosclerotic plaques by MRI in asymptomatic subjects is not yet possible in the near future.

Cardiovascular risk factors like C-reactive protein, homocysteine and fibrinogen are associated with an increased risk of coronary heart disease.[5-7,50-59] Recently, Lp-PLA2 has been suggested as a potential new risk factor for cardiovascular disease.[43,44] Studies on the association between Lp-PLA2 and coronary heart disease are conflicting. While some showed that Lp-PLA2 is an independent predictor of coronary heart disease, others failed to do so.[41,42,44] Further studies are needed in several directions. If an independent association with risk of cardiovascular disease can be confirmed, measurement of Lp-PLA2 may help in risk prediction. In fact, it has been suggested that measurement of Lp-PLA2 may be useful in detecting subjects at high risk of coronary heart disease among those with a low LDL-cholesterol.[41] The study described in chapter 5.1, however, shows that Lp-PLA2 is an independent predictor

for coronary heart disease over the entire range of LDL-cholesterol levels suggesting that Lp-PLA2 may be useful in detecting high-risk subjects regardless of their LDL-cholesterol level. Furthermore, future studies should elucidate the mechanism through which Lp-PLA2 exerts its effect on coronary heart disease and stroke. It is known that Lp-PLA2 hydrolyses oxidized phospholipids leading to lysophosphatidylcholine and free fatty acids,[8] but the specific pathways through which Lp-PLA2 exerts its increased risk of coronary heart disease and stroke are not yet known. The absence of an independent association between Lp-PLA2 activity and measures of atherosclerosis suggests that atherosclerosis doesn't play an important role in the effect of Lp-PLA2 on cardiovascular disease. Finally, Lp-PLA2 is currently being considered a new target for pharmacological intervention. Potential drugs to reduce the Lp-PLA2 activity include statins,[60] fibrates[61] and a novel agent that inhibits Lp-PLA2 which is currently in phase II development.[62]

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Summary

Cardiovascular disease is and will remain one of the leading causes of death. Therefore, identification of asymptomatic subjects at high risk is of utmost importance. Traditional cardiovascular risk factors like elevated blood pressure, elevated cholesterol level, diabetes mellitus and cigarette smoking only explain 50% of the coronary heart disease events. Non-invasive measures of atherosclerosis like coronary calcification as detected by electron-beam tomography (EBT) may improve coronary heart disease risk prediction. The studies described in chapter 2, 3 and 4 focus on determinants and predictive value of coronary calcification as detected by EBT. Evidence is accumulating that inflammation plays a role in the development of cardiovascular disease. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a potential new cardiovascular risk factor with pro-inflammatory properties. Chapter 5 describes the role of Lp-PLA2 in cardiovascular disease. The studies described in this thesis have been performed in the Rotterdam Study.

Chapter 2 describes the association between cardiovascular risk factors and coronary calcification. Chapter 2.1 gives an overview of the literature. Known risk factors like obesity, elevated blood pressure, elevated cholesterol levels, diabetes and smoking are associated with the amount of coronary calcification. Although C-reactive protein is associated with coronary heart disease, it is not associated with the amount of coronary calcification. While some studies found an association between fibrinogen and coronary calcification, others failed to do so. In most studies on the association between homocysteine and the amount of coronary calcification no association was present. However, most studies did not include large numbers. Therefore, the absence of an association may well be due to lack of power. Chapter 2.2 describes the association between known cardiovascular risk factors and coronary calcification in a population of older subjects. Age and gender are the most important risk factors for coronary calcification. Calcium scores in men are 5 times higher than in women of the same age. Cardiovascular risk factors measured 7 years before scanning are strongly associated with the amount of coronary calcification. Associations with blood pressure and total cholesterol are weaker when measured at the time of EBT scanning which may be due to older age or use of medication. Although cardiovascular risk factors are strongly associated with the amount of coronary calcification, almost 30% of men and 15% of women without risk factors have extensive calcification. The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is suggested to be associated with coronary heart disease in the general population. Subjects with the DD and ID genotype are considered to have a higher risk of coronary heart disease than subjects with the II genotype. Chapter 2.3 describes a study on the ACE I/D polymorphism and coronary calcification. The ACE I/D polymorphism is not significantly associated with the amount of coronary calcification. This suggests that the ACE I/D polymorphism doesn't play an important role in the development of

coronary atherosclerosis.

It has long been known that atherosclerosis is a generalised process, which affects all the vessels of the vascular tree. Therefore, measures of extracoronary atherosclerosis like carotid intima media thickness, carotid plaques, aortic calcification and ankle-arm index have been used to predict coronary heart disease events. Chapter 3.1 describes to what extent those measures reflect coronary atherosclerosis. All measures of extracoronary atherosclerosis are strongly associated with the amount of coronary calcification. The stronger associations of carotid plaques and aortic calcification with coronary calcification can be ascribed to the measurement of a similar stage of atherosclerosis rather than a stronger location specific association. Chapter 3.2 describes the association between angina pectoris according to the Rose questionnaire and coronary calcification. The Rose questionnaire is a standardized questionnaire that has been used for decades to assess angina pectoris in population-based studies. While men with Rose questionnaire angina pectoris compared to those without angina pectoris have a 13-fold increased risk of a calcium score above 1000, women only have a five-fold increased risk of a calcium score above 1000. After adjustment for the large sex difference in the distribution of the calcium score, by using sex-adjusted percentiles, this sex difference remained. The results suggest that the Rose questionnaire more often misclassifies chest pain in women than in men.

Whether measurement of coronary calcification is additive to conventional risk prediction in all age groups is unresolved. The study described in chapter 4.1 shows that coronary calcification measured by EBT is a strong predictor of coronary heart disease in a general population of older subjects. Subjects with a calcium score >1000 had an eight times increased risk of coronary heart disease compared to those with a calcium score of 0 to 100. Furthermore, it shows that coronary calcification predicts coronary heart disease events in all strata of Framingham risk.

Lp-PLA2, an enzyme which is mainly bound to LDL-cholesterol and HDL-cholesterol, is a potential new risk factor for cardiovascular disease with pro-inflammatory properties. Chapter 5.1 describes the association between Lp-PLA2 and risk of coronary heart disease and stroke. Lp-PLA2 is associated with risk of coronary heart disease and stroke independent of known cardiovascular risk factors, including total cholesterol. To investigate whether atherosclerosis is an intermediate in the pathway, we studied the association between Lp-PLA2 and several measures of atherosclerosis. The results described in chapter 5.2 suggest that the association between Lp-PLA2 and measures of extracoronary atherosclerosis is weak or absent. This suggests that atherosclerosis is not an important pathway through which the effects of Lp-PLA2 on cardiovascular disease are exerted.

Samenvatting

Hart- en vaatziekten is een van de belangrijkste doodsoorzaken. Om deze reden is identificatie van mensen zonder klachten met een hoog risico op hart- en vaatziekten van groot belang. Traditionele risicofactoren zoals hoge bloeddruk, hoog cholesterol, suikerziekte en roken verklaren slechts 50% van de gevallen van coronaire hartziekte. Niet-invasieve maten van atherosclerose zoals coronaire verkalking gemeten met elektronenbundeltomografie (EBT) kunnen bijdragen aan het verbeteren van risicopredictie. De onderzoeken in hoofdstuk 2, 3 en 4 beschrijven determinanten en voorspellende waarde van coronaire verkalking gedetecteerd met EBT. Er komt steeds meer bewijs dat ontsteking een rol speelt in het ontstaan van hart- en vaatziekten. Lipoproteine-gebonden fosfolipase A2 (Lp-PLA2) is een potentiële nieuwe risicofactor voor hart- en vaatziekten met ontstekingsbevorderende eigenschappen. Hoofdstuk 5 beschrijft de rol van Lp-PLA2 bij de ontwikkeling van hart- en vaatziekten. Alle onderzoeken zijn uitgevoerd in het Erasmus Rotterdam Gezondheids Onderzoek (ERGO), een vervolgonderzoek onder bijna 8000 ouderen in de Rotterdamse wijk Ommoord.

Hoofdstuk 2 beschrijft de relatie tussen cardiovasculaire risicofactoren en coronaire verkalking. Hoofdstuk 2.1 geeft een overzicht van de literatuur. Bekende risicofactoren zoals overgewicht, hoge bloeddruk, hoog cholesterol, suikerziekte en roken zijn gerelateerd met de hoeveelheid coronaire verkalking. Hoewel de ontstekingsmarker C-reactief proteïne geassocieerd is met coronaire hartziekte, is het niet geassocieerd met de hoeveelheid coronaire verkalking. Voor fibrinogeen en homocysteine worden wisselende resultaten gevonden maar de meeste onderzoeken konden geen verband aantonen. Echter, de meeste onderzoeken hadden relatief kleine aantallen, wat de afwezigheid van een significant verband kan verklaren. Hoofdstuk 2.2 beschrijft de relatie tussen bekende cardiovasculaire risicofactoren en coronaire verkalking in een populatie van oudere mensen. Leeftijd en geslacht zijn de belangrijkste risicofactoren voor coronaire verkalking. Kalkscores bij mannen zijn 5 keer zo hoog als kalkscores bij vrouwen van dezelfde leeftijd. Cardiovasculaire risicofactoren gemeten 7 jaar voor de EBT scan zijn sterk geassocieerd met de hoeveelheid coronaire verkalking. Relaties zijn zwakker voor bloeddruk en cholesterol wanneer deze gemeten worden ten tijde van de EBT scan, wat mogelijk komt door de hogere leeftijd op het latere bezoek of het toegenomen gebruik van medicatie. Hoewel cardiovasculaire risicofactoren sterk geassocieerd zijn met de hoeveelheid coronaire verkalking heeft bijna 30% van de mannen en 15% van de vrouwen zonder risicofactoren een hoge mate van verkalking. Diverse onderzoeken suggereren dat het D-allel van het insertie/deletie (I/D) polymorfisme op het angiotensin-converting enzyme gen (ACE) de kans op coronaire hartziekte in de algemene bevolking verhoogt. Echter, het ACE I/D polymorfisme is niet geassocieerd met de hoeveelheid coronaire verkalking (hoofdstuk 2.3). Dit suggereert dat het ACE I/D polymorfisme geen belangrijke rol speelt in de ontwik-

keling van coronaire atherosclerose.

Het is reeds lange tijd bekend dat atherosclerose een gegeneraliseerd proces is. Om deze reden worden maten van extracoronaire atherosclerose zoals intima media dikte van de halsslagader, plaques van de halsslagader, aorta verkalking en enkel-arm index gebruikt om het risico van coronaire hartziekte te voorspellen. Hoofdstuk 3.1 beschrijft in welke mate deze maten van extracoronaire atherosclerose coronaire atherosclerose weerspiegelen. Alle bovengenoemde maten van extracoronaire atherosclerose zijn sterk geassocieerd met de hoeveelheid coronaire verkalking. De verbanden van plaques van de halsslagader en aorta verkalking met coronaire verkalking zijn sterker dan voor de andere 2 maten van atherosclerose. Dit kan waarschijnlijk beter toegeschreven worden aan het meten van dezelfde stadia van atherosclerose dan aan locatiespecifieke verschillen. Hoofdstuk 3.2 beschrijft hoe angina pectoris volgens de Rose questionnaire met coronaire verkalking samenhangt. De Rose questionnaire is een gestandaardiseerde vragenlijst die gebruikt wordt om angina pectoris vast te stellen in epidemiologisch vervolgonderzoek. Terwijl mannen met angina pectoris volgens de Rose questionnaire een 13 keer verhoogde kans hebben op een hoge kalkscore (kalkscore >1000), hebben vrouwen met angina pectoris een 5 keer verhoogde kans op een hoge kalkscore. Na correctie voor het grote geslachtsverschil in kalkscore, door geslachtsgecorrigeerde percentielen te gebruiken, bleef het verschil tussen mannen en vrouwen bestaan. De resultaten suggereren dat de Rose questionnaire vaker pijn op de borst bij vrouwen misclassificeert dan bij mannen.

Of meting van coronaire verkalking toevoegt aan de predictie van het optreden van coronaire hartziekte is onbekend. Het onderzoek beschreven in hoofdstuk 4.1 laat zien dat coronaire verkalking gemeten met EBT een sterke voorspeller is van coronaire hartziekte in een algemene populatie van ouderen. Personen met een kalkscore boven de 1000 hadden een 8 keer verhoogde kans op het ontwikkelen van coronaire hartziekte in vergelijking met personen met een kalkscore van 0-100. Bovendien voorspelt coronaire verkalking de kans op coronaire hartziekte onafhankelijk van bekende cardiovasculaire risicofactoren.

Lp-PLA2, een enzym dat gebonden is aan LDL-cholesterol, is een potentiële nieuwe risicofactor voor hart- en vaatziekten met pro-inflammatoire eigenschappen. Hoofdstuk 5.1 beschrijft dat een hoge activiteit van Lp-PLA2 de kans op coronaire hartziekte en beroerte met meer dan 70% verhoogt onafhankelijk van bekende cardiovasculaire risicofactoren inclusief cholesterol. Om te onderzoeken wat de rol van atherosclerose is, bestudeerden we de relatie tussen Lp-PLA2 en verschillende maten van atherosclerose. De resultaten beschreven in hoofdstuk 5.2 laten zien dat het verband tussen Lp-PLA2 en maten van extracoronaire atherosclerose zwak of afwezig is. Dit suggereert dat atherosclerose geen belangrijke rol speelt in de relatie tussen Lp-PLA2 en hart- en vaatziekten.

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List of publications

Duncker DJ, Oei HHS, Hu F, Stubenitsky R, Verdouw PD. Role of K(ATP)(+) channels in regulation of systemic, pulmonary, and coronary vasomotor tone in exercising swine. Am J Physiol Heart Circ Physiol 2001;280:H22-33.

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