Suzette Elias-Smale

# Coronary artery calcification and cardiovascular risk prediction

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Suzette E. Elias-Smale

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# Coronary artery calcification and cardiovascular risk prediction

Verkalking in de kransslagaderen
en cardiovasculaire risico predictie

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*Aan mijn ouders,*
*voor Sjoerd, Josefien en Quirijn*

## MANUSCRIPTS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

**Chapter 2.1**
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**Chapter 4.2**
Vliegenthart-Proença R, Elias-Smale SE, van Rooij F, Hofman A, Witteman JCM, Oudkerk M. Coronary calcification has a very high negative predictive value in older adults; The Rotterdam Study. Submitted.

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Elias-Smale SE, Kavousi M, Verwoert GC, Koller MT, Steyerberg EW, Mattace-Raso FUS, Hofman A, Hoeks APG, Reneman RS, Witteman JCM. Common Carotid intima-media thickness in cardiovascular risk stratification of older persons; The Rotterdam Study. Eur J Cardiovasc Prev Rehabil 2011;June 22 (Epub ahead of print).

**Chapter 4.4**

Elias-Smale SE, Wieberdink RG, Odink AE, Hofman A, Hunink MGM, Koudstaal PJ, Krestin GP, Breteler MMB, van der Lugt A, Witteman JCM. Burden of atherosclerosis predicts coronary heart disease but not cerebrovascular events; The Rotterdam Study. Eur Heart J 2011;32:2050-8.

**CONTENTS**

# CHAPTER 1

## Introduction

## INTRODUCTION

Prevention of cardiovascular events starts with predicting one's cardiovascular risk, to be used as a solid base for the required level of intensity of preventive measures. Hence, the accuracy of our risk prediction is vital. Risk assessment algorithms, based on traditional risk factors, such as the U.S. Framingham Risk Score[1] and its European counterpart SCORE (Systemic COronary Risk Evaluation),[2] are widely used to estimate absolute 10-year risk of cardiovascular events. Traditional cardiovascular risk factors like age, gender, blood pressure and cholesterol are clearly related to the severity of atherosclerosis, the underlying mechanism of cardiovascular disease (CVD). However, at every level of risk factor exposure, there is substantial variation in the quantity of atherosclerosis. This variation in disease is probably due to interactions between risk factors, duration of exposure to the specific level of the risk factors, genetic disparity and aspects as biological and laboratory variability in the risk factor. Therefore, measuring the amount of atherosclerosis, representing the end result of risk exposures, might be useful to improve CVD risk prediction.

The amount of arterial calcification has been shown to adequately reflect a person's burden of atherosclerosis.[3, 4] Assessment of arterial calcification is typically performed by electron beam- or multi-detector-computed tomography (EBCT/MDCT). An advantage of this modality is that it can measure atherosclerosis in multiple vascular beds simultaneously. Since atherosclerosis is a systemic process, with a substantial number of persons afflicted with disease in more than one vascular bed, risk prediction is expected to benefit from targeting various cardiovascular events (i.e. myocardial infarction and stroke) simultaneously.[5, 6] Until now, research primarily focused on coronary calcification in relation to coronary heart disease (CHD). The relation of coronary calcium with stroke is still unclear. Furthermore, the value of assessment of arterial calcification in other vascular beds has only scarcely been studied.

The purpose of this thesis was to expand our knowledge of coronary calcification and explore the value of calcification of the thoracic aorta and carotid arteries for the improvement of cardiovascular risk prediction beyond traditional risk factors.

Most studies were conducted within the Rotterdam Study, a population-based cohort study among 7,983 men and women aged 55 years and older, living in a well-defined suburb of Rotterdam, The Netherlands.[7] The baseline visit took place from 1990-1993. In 2000-2001, the cohort was extended with 3,011 persons who had reached the age of 55 or moved into the suburb after start of the study. Study Center visits took place every three years, where cardiovascular risk factors and measurements of atherosclerosis were performed. In 1999-2000, 2,292 participants underwent an EBCT scan to assess their amount of coronary calcification. In 2003-2006, another 2,524 participants underwent an MDCT scan to measure their amount of coronary, aortic arch and carotid calcium.

Both sub-cohorts were followed-up for occurrence of coronary heart disease and cerebrovascular disease events.

Chapter 2 of this thesis focuses on the association of arterial calcification with other novel risk markers of cardiovascular disease. Chapter 2.1 describes the relation of C-reactive protein, a marker of inflammation, with arterial calcification and other measures of atherosclerosis. Chapter 2.2 is dedicated to the relation of resting heart rate with the amount of coronary calcification. This Study was performed within 1,014 healthy older participants of the ADVANCE study.[8]

Chapter 3 depicts the relation of arterial calcification with cardiovascular disease risk. In chapter 3.1, the relation of coronary calcification with the risk of heart failure is described, whereas chapter 3.2 focuses on coronary, aortic arch and carotid artery calcification in association with history of stroke.

Chapter 4 is dedicated to the additional value of arterial calcification beyond traditional risk factors in cardiovascular risk prediction. Chapter 4.1 focuses on the question whether coronary calcium scoring improves classification of coronary heart disease risk beyond traditional risk factors. Chapter 4.2 looks into the negative predictive value of coronary calcification for development of coronary heart disease. Chapter 4.3 answers the question whether carotid intima-media thickness, a non-calcification measure of atherosclerosis, improves cardiovascular risk prediction. Chapter 4.4 describes the value of coronary, aortic arch and carotid artery calcification in the risk prediction of coronary heart disease and cerebrovascular disease events. The general discussion of this thesis (Chapter 5) elaborates on the question whether coronary calcium scoring, to date the most established measure of arterial calcification with regard to its usefulness in cardiovascular risk prediction, is ready to be put into clinical practice.

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# CHAPTER 2

## Coronary calcification and other risk markers of cardiovascular disease

**Manuscript based on this chapter**

Elias-Smale SE, Kardys I, Oudkerk M, Hofman A, Witteman JCM. C-reactive protein is related to extent and progression of coronary and extra-coronary atherosclerosis; Results from the Rotterdam study. Atherosclerosis 2007;195:e195-202.

# CHAPTER 2.1

## C-reactive protein and extent and progression of (extra-)coronary atherosclerosis


**SUMMARY**

**Background:** Although prospective studies have unequivocally shown that C-reactive protein (CRP) is an independent predictor of future cardiovascular events, studies on the association between CRP and atherosclerosis have provided inconsistent results. We investigated the association of CRP with extent and progression of atherosclerosis in multiple vessel beds in a large, population-based cohort study.

**Methods:** In the Rotterdam Study, standardized measurements of coronary and extra-coronary atherosclerosis were performed in 1,962 persons and 6,582 persons, respectively. Progression of extra-coronary atherosclerosis during a mean follow-up period of 6.4 years was assessed in 3,757 persons.

**Results:** Independent and graded associations were found of CRP with the number of carotid plaques and carotid plaque progression ((OR 1.72; 95% CI 1.14–2.59) for severe progression in participants with CRP >3 mg/dl versus participants with CRP <1 mg/dl). Similarly, CRP was independently and graded related to ankle-brachial-index (ABI) and worsening ABI over the years ((OR 1.99; 95% CI 1.37–2.88) for severe progression in participants with CRP >3 mg/dl versus participants with CRP <1 mg/dl). Although CRP was independently related to the highest level of carotid intima-media thickness (cIMT), the association with change in cIMT was not significant. Furthermore, there was an

independent, graded relation between CRP and aortic calcification, but no independent association was observed with progression of aortic calcification, or with the amount of coronary calcification.

**Conclusion:** In this population-based study, independent and graded associations were present of CRP with extent and progression of carotid plaques and ABI, while associations with carotid IMT and aortic and coronary calcification were less pronounced.

## INTRODUCTION

Multiple prospective studies have unequivocally shown that C-reactive protein (CRP) is an independent predictor of future cardiovascular events including myocardial infarction, stroke and peripheral vascular disease.[1–3] Studies published on the association between CRP and established measures of atherosclerosis, however, have provided inconsistent results. In some studies, the association was independently present in the whole study population,[2,4–11] in women only[12] or in men only.[13–16] Other studies found a positive association that was lost after adjustment for cardiovascular risk factors.[13,17–19] Several studies did not find any relation between CRP and atherosclerosis.[20,21]

Studies performed thus far have several limitations. Firstly, most studies are cross-sectional rather than longitudinal while inflammation is thought to play an important role in the progression of atherosclerosis.[22] Secondly, some studies measured artery calcification which is considered to represent stable atherosclerosis, or increased intima-media thickness which is thought to reflect early atherosclerosis,[23] stages in which CRP may play a less important role.[22,24] Thirdly, most studies did not look at CRP in relation to graded levels of atherosclerosis. Finally, many studies had selected or small study populations and most studies examined one vessel bed only.

The Rotterdam Study is a large, prospective, population-based cohort study with standardized measurements of atherosclerosis. We studied the association of CRP with extent and progression of atherosclerosis in multiple vessel beds. We were able to substantially increase the number of subjects available for analysis compared to earlier work on this topic in a limited random sample of the Rotterdam Study,[6] thus increasing precision leading to stronger conclusions.

## METHODS

### Study population

The Rotterdam Study is a prospective, population-based cohort study including 7,983 men and women ≥55 years of age (response rate 78%). A detailed description of the rationale and design of the Rotterdam Study has been given elsewhere.[25]

Between visit 1 (1990–1993) and 3 (1997–1999), 1,859 (23.3%) participants died and 135 (1.7%) were lost to follow-up or not invited due to logistic reasons. Furthermore, 1,193 (14.9%) persons did not participate in the third visit. At the third visit, 2,063 subjects (response rate 61%) underwent an electron beam CT scan (EBCT).[26] The Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study, and all participants gave informed consent.

### Measurement of CRP

At visits 1 and 3, C-reactive protein was measured in serum using a nephelometric method (Immage, Beckman Coulter).This technique has a within-run precision <5.0%, a total precision <7.5%, and a reliability coefficient of 0.995. The serum was kept frozen at −20 °C (1990–1993) or −80 °C (1997–1999).

### Measures of extent 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).

The common carotid artery, carotid bifurcation, and internal carotid artery were examined on both the left and right sides for the presence of a plaque. Per location a point was added to a plaque score when this location showed the presence of atherosclerotic plaque. Thus, a total plaque score between 0 and 6 was obtained for each participant.[27] The categories of 0–6 plaques comprised 40.4%, 15.2%, 18.3%, 8.9%, 9.9%, 3.7%, and 3.6% of the study population, respectively.

Common carotid IMT was determined as the average of near- and far-wall measurements, and the average of left and right common carotid IMT was computed.[28] For the analyses, cIMT was categorized into deciles (cut-off values (mm): 0.62, 0.66, 0.70, 0.74, 0.78, 0.81, 0.86, 0.91, and 1.00).

Aortic calcification was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. Calcification of the posterior abdominal aortic wall was scored according to the length of the involved area along the lumbar spine (L1–L4) with scores of 0–5 corresponding to 0, ≤1, 1.1–2.4, 2.5–4.9, ≥5.0–9.9, and ≥10 cm, respectively. The categories of 0–5 plaques comprised 33.1%, 9.4%, 26.9%, 17.7%, 10.6%, and 2.2% of the study population, respectively.

The ratio of the systolic blood pressure at the ankle (8-MHz continuous-wave Doppler probe (Huntleigh 500D, Huntleigh Technology)) to the systolic blood pressure at the arm (random-zero sphygmomanometer) was computed to obtain the ABI.[4] Because arterial rigidity prevents arterial compression leading to spuriously high values of the ABI, an ABI > 1.50 was considered invalid.[29] For the analyses, we used the leg with the lowest value of ABI and categorized ABI into deciles (cut-off values: 1.29, 1.23, 1.18, 1.13, 1.09, 1.05, 0.99, 0.91, and 0.76).

Coronary calcifications in the epicardial coronary arteries were detected on EBCT scans. Imaging was performed with a C-150 Imatron scanner (GE-Imatron) as described previously.[26] Calcium scores were calculated according to Agatston's method[30] and divided into five categories: 0–10, >10–100, >100–500, >500–1000, and >1000. The median duration between visit 3 and EBCT scanning was 50 days.

### Measures of progression of atherosclerosis

Progression of atherosclerosis was computed for each of the four extra-coronary measures by subtracting the extent of atherosclerosis at visit 1 from the extent at visit 3. We categorized this new variable into no, mild, moderate and severe progression. To ensure comparable categories between the different measures of atherosclerotic progression (i.e. progression of aortic calcification, carotid plaques, cIMT and ABI), we choose cut-off points for each variable that resulted in more or less the same number of subjects in that category (i.e. no, mild, moderate or severe progression) for all progression variables. We chose to categorize the progression variables instead of analyzing the data in a continuous way because we expected a non-linear relation with

CRP, assuming the strongest relation with severe progression.[22,33]

For progression of aortic calcification, baseline and follow-up X-ray films were examined in pairs. We defined no, mild, moderate, and severe progression of aortic calcification as a progression of 0, ≤1, 1.1–2.4, and ≥2.5 cm of aortic calcification along the lumbar spine. None of the participants showed a decrease in the extent of aortic calcification. We defined no, mild, moderate, and severe progression of carotid plaques as an increase of 0, 1, 2, or ≥3 plaque locations, respectively. Participants with a decrease in plaque score were added to the group with no progression. We based the categories of no, mild, moderate, and severe progression of the continuous variables cIMT and ABI (leg with largest decrease) on the 30th, 60th, and 90th percentile of the sample distribution. The mean interval between extra-coronary measurements at visits 1 and 3 was 6.4±0.4 years.

### Assessment of covariables

At visits 1 and 3, covariates were ascertained using standard procedures as described previously.[26,28] Diabetes mellitus was considered to be present when fasting blood

glucose exceeded 7.0 mmol/L, non-fasting glucose exceeded 11.0 mmol/L, and/or antidiabetic medication was used. History of cardiovascular disease included history of myocardial infarction, stroke or presence of peripheral artery disease according to the Rose criteria.[31]

### Populations for analyses

The following participants were included: (A) 6,582 persons in whom CRP and at least one measure of extra-coronary atherosclerosis were assessed at visit 1. Within this group, measurements of carotid plaques, cIMT, aortic atherosclerosis, and ABI were available for 5,267, 4,385, 5,429, and 5,959 participants, respectively. (B) 3,757 participants in whom information on CRP at visit 1 and at least one measure of atherosclerosis at visit 1 and visit 3 were available. Within this group, information on progression of carotid plaques, cIMT, aortic calcification and ABI was available for 2,661, 2,301, 2,565, and 3,298 participants, respectively. (C) 1,962 participants of visit 3 in whom both CRP and a coronary calcification score were obtained.

### Statistical analyses

We performed analyses of variance to compute geometric means of CRP for categories of atherosclerosis and used a Student *t*-test to compare categories against the reference group (lowest amount of atherosclerosis). Linear regression analysis was used as a test for trend. Because the distribution of CRP was highly skewed, log CRP was used for analysis of variance and linear regression analysis. Outliers (values >3*standard deviation (SD) of the population distribution of log CRP; study population A, n=25; B, n=4; C, n=6) were excluded.

Using multinomial logistic regression analysis, we examined the association of CRP measured at visit 1 with mild, moderate, and severe progression of atherosclerosis. CRP was divided in categories of <1 mg/L, 1–3 mg/L, and >3 mg/L [32]. These categories comprised 34%, 42%, and 24% of the study population, respectively. CRP <1 mg/L served as the reference category. Participants who already had the maximum number of carotid plaques at visit 1 were excluded because of lacking ability of progression (n=52).

In all analyses, we used two models. In model 1, analyses were adjusted for age and sex. In model 2, we additionally adjusted for smoking status and number of pack-years, systolic and diastolic blood pressure, anti-hypertensive medication, total cholesterol, high-density lipoprotein (HDL) cholesterol, cholesterol-lowering medication, body-mass-index (BMI), hormone replacement therapy (for women), cardiovascular history and, in case of analyses of progression, duration of follow-up and baseline level of atherosclerosis. To test whether the relation between CRP and extent or progression of atherosclerosis was different for men and women, we added an interaction term to the regression model (atherosclerosis measure × gender for the linear regression, CRP × gender for the multinomial regression). If the interaction term was statistically significant ($P<0.05$), we conducted analyses for men and women separately.

Missing data on covariates were imputed by single imputation using the Expectation Maximization (EM) algorithm. Values for cardiovascular risk factors were missing mainly due to logistic reasons (e.g. absence of sonographers). Analyses were performed with SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

## RESULTS

Table 1 displays the baseline characteristics of the three study populations. Baseline characteristics of the populations with measurements of extra-coronary extent and progression of atherosclerosis were assessed at visit 1 (1990–1993) while characteristics of the population with coronary calcification measurement were assessed at visit 3 (1997–1999).

**Table 1.** Baseline characteristics of the study populations

| Variable | Visit 1 | | Visit 3 |
|---|---|---|---|
| | Extra-coronary Atherosclerosis (n=6,582) | Progression of Atherosclerosis (n=3,757) | Coronary Atherosclerosis (n=1,962) |
| Age, y | 69.5±9.2 | 65.7±6.9 | 71.3±5.7 |
| Male, % | 40.6 | 42.5 | 46.6 |
| Body-mass-index, kg/m$^2$ | 26.3±3.7 | 26.2±3.5 | 27.0±3.9 |
| Systolic blood pressure, mmHg | 139±22 | 136±21 | 143±21 |
| Diastolic blood pressure, mmHg | 74±12 | 74±11 | 76±11 |
| Total cholesterol, mmol/L | 6.6±1.2 | 6.7±1.2 | 5.7±1.0 |
| HDL-cholesterol, mmol/L | 1.3±0.4 | 1.4±0.4 | 1.3±0.4 |
| Smokers, % | | | |
| Current | 22.9 | 21.5 | 16.0 |
| Past | 42.0 | 45.2 | 54.4 |
| Pack-years smokers | 22.5 (9.0-40.0) | 21.5 (9.0-37.5) | 18.5 (8.0-32.2) |
| Diabetes mellitus, % | 11.0 | 7.2 | 12.1 |
| Cardiovascular history,* % | 16.1 | 11.2 | 17.0 |
| Anti-hypertensive medication, % | 17.8 | 16.2 | 28.7 |
| Cholesterol-lowering medication, % | 3.3 | 4.2 | 19.2 |
| Hormone-replacement therapy, % | 15.6 | 18.8 | 20.7 |
| Carotid plaque score >0, % | 59,6 | 42,4 | - |
| Intima-media thickness, mm | 0.80±0.16 | 0.76±0.14 | - |
| Aortic plaque score >0, % | 66,9 | 69,2 | - |
| Ankle-brachial-index | 1.05±0.23 | 1.11±0.18 | - |
| Coronary calcification score >0, % | - | - | 77,0 |
| CRP, mg/L | 1.87 (0.91-3.64) | 1.62 (0.79-3.06) | 2.44 (1.26-4.45) |

Categorical variables are presented as percentage. Continuous values are expressed as mean ± standard deviation. Median (inter-quartile range) is presented in case of skewed distribution. *History of cardiovascular disease includes history of myocardial infarction, stroke and/or peripheral artery disease.

Geometric mean CRP levels (mg/L) for categories of measures of coronary and extra-coronary atherosclerosis are presented in Fig. 1. After adjusting for age and sex (model 1), a strong and graded increase in CRP level was observed with increasing number of carotid plaques, whereas CRP levels only rose across the highest 3 deciles of carotid IMT, corresponding to a cIMT above 0.86 mm. A graded increase of CRP levels was also seen across incremental categories of aortic calcification. Furthermore, CRP levels gradually rose with decreasing ABI, but most pronounced in the highest 2 deciles, corresponding to an ABI below 0.91. With increasing level of coronary artery calcification, a gradual rise in CRP was observed, but only modestly compared to the extra-coronary measures. After multivariable adjustment (model 2), the association between CRP level and extent of atherosclerosis was attenuated in the extra-coronary measures but remained statisti-

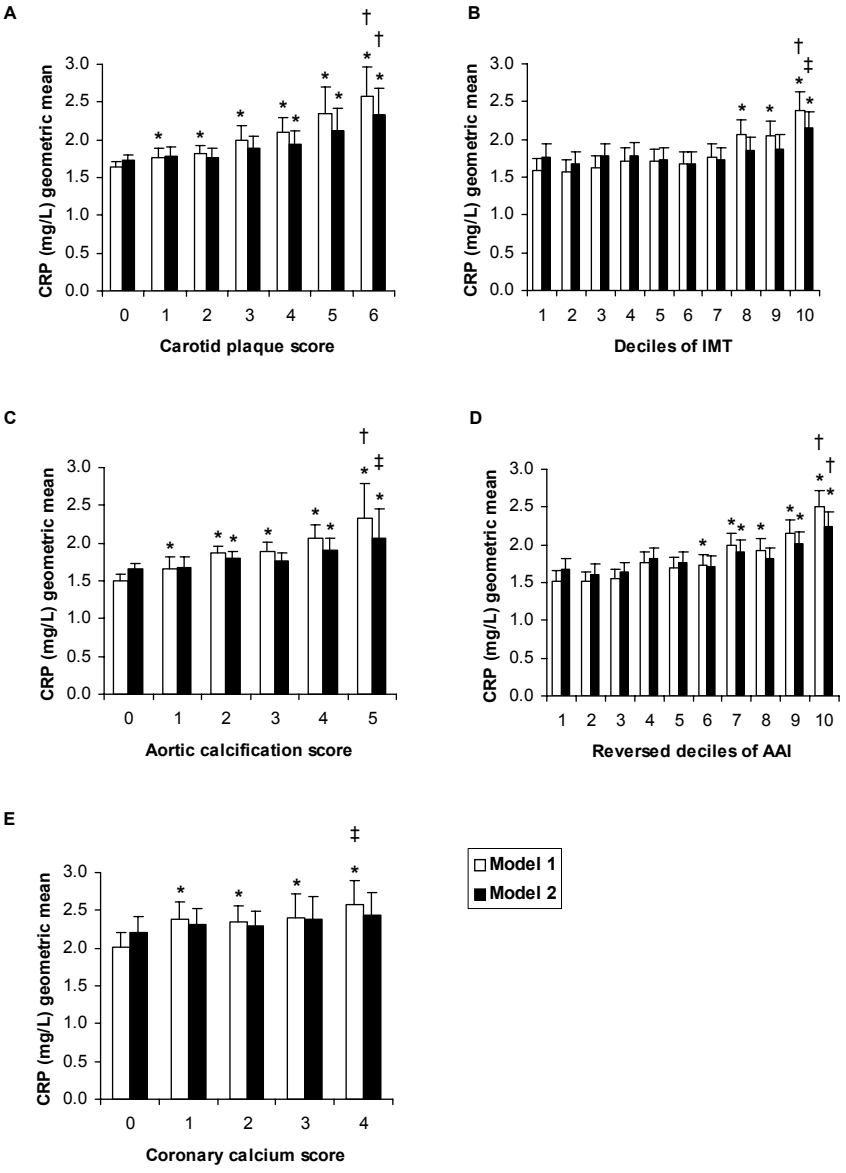


**Figure 1.** Geometric mean CRP level (mg/L) for categories of carotid plaque, cIMT, aortic calcification, ABI and coronary calcification.

CRP: C-reactive protein, cIMT: carotid intima-media thickness, ABI: ankle-brachial-index. Model 1: age and sex adjusted. Model 2: additionally adjusted for BMI, systolic and diastolic blood pressure, anti-hypertensive medication, total cholesterol, HDL- cholesterol, cholesterol-lowering medication, smoking status, number of pack-years, serum glucose, anti-diabetic medication, hormone replacement therapy (for women) and cardiovascular history. * Significant higher geometric mean CRP level as compared to the reference category (no atherosclerosis) ($p<0.05$). † P for trend <0.001. ‡ P for trend <0.05.

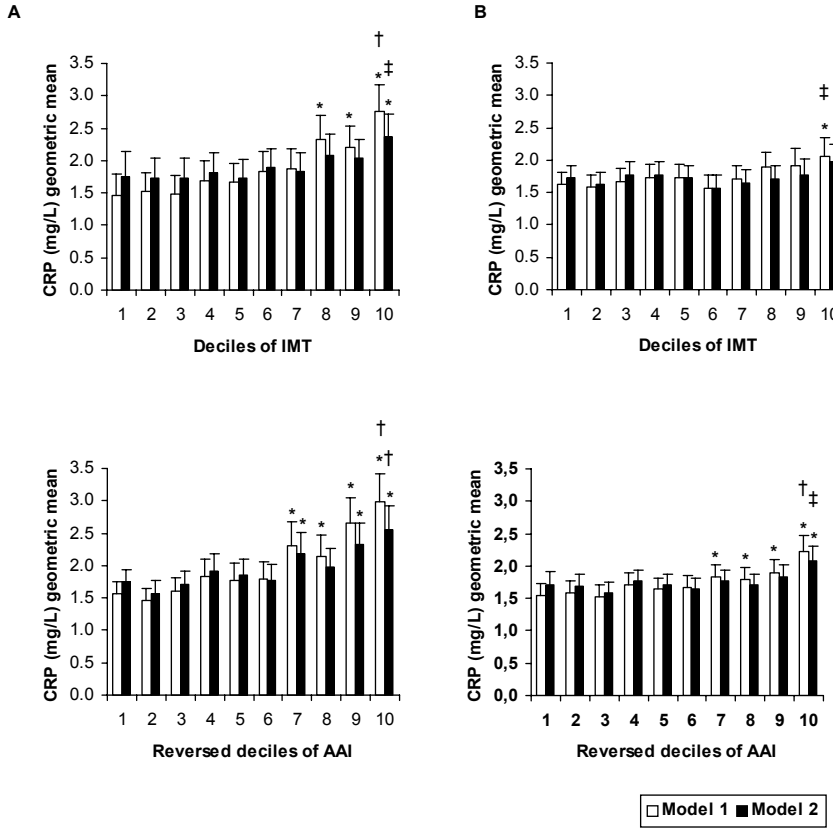


**Figure 2.** Geometric mean CRP level (mg/L) for deciles of cIMT and ABI in men (A) and women (B). CRP: C-reactive protein, cIMT: carotid intima-media thickness, ABI: ankle-brachial-index. Model 1: age and sex adjusted. Model 2: additionally adjusted for BMI, systolic and diastolic blood pressure, anti-hypertensive medication, total cholesterol, HDL-cholesterol, cholesterol-lowering medication, smoking status, number of pack-years, serum glucose, anti-diabetic medication, hormone replacement therapy (for women) and cardiovascular history. * Significant higher geometric mean CRP level as compared to the reference category (no atherosclerosis) ($P<0.05$). † P for trend <0.001. ‡ P for trend <0.05.

cally significant for the highest levels of atherosclerosis. No independent association was found between CRP and coronary artery calcification. Tests for trend were all significant except for coronary calcification after multivariable adjustment (Fig. 1).

Since the interaction term of gender × atherosclerosis level was statistically significant for carotid IMT and ABI, we analyzed the relation of CRP with cIMT and ABI for men and women separately. For both measures, the association between CRP and extent of atherosclerosis showed a similar pattern compared to the overall results on cIMT and ABI, but was stronger in men than in women (Fig. 2). The tests for trend were all significant except for cIMT of women after multivariable adjustment (beta's, 95%CI) for model 2

were (0.03, 0.01–0.05) for men and (0.01, –0.01 to 0.03) for women and for ABI (0.04, 0.03–0.06) for men and (0.02, 0.01–0.03) for women (Fig. 2).

Table 2 shows the odds ratios for risk of mild, moderate, and severe progression of atherosclerosis in comparison to no progression for categories of CRP level. CRP levels >3 mg/L were independently related to severe carotid plaque progression and severe decrease in ABI over time. The association with progression of aortic calcification was weaker and there was no clear relation between CRP and change in carotid IMT. Risk estimates did not change when baseline level of atherosclerosis was not included in the model.

**Table 2.** CRP in relation to progression of atherosclerosis during 6.4 years of follow-up

| Degree of Progression | N | Odds Ratios (95% CI) Model 1 | | | Odds Ratios (95% CI) Model 2 | | |
|---|---|---|---|---|---|---|---|
| | | CRP (mg/L) | | | CRP (mg/L) | | |
| **Carotid plaques** | | <1 | 1-3 | >3 | <1 | 1-3 | >3 |
| Mild | 581 | 1.0 | 1.06 (0.84-1.33) | 1.29 (1.00-1.67) | 1.0 | 1.01 (0.80-1.27) | 1.24 (0.94-1.63) |
| Moderate | 318 | 1.0 | 1.14 (0.86-1.51) | 1.10 (0.79-1.54) | 1.0 | 1.03 (0.77-1.38) | 1.02 (0.71-1.46) |
| Severe | 229 | 1.0 | 1.36 (0.96-1.93) | 1.99 (1.37-2.90) | 1.0 | 1.12 (0.78-1.62) | 1.72 (1.14-2.59) |
| **Carotid IMT** | | | | | | | |
| Mild | 690 | 1.0 | 1.31 (1.03-1.68) | 1.25 (0.94-1.66) | 1.0 | 1.35 (1.04-1.75) | 1.38 (1.01-1.88) |
| Moderate | 690 | 1.0 | 1.07 (0.84-1.36) | 1.06 (0.80-1.40) | 1.0 | 1.01 (0.78-1.31) | 1.05 (0.77-1.44) |
| Severe | 229 | 1.0 | 1.21 (0.85-1.73) | 1.41 (0.95-2.10) | 1.0 | 0.99 (0.68-1.45) | 1.16 (0.74-1.79) |
| **Aortic calcification** | | | | | | | |
| Mild | 867 | 1.0 | 0.95 (0.76-1.19) | 1.06 (0.82-1.38) | 1.0 | 0.88 (0.69-1.12) | 0.92 (0.68-1.24) |
| Moderate | 731 | 1.0 | 1.13 (0.89-1.43) | 1.37 (1.05-1.81) | 1.0 | 1.01 (0.78-1.32) | 1.14 (0.83-1.57) |
| Severe | 174 | 1.0 | 1.38 (0.93-2.04) | 1.36 (0.86-2.15) | 1.0 | 1.35 (0.88-2.05) | 1.37 (0.82-2.29) |
| **ABI** | | | | | | | |
| Mild | 989 | 1.0 | 0.94 (0.76-1.15) | 0.98 (0.78-1.24) | 1.0 | 1.07 (0.86-1.33) | 1.29 (0.99-1.69) |
| Moderate | 989 | 1.0 | 0.94 (0.76-1.15) | 0.96 (0.76-1.22) | 1.0 | 1.07 (0.85-1.36) | 1.32 (1.00-1.75) |
| Severe | 329 | 1.0 | 1.04 (0.77-1.42) | 1.42 (1.02-1.98) | 1.0 | 1.21 (0.87-1.68) | 1.99 (1.37-2.88) |

Risk estimates are odds ratios (95% confidence intervals) for categories of high CRP. The category of CRP <1 mg/L serves as a reference. Categories of no progression for carotid plaque, carotid IMT, aortic calcification and ankle-brachial-index (ABI) comprise 1,480, 689, 789 and 988 persons, respectively. Model 1: age and sex adjusted. Model 2: additionally adjusted for BMI, systolic and diastolic blood pressure, anti-hypertensive medication, total cholesterol, HDL-cholesterol, cholesterol-lowering medication use, smoking status, number of pack-years, serum glucose, anti-diabetic medication use, hormone replacement therapy (for women), cardiovascular history, extent of baseline atherosclerosis and follow-up time.

## DISCUSSION

We found an independent, graded association of CRP with extent and progression of carotid plaques and ABI. CRP was independently related to the highest level of carotid IMT, while the association with change in cIMT was not significant. Although there was an independent, graded relation between CRP and aortic calcification, no independent association was found with progression of aortic calcification, or with the amount of coronary calcification.

### Relation of CRP with measures of atherosclerosis

Inflammation plays a role in all stages of atherosclerosis. In early stages, it is involved in the formation and progression of atherosclerotic lesions in the intima of the arterial wall. In more advanced stages, inflammatory processes become more pronounced and are thought to play an important role in plaque vulnerability, rapid progression of plaques, and thrombotic complications.[22,33] We found no independent association between CRP and coronary calcification. Research on coronary plaque morphology shows that stable plaques are most often calcified, while vulnerable plaques are typically not.[34] The lack of an independent relation between CRP and coronary calcification, a feature of plaque stability, is in line with the finding that CRP plays an important role in plaque vulnerability.[24,35]

In our study, CRP was only related to the highest levels of cIMT. This agrees with the view that intima-media-thickness represents early stages of atherosclerosis.[23] In case of carotid plaques and ABI, we found that CRP was most strongly related to advanced stages of atherosclerosis and to more severe categories of progression. This is in accordance with the view that the role of inflammation is more pronounced in advanced stages of atherosclerosis.

In the gender specific analyses, we observed a stronger relation of CRP with carotid IMT and ABI in men as compared to women. Whereas the difference could be at least partly explained by the fact that women had lower cIMT levels than men, ABI levels were comparable between both sexes (data not shown).

### Results of prior population-based studies

Only a few large population-based studies focused on CRP in relation to quantity of carotid plaque. An independent relation was found between CRP and grade of carotid artery stenosis.[12] and between CRP and amount of carotid plaque.[14] The latter study found an association in men only. A Danish population-based study did not show an independent relation between CRP and number of carotid plaques.[21]

One population-based study investigated the relation between CRP and levels of carotid IMT and found an independent association between CRP and the highest decile

of IMT.[21] Large population-based studies on the relation between quartiles of CRP and carotid IMT found associations that were lost after adjustment for cardiovascular risk factors.[8,12–14,19] One of these studies showed an independent relation between CRP and internal but not common carotid IMT.[12]

Studies on the relation between CRP and levels of ABI are scarce. Folsom et al.[13] showed an independent, inverse relation between CRP and ABI in men only. However, several large population-based studies showed an independent association between CRP and presence of peripheral artery disease (ABI <0.9).[7–10]

The only large, population-based study on the relation between CRP and amount of coronary calcification did not find an independent association.[18]

Relatively few studies have been performed on the association between CRP and progression of atherosclerosis. In a population-based study on carotid artery plaque progression, CRP was only related to early stages of plaque progression after 5 years, but not to advanced stages of progression. However, the number of participants with advanced progression was limited.[11] A large study on carotid IMT progression over a period of 2 years showed an association between CRP and cIMT progression that was lost after adjustment for cardiovascular risk factors.[17] A study on the effect of CRP on decrease in ABI showed an independent association of borderline significance after 5 years and a clear independent association after 12 years.[5] Furthermore, a previous study on the relation between CRP and progression of atherosclerosis among a subgroup of 773 participants of the Rotterdam Study showed an independent relation between CRP and progression of carotid plaques, but not with change in aortic calcification and ABI over a mean period of 6.4 years.[6]

In summary, population-based studies on the relation between CRP and quantity of atherosclerosis, although limited in number, generally support independent relations with extent and progression of carotid plaques and ABI. Furthermore, CRP is found to be associated with the highest level of carotid IMT, but not with progression of cIMT, or with the amount of coronary calcification. Our findings are generally in line with these studies.

### Strengths and limitations

This is the largest population-based study to date in which the relation of CRP with extent and progression of atherosclerosis is examined in multiple vessel beds. However, some methodological issues need to be addressed. Firstly, analyses were conducted among different study populations. In analyses of progression of atherosclerosis, responders were younger (mean age difference 8.8 years), consisted of a higher percentage of men (42.5% versus 38.2%) and generally had lower levels of cardiovascular risk factors compared to the non-responders. Although the lower amount of cardiovascular risk factors may have somewhat limited the range of baseline levels of atherosclerosis,

it is unlikely that it has affected the validity of the risk estimates. The population with measurements of coronary calcification consisted of 61% of the eligibles. A difference between responders and non-responders was found in the

percentage of men (46.6% versus 37.8%), however no differences were present in levels of cardiovascular risk factors.[26] Secondly, we attributed differences in the association

between CRP and atherosclerosis to differences in applied measures of atherosclerosis, based on pathophysiological views as described above. However, we cannot exclude possible effects of differences in vessel beds. Finally, in the study on progression, we found the strongest relation between CRP and severe progression of atherosclerosis.

However, categories of mild and moderate progression are most susceptible to misclassification. We cannot rule out that the association of CRP with these categories maybe mitigated by misclassification.

## Conclusion

This population-based study shows graded associations of CRP with extent and progression of atherosclerosis. However, the strength of the associations depends on the applied measure of atherosclerosis. We found an independent, graded association between CRP and extent and progression of carotid plaques and ABI. Furthermore, CRP was independently related to the highest level of carotid IMT, while the association with change in cIMT was not significant. Although there was an independent, graded relation between CRP and aortic calcification, no independent association was found with progression of aortic calcification, or with the amount of coronary calcification. Our findings are generally supported by previous studies on the relation between

CRP and quantity of atherosclerosis. The inconsistency in the literature on the relation between CRP and atherosclerosis may, at least partly, be explained by differences in

applied measures of atherosclerosis and lack of quantification.

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**Manuscript based on this chapter**

Elias-Smale SE, Fair JM, Varady AN, Iribarren C, Go AS, Hlatky MA, Rubin GD, Witteman JCM, Fortmann SP. Is elevated heart rate related to severity or progression of coronary atherosclerosis in healthy older adults? Submitted.

# CHAPTER 2.2

## Elevated heart rate and extent and progression of coronary atherosclerosis


**SUMMARY**

**Background:** Although elevated heart rate is an established predictor of cardiovascular disease, it is still under debate whether heart rate is a true risk factor for atherosclerosis, rather than a risk marker through underlying sympathetic over-activity. This study investigated the association of elevated heart rate with severity and progression of coronary atherosclerosis and examined whether this association could be explained by cardiovascular risk factors, or heart rate variability, a commonly used proxy for autonomic nervous system dysfunction.

**Methods:** In 1,014 healthy older adults (60-72 years, 62% male), coronary calcification was measured using a multi-detector computed tomographic (MDCT) scan. A total of 909 persons underwent a second MDCT scan on average 23.5±1.4 months later.

**Results:** Compared to persons with the most common heart rate (third quintile, 62-66 beats/min), persons with a high resting heart rate (highest quintile, ≥74 beats/min) had a 62% higher chance of having relatively severe coronary calcification (OR 1.62; 95% CI 1.01-2.60, $P<0.05$), independent of cardiovascular risk factors and heart rate variability. However, resting heart rate did not appear to be related to the rate of progression of coronary calcification.

**Conclusion:** In healthy older persons, elevated heart rate was directly related to severity, but not to short-term progression of coronary calcification.

## INTRODUCTION

Numerous studies have indicated that elevated heart rate is an important predictor of cardiovascular morbidity and mortality in patients with stable coronary artery disease and in the general population.[1-5] The association between heart rate and cardiovascular disease has been commonly regarded as a result of increased sympathetic or reduced parasympathetic nervous system activity (or both). This would imply that high heart rate is only a marker of autonomic dysfunction and that its reduction would not grant substantial benefit.[6] However, corroborative evidence indicates that heart rate is a true, independent risk factor for cardiovascular disease[5, 7] and recent evidence of randomized clinical trials in patients with coronary artery disease (CAD) support the idea that reduction of heart rate leads to improvement of cardiovascular disease outcomes.[8, 9]

Independent of autonomic dysfunction, elevated heart rate could be directly related to acceleration of coronary atherosclerosis by several mechanisms promoting pro-atherogenic alteration of the coronary micro-environment.[6, 10] This hypothesis is supported by animal studies showing that high heart rate is related to more severe coronary and carotid atherosclerosis and that experimental lowering of heart rate reduced atherosclerosis.[6] However, human studies on the association of elevated heart rate with severity and progression of atherosclerosis are scarce.[11-14] Moreover, these studies mainly focused on patients with cardiovascular disease or hypertension and did not particularly examine whether the association can be explained by underlying autonomic dysfunction.

In a healthy cohort of older adults, we examined the association of elevated heart rate with severity and progression of coronary atherosclerosis and investigated whether this association could be explained by cardiovascular risk factors, or heart rate variability, a commonly used proxy for autonomic nervous system dysfunction.

## METHODS

### Study population

This study was performed in the healthy older control participants in the Atherosclerotic Disease, Vascular Function and Genetic Epidemiology (ADVANCE) Study. Details of the full study recruitment were published previously.[15-17] The older control cohort included persons aged 60 to 72 years identified from the automated databases of Kaiser Permanente of Northern California, a large integrated health care plan, and consisted of 1,023 persons (638 men and 385 women) free of diagnosed cardiovascular disease. At study baseline, all subjects completed a comprehensive clinic visit and all but nine persons underwent a multi-detector coronary computed tomography (MDCT) scan. Thus, analyses on the relation between heart rate and extent of atherosclerosis were based on 1,014

persons. Analyses on progression of atherosclerosis were based on the 909 persons who underwent a second MDCT scan after a median follow-up time of 23.5±1.4 months. The Institutional review boards from the Kaiser Foundation Research Institute and Stanford University approved this study. All participants gave informed consent.

### Heart rate measurement

Heart rate was measured in the right arm after the participant had been sitting quietly for 5 minutes. The radial pulse was palpated and the rate was counted for 30 seconds.

### Coronary calcium measurement

Detection and quantification of coronary calcium was performed using 4-, 16-, or 64-row MDCT scan (Siemens Medical Solutions, Erlangen, Germany or General Electric Medical Systems, Milwaukee, Wisconsin), as described previously.[18] An electrocardiographically-triggered breath-held scan through the heart was performed at 80% of the RR interval. Two identical scans were acquired sequentially. The Agatston scoring method was used to quantify coronary calcium.[19] An attenuation threshold of 130 HU was applied for differentiating calcification from soft tissues. A calcific lesion was defined as >2 contiguous pixels with attenuation of ≥130 Hounsfield units (HU). Measurements were made on the 2 scans, and Agatston total scores reported are the average of scores from each of the 2 scans.

### Cardiovascular risk factors and medication use

Information on sociodemographic characteristics and personal and family medical history was obtained from the self-administered health survey. Risk factors were measured using standardized protocols and a standardized and validated questionnaire was used to collect data on physical activity, as described previously.[17] Family history of coronary artery disease was defined as percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) or myocardial infarction (MI) in first degree male relatives <55 of age and female relatives <65 years of age. Each participant was asked to bring her/his medications and supplements to the scheduled clinic visit. All medication names, dose, frequency and route were reviewed with each participant.

### Heart rate variability

Heart rate variability (HRV) was determined by power spectral analysis from a 10 minute beat-to-beat heart rate recording using wrist electrodes placed over the left and right radial pulses. Data were collected after a 10 minute rest period using Biocom 1500 ECG Interface Unit with heart scanner software. An analysis file which included an EKG tracing was visually reviewed and manually corrected for ectopy and artifact. HRV data variables include time domain measure SDNN (standard deviation of normal-to-normal

beats) and frequency domain measures calculated by Fast Fortier transformations (very low frequency, low frequency, high frequency and total power).[20]

### Statistical analyses

We performed analyses of covariance (ANCOVA) to compute means of resting heart rate for categories of coronary artery calcium (CAC) and used a Student *t* test to compare categories against the reference group. A total of 165 of 383 women (43.1%) and 80 of 631 men (12.7%) had a coronary calcium score of zero. These 245 persons (24.1% of the total population) were assigned to the reference group. All persons with a calcium score of >0 were ranked into gender-specific tertiles, so that each CAC group comprised about a quarter of the study population. In men, the first, second and third tertile comprised calcium scores of 1-60, 61-378 and 380-6719 Agatston units, respectively. In women, corresponding calcium scores were: 1-13, 14-104, and 104-3142. Using logistic regression analysis, we examined the association of quintiles of heart rate with the risk of being in the highest category of coronary calcium versus all other categories of CAC. We chose to use quintiles of heart rate and to define high resting heart rate from the lower limit of the upper quintile as many epidemiologic studies on heart rate and coronary heart disease have done.[7] The absolute cut-offs of the heart rate quintiles were: 32-56, 58-60, 62-66, 68-72, and 74-128, respectively. The third quintile of heart rate (62-66 beats/min) was chosen as the reference category, since it comprises the mean heart rate, and thus represents the most common heart rate.

We performed ANCOVA to compute means of resting heart rate for gender-specific quartiles of progression of coronary calcium and used a Student *t* test to compare categories against the reference group. Logistic regression was used to examine the association of quintiles of heart rate with the risk of being in the highest quartile of change in coronary calcium score versus the lower three quartiles. Although the population for analyses comprised 909 instead of 1,014 persons, the absolute cut-offs of the heart rate quintiles were found to be the same as for the whole population. The third quintile of heart rate (62-66 beats/min) was again chosen as the reference category.

In model 1, analyses were adjusted for age, gender and race/ethnicity. In model 2, we additionally adjusted for the following cardiovascular risk factors: systolic and diastolic blood pressure, anti-hypertensive medication use, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, cholesterol-lowering medication use, triglycerides, smoking status (current/former/never), fasting glucose, anti-diabetic medication use, body-mass-index, physical activity and family history of CAD. In model 3, we added the time and frequency domain measures of HRV, namely: SDNN and very low frequency, low frequency, high frequency, and total power to the covariables of model 2 to test whether associations were independent of autonomic nervous system

**Table 1.** Baseline characteristics

| Variable | Extent of atherosclerosis n=1,014 | Progression of atherosclerosis n=909 |
|---|---|---|
| Age, years | 65.8±2.8 | 65.8±2.8 |
| Male, % | 62.2 | 62.4 |
| Race/ethnicity, % | | |
| White | 67.4 | 69.1 |
| African-American | 8.2 | 7.5 |
| Asian | 7.7 | 7.9 |
| Hispanic | 6.0 | 5.9 |
| Other | 10.7 | 9.6 |
| Systolic blood pressure, mmHg | 132±17 | 132±17 |
| Diastolic blood pressure, mmHg | 74±9 | 74±9 |
| Anti-hypertensive medication, % | 48.8 | 48.3 |
| Beta-blockers | 17.1 | 16.9 |
| Calcium channel blockers | 8.2 | 8.1 |
| LDL-cholesterol, mg/dl | 124±32 | 124±31 |
| HDL-cholesterol, mg/dl | 54±16 | 54±16 |
| Triglycerides, mg/dl | 139±86 | 133±66 |
| Cholesterol-lowering medication, % | 22.7 | 22.3 |
| Smokers, % | | |
| Current | 7.5 | 7.6 |
| Former | 51.0 | 50.2 |
| Fasting glucose, mg/dl | 105±24 | 105±24 |
| Anti-diabetic medication, % | 11.3 | 10.4 |
| BMI, kg/m2 | 28.4±5.2 | 28.3±5.1 |
| Physical activity, kcal/kg/day | 34.7±3.3 | 34.7±3.2 |
| Family history of CAD*, % | 14.0 | 14.6 |
| Heart rate variability, $ms^2$ | | |
| Total power | 440 (217-798) | 441 (219-797) |
| Very low frequency power | 236 (113-450) | 239 (115-450) |
| Low frequency power | 110 (48-238) | 111 (49-238) |
| High frequency power | 51 (24-117) | 50 (24-117) |
| SDNN†, ms | 35 (25-46) | 35 (25-46) |
| Resting heart rate, beats/min | 65±11 | 65±11 |
| Calcium score, Agatston | 42 (1-266) | 39 (1-248) |
| Persons without coronary calcium, % | 24.2 | 25.0 |
| Progression, Agatston | - | 3 (0-33) |

Variables are expressed as mean values ± standard deviation. In case of skewed distribution median (inter-quartile range) is presented. LDL : low-density lipoprotein, HDL: high-density lipoprotein, BMI: body-mass-index. * CAD: coronary artery disease comprising percutaneous coronary intervention, coronary artery bypass grafting and/or myocardial infarction in first degree male relatives <55 years or first degree male relatives <65 years of age. † SDNN: standard deviation of normal to normal R-R intervals.

dysfunction. All analyses on progression were additionally adjusted for baseline calcium score and time interval between scans.

Since heart rate is especially lowered by beta-blocking agents and calcium-channel blockers, we repeated the model 2 and 3 analyses replacing the covariable "use of antihypertensive medication" by the covariables "use of beta-blocking agents" and "use of calcium-channel blockers". Furthermore, analyses were checked for collinearity between heart rate and HRV measures using collinearity diagnostics (Tolerance and Variance Inflation Factor (VIF). To get further insight in the association between HRV and CAC, we repeated logistic regression analyses using heart rate variability measures as univariable factors. To test whether the relation between heart rate and coronary calcium was different for men and women, we added an interaction term to the regression model (coronary calcium x gender for the ANCOVA analyses and heart rate x gender for the logistic regression analyses). Because none of the interaction terms were statistically significant (P<0.05), we conducted analyses for men and women together.

Data on cardiovascular risk factors and medication use were missing in up to 2.4% and 9.3% of persons, respectively. Data on heart rate variability were missing in 4.7% of the study population. Missing data were imputed by single imputation using the Expectation Maximization (EM) algorithm. Analyses were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

## RESULTS

Baseline characteristics of the populations under study for severity (n=1,014) and progression (n=909) of coronary calcification are presented in Table 1. Characteristics of the two groups were highly comparable.

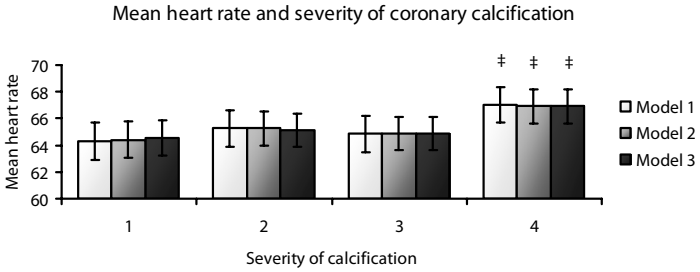


**Figure 1.** Mean resting heart rate per category of severity of coronary calcification.
Model 1 is adjusted for age, gender and race/ethnicity. Model 2 is model 1 plus adjustment for cardiovascular risk factors. Model 3 is model 2 plus adjustment for heart rate variability.
Group 1 (n=245) indicates persons with no detected calcium, groups 2 (n=255), 3 (n=257) and 4 (n=257) are gender-specific tertiles of persons with a calcium score > 0. ‡ P< 0.05 compared to group 1.

Mean heart rate per category of coronary calcification is presented in Figure 1. Compared to persons with no detected coronary calcium, persons with highest calcium scores had a significantly higher mean heart rate, independent of cardiovascular risk factors (66.9 versus 64.4, P=0.011, model 2) and heart rate variability (66.9 versus 64.5, P=0.014, model 3). There were no significant differences in heart rate between persons in the first and second tertile of calcium scores compared to persons with no coronary calcification.

The risk of having a high calcium score (i.e. a score within the highest tertile of non zero calcium scores compared to no calcium) per quintile of heart rate is displayed in Table 2. Compared to persons with the most common heart rate (third quintile, 62-66 beats/min), persons with a high heart rate of (highest quintile, ≥74 beats/min) had a 62% increased risk of being in the highest calcium score group, independent of cardiovascular risk factors (OR 1.62; 95% CI 1.02-2.59, P=0.043, model 2). Heart rate variability (OR 1.62; 95% CI 1.01-2.60, P=0.044, model 3) did not substantially modify his relation. No significant relation was found between lower heart rates and coronary calcification.

Mean heart rate did not significantly differ between quartiles of progression (Figure 2). Furthermore, as displayed in Table 3, resting heart rate did not appear to be significantly related to the risk of having a high progression calcium score (i.e. a score within the highest quartile of progression of coronary calcification).

**Table 2.** Risk of severe coronary artery calcification per quintile of heart rate (n=1,014)

| Heart rate | | N | Odds ratio (95% CI) | | |
|---|---|---|---|---|---|
| Quintile | Range | | Model 1 | Model 2 | Model 3 |
| 1 | ≤56 | 226 | 1.06 (0.69-1.64) | 1.04 (0.65-1.64) | 1.02 (0.64-1.62) |
| 2 | 58-60 | 153 | 0.93 (0.56-1.53) | 0.94 (0.57-1.58) | 0.95 (0.57-1.59) |
| 3 | 62-66 | 225 | Reference | Reference | Reference |
| 4 | 68-72 | 211 | 1.03 (0.66-1.62) | 1.03 (0.64-1.65) | 1.04 (0.64-1.67) |
| 5 | ≥74 | 199 | 1.65 (1.06-2.55)‡ | 1.62 (1.02-2.59)‡ | 1.62 (1.01-2.60)‡ |

Model 1 is adjusted for age, gender and race/ethnicity. Model 2 is model 1 plus adjustment for cardiovascular risk factors. Model 3 is model 2 plus adjustment for heart rate variability.
‡ P<0.05.

Repetition of analyses with specific correction for use of beta-blockers and calcium channel blockers did not change our results (data not shown). No collinearity was detected between heart rate and measures of heart rate variability (Tolerance was no lower than 0.9, VIF was not higher than 1.1). Logistic regression analyses on severity and progression of coronary calcification using measures of HRV alone showed no significant association between any measure of HRV and (progression of) CAC. (All P>0.05).

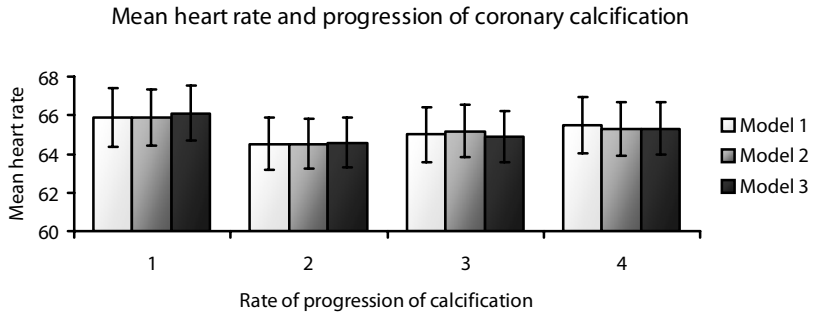


**Figure 2.** Mean resting heart rate per category of progression of coronary calcification.
Model 1 is adjusted for age, gender and race/ethnicity. Model 2 is model 1 plus adjustment for cardiovascular risk factors. Model 3 is model 2 plus adjustment for heart rate variability. All models are adjusted for baseline calcium score and time interval between scans.
Groups 1 to 4 indicate gender-specific quartiles of progression of coronary calcification over a median time period of 24 months.

**Table 3.** Risk of highest progression of coronary calcification per quintile of heart rate (n=909)

| Heart rate | | N | Odds ratio (95% C.I.) | | |
|---|---|---|---|---|---|
| Quintile | Range | | Model 1 | Model 2 | Model 3 |
| 1 | ≤56 | 211 | 1.16 (0.72-1.87) | 1.11 (0.68-1.81) | 1.11 (0.67-1.82) |
| 2 | 58-60 | 132 | 0.94 (0.54-1.63) | 0.85 (0.48-1.51) | 0.86 (0.48-1.52) |
| 3 | 62-66 | 201 | Reference | Reference | Reference |
| 4 | 68-72 | 189 | 1.40 (0.87-2.25) | 1.27 (0.77-2.08) | 1.27 (0.78-2.08) |
| 5 | ≥74 | 176 | 1.22 (0.75-1.99) | 1.07 (0.64-1.79) | 1.09 (0.65-1.82) |

Model 1 is adjusted for age, gender and race/ethnicity. Model 2 is model 1 plus adjustment for cardiovascular risk factors. Model 3 is model 2 plus adjustment for heart rate variability. All models are adjusted for baseline calcium score and time interval between scans.

## DISCUSSION

In this cohort of healthy elderly, persons with a high resting heart rate (highest quintile, ≥74 beats/min) had a 62% higher chance of having relatively severe coronary calcification after adjustment for cardiovascular risk factors and heart rate variability, a measure of autonomic nervous system dysfunction. However, resting heart rate did not appear to be related to the rate of progression of coronary calcification over a mean time period of 23.5±1.4 months.

### Heart rate and severity of coronary atherosclerosis

Two previous studies examined heart rate in association with severity of atherosclerosis. A case-control study among 185 hypertensive older adults and 181 normotensive con-

trols showed that hypertensive persons with a high resting heart rate (highest quintile, ≥80 beats/min) had a 3.2 times higher risk of ≥50% carotid stenosis as assessed by duplex, compared to persons with a heart rate of <60 beats/min (P=0.02). This relation was independent of cardiovascular risk factors. Unfortunately, power was too limited to examine the relation in the normotensive persons. However, re-analysis on the total population, additionally adjusting for hypertension, showed that a heart rate of ≥80 beats/min (12% of the population) was associated with a 2.8 times higher risk of stenosis (P=0.01).[14] A study by Perski et al. among 56 young, male myocardial infarction patients, reported that a higher minimum (during sleep) and mean heart rate, measured during a 24-hour period, were positively related to severity of diffuse atherosclerosis and distinct stenoses on coronary angiogram, independent of classical cardiovascular risk factors. Patients with a heart rate above the median value of the minimum heart rate had a 2 times higher atherosclerosis score and a 1.5 times higher stenosis score than persons with a heart rate below the median.[12, 13]

Although both studies used different definitions of elevated heart rate and atherosclerosis then we did, our results appear to be generally in line with their findings, indicating that elevated heart rate is related to severity of atherosclerosis, independent of cardiovascular risk factors. In addition, we found that this association cannot be explained by underlying autonomic nervous system dysfunction.

### Heart rate and progression of coronary atherosclerosis

The study by Perski et al. was extended to examine progression of atherosclerosis. After an average of 61.6 months (range 42 to 82 months) re-angiogaphy was performed. Minimum but not mean or maximal heart rate was found to be independently related to the rate of progression of diffuse and stenotic atherosclerosis. Patients with a heart rate above the median value of minimum heart rate had a 1.7 times higher atherosclerosis progression score and a 3.4 times higher stenosis progression score than persons with a heart rate below the median.[13] A study by Huikuri et al., primarily examining the association between heart rate variability and progression of coronary atherosclerosis, repeated angiograms an average of 32 months later in 265 male CABG patients (average age 59 years). In this study, univariable analysis showed that elevation of the lowest heart rate within 24 hours was related to the highest tertile of progression of discrete coronary stenoses, but not to progression of diffuse disease. This relation was abolished in a multivariable model including cardiovascular risk factors and heart rate variability parameters. Maximal heart rate did not show any relation with progression of atherosclerosis.[11] In contrast to the other two studies, we did not find any relation between heart rate and progression of atherosclerosis. This might partly be explained by the fact that we measured coronary calcification which can be regarded as a measure of diffuse rather than stenotic atherosclerosis. It is likely that this contrast was amplified by the

relatively short time period between our atherosclerosis measurements (24 months), especially as compared to the study by Perski et al. (62 months). The fact that we studied a healthy population instead of CAD patients might also play a role. Another issue is that the other study groups found a relation of minimal (sleep), but not mean (Perski) and maximal (Perski and Huikuri) heart rate with progression of atherosclerosis. We measured resting heart rate, which can probably be ranked between minimal and average heart rate.

### Heart rate variability and coronary calcification

Thus far, only two studies published on the relation between HRV and CAC. Colhoun et al. performed a study in 160 type 1 diabetes patients and 163 controls and found in the total population that, per tertile lower total spectral power, the risk of presence of CAC increased 1.5-fold ($P < 0.01$). However, this association was no longer statistically significant after adjustment for cardiovascular risk factors.[21] A study among 79 postmenopausal women found that presence of CAC was related to a greater reduction in HF-HRV after a psychological stressor, independent of cardiovascular risk factors. No independent relation was found with aortic calcification. Besides the small sample size, this study was hampered by the fact that calcification was measured at a median of 282 (range 0-754) days after HRV measurement.[22] In our study we did not find any relation between measures of HRV and the severity and progression of coronary calcification. Thus, until now, no clear, independent relation was found between HRV and coronary calcification.

### Pathophysiological mechanisms

The fact that the relation of HR with CAC did not depend on cardiovascular risk factors or on HRV supports the hypothesis that elevated heart rate can directly stimulate atherogenesis. In a recent review, Giannoglou et al. elaborated on hemodynamic mechanisms that can directly link elevated heart rate to coronary atherosclerosis. Mechanisms include enhancement of the magnitude and frequency of tensile stress imposed on the arterial wall, prolongation of the exposure of coronary endothelium to shear stress and intensification of the periodically changing coronary geometry, which in turn affects the local hemodynamical environment. All these processes induce structural and functional changes of the endothelial cells, resulting in promotion of atherosclerosis in atherosclerosis-prone regions.[6]

The stronger relation of elevated heart rate to progression of discrete stenotic lesions compared to diffuse atherosclerosis might be due to a closer relation of hemodynamic factors to geometric aspects of vulnerable areas, i.e. discrete lesions, than to diffuse disease.[6, 11]

The fact that only a high minimal heart rate (during sleeping hours), was found to be related to progression of atherosclerosis suggests that not only hemodynamical

mechanisms, but also over-activation of the sympathetic nervous system or impairment of the vagal innervation is involved.[23] That seems to be contradictory to our finding that the association between elevated heart rate and the severity of atherosclerosis was not affected by heart rate variability. However, we cannot completely rule out an effect of autonomic nervous function (See study limitations).

**Strengths and limitations**

The strongest points of our study are population-based sampling and the large array of standardized, measured cardiovascular risk factors that enabled us to investigate a direct relation between heart rate and coronary atherosclerosis. However, to be able to appreciate our results, some limitations should also be addressed. First, resting heart rate was only measured once, which could have led to some misclassification resulting in an underestimation of the relations under study. Second, although we adjusted our analyses for parameters of heart rate variability, we cannot completely rule out an effect of autonomic nervous function. HRV measurements are only a proxy for autonomic nervous system dysfunction. Furthermore, the autonomic nervous system is thought to play a role in the pathogenesis of atherosclerosis via induction of both hemodynamic (e.g., tachycardia, hypertension) and metabolic (e.g.hyperglycemia, dyslipidemia, obesity) changes.[6] Because we also adjusted our model for most of these cardiovascular risk factors, the effect of HRV could have been abolished. However, in univariable analyses, we did not find a relation between HRV parameters and severity and progression of coronary calcification. The scarce studies performed thus far did not find a clear, independent association of HRV with coronary calcification but future research on this topic is warranted. Third, only 909 of 1,014 persons underwent a second CT scan. However, baseline characteristics were not substantially different between the two populations under study. Therefore, we have no reason to expect our results on the progression analyses to be biased. Fourth, as already discussed, the relatively short time period between the two CT scans might have contributed to the fact that we did not find a relation between elevated heart rate and progression of atherosclerosis. Fifth, although various mechanisms implicate a direct effect of elevated heart rate on atherosclerosis, we performed a cross-sectional study, and therefore cannot draw conclusions concerning causal inference. Last, our study participants were elderly. Therefore, our results cannot automatically be generalized to a younger population.

**Conclusion**

In this cohort of healthy elderly, a high resting heart rate (highest quintile, ≥74 beats/min) was related to more severe coronary calcification, independently of cardiovascular risk factors and autonomic nervous system function. However, no association was found between resting heart rate progression of coronary calcification over a mean time period of 24 months.

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# CHAPTER 3

## Coronary calcification and risk of cardiovascular disease

**Manuscript based on this chapter**

Leening MJG, Elias-Smale SE, Felix JF, Vliegenthart-Proença R, Deckers JW, Hofman A, Oudkerk M, Stricker BHCh, Witteman JCM. Coronary calcification and the risk of heart failure in the elderly; The Rotterdam Study. Submitted.

# CHAPTER 3.1

## Coronary calcification and risk of heart failure


**SUMMARY**

**Background:** In elderly, heart failure is often observed as a first manifestation of coronary atherosclerosis rather than a sequel from overt coronary heart disease (CHD). Numerous studies showed that coronary artery calcification (CAC) is a strong predictor of myocardial infarction and mortality, but the association between CAC and incident heart failure has never been studied. The aim of this study was to determine this association in the elderly, and examine its independence of overt CHD.

**Methods:** Within the population-based Rotterdam Study, 1,897 asymptomatic participants (mean age 69.9 years, 42% men) underwent CAC scoring. Adjusted for cardiovascular risk factors, hazard ratios (HR) for developing heart failure were computed. Analyses were repeated after censoring participants at the occurrence of CHD.

**Results:** During a median follow-up of 6.8 years, 78 cases of heart failure occurred. Increasing CAC scores were associated with heart failure (P for trend 0.002), with a HR (95% CI) of 4.2 (1.7-10.4) for CAC scores >400 compared to CAC scores of 0-10. After censoring participants for CHD, increasing extent of CAC remained associated with heart failure (P for trend 0.035), with a HR (95% CI) of 3.1 (1.2 to 8.1) for CAC scores above 400.

**Conclusion:** CAC was clearly associated with the risk of heart failure, independently of overt CHD. Since heart failure is highly prevalent in the elderly, it might be worthwhile to include heart failure as an outcome in future risk assessment programs incorporating CAC screening.

## INTRODUCTION

Coronary artery calcification, an established measure of subclinical coronary atherosclerosis, is a strong and independent predictor of future CHD.[1-3] Furthermore, calcium scoring appears to improve CHD risk prediction beyond risk scoring algorithms such as the Framingham Risk Score and is considered useful in persons at intermediate risk of CHD (absolute 10-year risk of 10-20%).[1, 4-6]

It is well known that heart failure is a highly prevalent disease in the elderly, associated with reduced life expectancy and ever increasing costs.[7-9] Especially in the elderly, coronary atherosclerosis is the current leading cause of heart failure and heart failure is often observed as a first manifestation of coronary atherosclerosis rather than a sequel from overt coronary insufficiency or myocardial infarction (MI).[10, 11]

In this light, heart failure could be considered as an additional outcome in cardiovascular risk assessment programs using CAC. As a prerequisite, it is important to examine the strength of the association between CAC and incident heart failure, independent of cardiovascular risk factors and overt CHD. Within the Rotterdam Study, a prospective population-based cohort study among elderly, we investigated the association between CAC and the risk of heart failure and examined whether this relation is independent of incident overt CHD.

## METHODS

### Study population

This study was embedded in the Rotterdam Study, a prospective, population-based cohort study among persons ≥55 years of age that started in 1990. In 2000, the original cohort was extended with a second cohort of inhabitants who reached the age of 55 years and persons who had been migrated into the research area. The rationale and design of the Rotterdam Study have been described elsewhere.[12]

For both cohorts, identical examinations took place from 1997 to 2001 and participants through 85 years of age were invited to undergo an electron beam CT (EBCT) scan in a separate visit. Scans were obtained for 2,349 participants (response rate 61%). Due to several causes image acquisition data could not be analyzed in 57 persons. Therefore, data were available for 2,292 participants. For the present study we excluded 79 persons with known heart failure at the moment of CT scanning and ten persons with incomplete data concerning heart failure status at the moment of CT scanning. Furthermore we excluded the subset of the study population with a documented history of CHD (n=306), defined as a recognized or unrecognized MI, percutaneous coronary intervention (PCI), or coronary artery bypass grafting (CABG). This left a total of 1,897

coronary asymptomatic subjects eligible for the present study. The median duration between the examination at the Rotterdam Study research center and CT scanning was 44 days. Follow-up started at the date of CT scanning and for the present study ended on December 13, 2006. The study was approved by the Medical Ethics Committee of the Erasmus MC, The Netherlands. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

### Assessment of coronary calcification

We assessed CAC in the epicardial coronary arteries detected on electron-beam CT scans as described in detail previously.[2] Briefly, imaging was performed with a C-150 Imatron scanner (GE-Imatron). From the level of the root of the aorta through the heart, 38 images were obtained with 100 milliseconds (ms) scan time and 3 millimeter (mm) slice thickness. A calcification was defined as a minimum of two adjacent pixels (area, 0.65 $mm^2$) with a density of over 130 Hounsfield units. CAC scores were calculated according to Agatston's method.[13] Conform the protocol outlines approved by the Medical Ethics Committee, participants were not informed about their CAC score, nor were their treating physicians.

### Assessment of risk factors

In the Rotterdam Study, assessment of anthropometrics, cardiovascular risk factors and use of medication has been described previously.[14] We defined diabetes mellitus as a fasting serum glucose level >7.0 mmol/L, a non-fasting serum glucose level >11.0 mmol/L (only if fasting serum was unavailable), or the use of oral blood glucose-lowering drugs or insulin. We computed the serum non-high-density lipoprotein (non-HDL-) cholesterol level by subtracting the serum HDL-cholesterol level from the serum total cholesterol level. A family history of MI was considered positive when a parent, sibling, or child had been diagnosed with MI before 65 years of age.

### Assessment of outcomes

Cases of incident heart failure and CHD were obtained by continuously monitoring participants of the Rotterdam Study during follow-up as described previously.[7] The date of incident heart failure was defined as the day of the first occurrence of symptoms suggestive for heart failure as obtained from the medical records, or as the day of receipt of a first prescription for a loop diuretic or an angiotensin-converting enzyme inhibitor for this indication, whichever came first. The diagnosis of heart failure was classified as definite, probable, possible, or unlikely.[7] In accordance with the criteria of the European Society of Cardiology, only definite and probable cases were included in our analyses.[15] CHD events were defined as MI, PCI, CABG, or cardiac mortality. Assessment of CHD events has been described in detail previously.[2]

### Statistical analyses

CAC scores were divided into 5 categories: 0-10, 11-100, 101-400, and above 400, as proposed by Rumberger et al.[16] We used Cox proportional hazards models in order to construct age- and gender-adjusted heart failure-free survival curves and to calculate hazard ratios for the risk of heart failure for the different CAC score categories.[17] In model 1 we adjusted for age and gender. In model 2, we additionally adjusted for the following cardiovascular risk factors: systolic blood pressure, use of anti-hypertensive medication, diabetes mellitus, current smoking, non-HDL-cholesterol, and family history of MI. Participants with a CAC score of 0-10 served as the reference category. P for trend was obtained by entering CAC score categories into the Cox models as a continuous variable. Participants were censored at the date of death, loss to follow-up, or the end of the study period. In addition, we repeated the analyses after censoring participants when incident clinical non-fatal CHD occurred during follow-up (model 3 and additionally adjusted for cardiovascular risk factors in model 4). This was done to examine the association between CAC and risk of future heart failure, independent of incident CHD.

We computed the probability values of the interaction terms between gender and the natural logarithm of the CAC scores (log(CAC+1)) in all 4 models, which were all non-significant (all $P>0.28$). We added 1 to all CAC score values in order to deal with participants who had a CAC score of 0. Missing covariates were handled by single imputation using an expectation-maximization algorithm.[18] All HR are presented with 95% confidence intervals (CI). We used the level of significance of $P<0.05$ for P for trend and interaction terms. Data were analyzed using the SPSS statistical package, version 15.0 (SPSS Inc., Chicago, Illinois, USA).

## RESULTS

Table 1 shows the baseline characteristics of the study population, by category of CAC. Mean age and systolic blood pressure were higher in increasing CAC score categories. Furthermore, male gender, use of anti-hypertensive medication, diabetes mellitus, and smoking were noted more frequently with increasing CAC scores. For the total population the median (inter-quartile range) CAC score was 78 (7-351). During a median (inter-quartile range) follow-up time of 6.8 (6.3-7.5) years, 78 cases of incident heart failure, 76 cases of non-fatal incident CHD (46 MI, 19 PCI, and 11 CABG), and 29 cases of fatal incident CHD occurred. These 183 events occurred in 160 participants. Non-fatal incident CHD preceded heart failure in 14 out of 78 (18%) participants. Heart failure represented 64 out of 160 (40%) of the first cardiac events observed. In all but one participant who developed heart failure during follow-up coronary calcifications were detected (98.7%).

Figure 1 shows the association between CAC score categories and incident heart failure, adjusted for age and gender. The event-free survival decreased with increasing

**Table 1.** Baseline characteristics of the study population

| | All | CAC score | | | |
|---|---|---|---|---|---|
| | | 0-10 | 11-100 | 101-400 | >400 |
| N | 1,897 | 528 | 498 | 435 | 436 |
| Age, years | 69.9±6.5 | 67.3±6.0 | 69.8±6.3 | 70.9±6.2 | 72.3±6.2 |
| Men, % | 41.9 | 24.1 | 39.4 | 47.6 | 60.6 |
| SBP, mmHg | 143±21 | 138±21 | 144±21 | 144±22 | 147±21 |
| DBP, mmHg | 76±11 | 75±10 | 77±11 | 76±11 | 77±11 |
| hypertension medication, % | 32.4 | 24.4 | 29.7 | 34.5 | 43.3 |
| Diabetes mellitus | 11.5 | 6.5 | 9.8 | 14.4 | 16.6 |
| Smokers, % current | 16.9 | 12.0 | 15.9 | 20.3 | 20.6 |
| former | 51.4 | 45.8 | 50.8 | 51.6 | 58.8 |
| never | 31.7 | 42.4 | 33.3 | 28.1 | 20.6 |
| Total cholesterol, mmol/L | 5.9±0.9 | 5.9±1.0 | 5.9±0.9 | 5.9±1.0 | 5.8±0.9 |
| HDL-cholesterol, mmol/L | 1.4±0.4 | 1.5±0.4 | 1.4±0.4 | 1.4±0.3 | 1.4±0.4 |
| Non-HDL-cholesterol, mmol/L | 4.5±1.0 | 4.4±1.0 | 4.5±0.9 | 4.5±0.9 | 4.5±1.0 |
| Body-mass-index, kg/m2 | 27.1±4.0 | 26.5±3.6 | 27.6±4.2 | 26.9±4.1 | 27.1±3.9 |
| Family history of MI < age 65 | 18.5 | 15.0 | 19.5 | 20.7 | 19.3 |
| Calcium score | 78 (7-351) | 1 (4) | 39 (47) | 193 (122) | 808 (840) |
| Calcium score >0 | 89.4 | 61.7 | 100 | 100 | 100 |

Categorical variables are presented as percentage. Continuous values are expressed as mean ± standard deviation. Median (inter-quartile range) is presented in case of skewed distribution. HDL: high-density lipoprotein, MI: myocardial infarction.

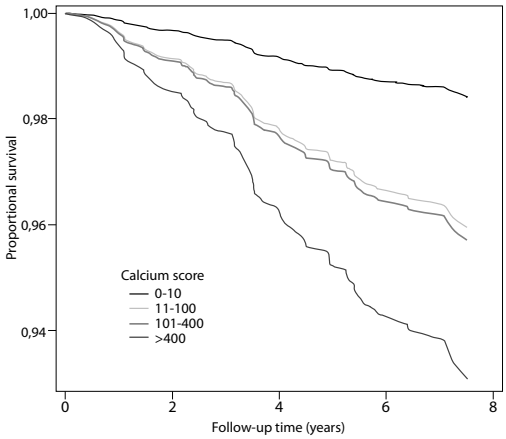


**Figure 1.** Heart failure-free survival curves by coronary calcium score category.

| Follow-up time (yrs) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Participants at risk | 1,897 | 1,869 | 1,800 | 1,730 | 1,645 | 1,571 | 1,469 | 804 | 207 |
| Cumulative HF* | 0 | 8 | 20 | 30 | 49 | 61 | 71 | 74 | 78 |

* Cumulative number of heart failure events.

**Table 2.** Hazard ratios for developing heart failure, by CAC score category

| CAC score | Hazard ratios (95% CI) | | | | | |
|---|---|---|---|---|---|---|
| | N/events | Model 1 * | Model 2 † | N / n | Model 3 *‡ | Model 4 †‡ |
| 0-10 | 528/6 | reference | reference | 528/6 | reference | reference |
| 11-100 | 498/19 | 2.6 (1.0-6.6) | 2.6 (1.0-6.6) | 498/18 | 2.5 (1.0-6.3) | 2.5 (1.0-6.5) |
| 101-400 | 435/19 | 2.8 (1.1-7.1) | 2.6 (1.0-6.6) | 435/17 | 2.5 (1.0-6.5) | 2.5 (0.9-6.4) |
| >400 | 436/34 | 4.6 (1.9-11.2) | 4.2 (1.7-10.4) | 436/23 | 3.2 (1.2-8.1) | 3.1 (1.2-8.1) |
| P for trend | | 0.001 | 0.002 | | 0.027 | 0.035 |

CAC: coronary artery calcification, CI: confidence interval, HR: hazard ratio. * Adjusted for age and gender.† Adjusted for age, gender, systolic blood pressure, use of anti-hypertensive medication, diabetes mellitus, current smoking, non-high-density lipoprotein cholesterol, and family history of MI. ‡ After censoring participants at the occurrence of non-fatal coronary heart disease during follow-up.

CAC scores, with a cumulative incidence at 6 years of 1.4%, 3.3%, 3.5%, and 5.7% for CAC scores of 0-10, 11-100, 101-400, and >400, respectively.

Table 2 shows HR of developing heart failure by category of CAC. Increasing CAC score categories, adjusted for age and gender, were all significantly associated with heart failure (P for trend 0.001), with a HR (95% CI) of 4.6 (1.9-11.2) for CAC scores >400 (model 1). Additional adjustment for cardiovascular risk factors lowered these estimates slightly but remained significant (P for trend 0.002), with a HR (95% CI) of 4.2 (1.7-10.4) in the CAC score category >400 (model 2).

When repeating these analyses with additional censoring of participants at the occurrence of a non-fatal MI or coronary revascularisation procedure during follow-up, estimates of the HR for increasing CAC score categories decreased but remained associated (P for trend 0.027) and significant in the upper CAC score category (model 3). After additional adjustment for cardiovascular risk factors (model 4), increasing CAC score categories were still significantly associated with the risk of heart failure (P for trend 0.035). A CAC score >400 yielded a significantly higher risk of heart failure (HR (95% CI) 3.1 (1.2 to 8.1)) compared to the reference group (CAC score 0-10).

## DISCUSSION

Our results indicate a clear and graded association between the extent of CAC and the risk of heart failure in a general population of Dutch elderly. Persons in the highest CAC score category (>400) were over four times more likely to develop heart failure compared to persons with a zero or very low CAC score, independent of cardiovascular risk factors. After censoring participants at the occurrence of incident non-fatal CHD, a 3-fold increased risk of heart failure persisted for this CAC score category, indicating a clear association between CAC and risk of heart failure apart from overt CHD.

## Studies on CAC and risk of heart failure

Thus far, numerous large population-based studies investigating CAC and future cardiovascular disease only examined the risk of MI, coronary revascularisation, stroke, death, or a combination of these outcomes.[1-5] Recently, increasing CAC scores have been cross-sectionally associated with self-reported history of heart failure, with odds ratios (95% CI) adjusted for age and gender of 1.3 (0.7-2.2) for CAC scores of 10-99, 1.9 (1.1-3.3) for CAC scores of 100-399, and 2.2 (1.2-4.1) for CAC scores above 399.[19]

In our study, heart failure represented 40% (64 out of 160) of the first cardiac events observed. This is in concordance with numbers from a recent report of the Framingham Heart Study, showing in an elderly subpopulation that 48% of the participants with a cardiac event had heart failure as their initial presentation.[20] This reinforces the plea for heart failure as an additional outcome measure in studies on cardiovascular risk prediction in the elderly, besides hard coronary outcomes. Research indicates that CAC screening in the elderly has additive value on CHD risk prediction over traditional risk factors.[4,5] However, its effect on clinical outcomes and its cost-effectiveness in large-scale randomized trials is still awaited.[21] Given our study results, heart failure should be considered as an additional outcome measure in possible future cardiac risk assessment programs using CAC screening.

## Strengths and limitations

Strengths of our study include the standardized measurements of cardiovascular risk factors in a population-based setting with long and virtually complete follow-up. Furthermore, due to the fact that both participants of our study and their treating physicians were not informed about the CAC scores, our cohort is one of few in the world in which an unbiased association between CAC and future heart failure can be investigated. Awareness of a high CAC score has proven to motivate patients to adapt beneficial lifestyle changes and stimulates cardiologists to perform coronary angiography procedures, despite negative myocardial ischemia tests.[22, 23] However, our study also has some limitations that need to be addressed. First, heart failure diagnosis was based on the occurrence of symptoms and response to medical therapy, usually supported by concurrent echocardiography or chest X-ray. However, additional information was not present in all cases, especially not in nursing home residents. This might have led to some misclassification and thereby underestimation of the associations. Second, our results were obtained from an elderly population. Strength of associations of traditional cardiovascular risk factors diminish with increasing age, while increasing extent of CAC can be seen as a cumulative measurement of a lifetime exposure to cardiovascular risk factors.[24] Therefore our results should not automatically be generalized to middle-aged individuals.

## Conclusion

Our study showed that coronary calcification was clearly associated with the risk of heart failure, independently of overt CHD. In view of the high prevalence of heart failure in the elderly, it might be worthwhile to include heart failure as an outcome if future risk assessment programs will incorporate CAC screening.

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**Manuscript based on this chapter**

Elias-Smale SE, Odink AE, Wieberdink RG, Hofman A, Hunink MGM, Krestin GP, Koudstaal PJ, Breteler MMB, van der Lugt A, Witteman JCM. Carotid, aortic arch and coronary calcification are related to history of stroke: The Rotterdam Study. Atherosclerosis 2010;212:656-60.

# CHAPTER 3.2

## Carotid, aortic arch and coronary calcification in relation to history of stroke


**SUMMARY**

**Background:** Multi-detector computed tomography (MDCT), which has been mainly used to study coronary atherosclerosis, also enables non-invasive measurement of carotid and aortic atherosclerosis and might be suitable for screening in the general population. The aim of this study was to investigate the associations of carotid artery, aortic arch and coronary artery calcification as assessed by MDCT, with presence of stroke.

**Methods:** The study was embedded in the population-based Rotterdam Study and comprises 2,521 persons (mean age 69.7±6.8 years, 48% males) that underwent an MDCT scan. History of stroke was reported by 96 persons. We used multivariable logistic regression to investigate the associations of calcification in the carotid arteries, aortic arch, and coronary arteries with presence of stroke.

**Results:** We found strong and graded associations of prevalent stroke with carotid artery (OR quartile 4 versus 1 (95% CI): 5.0 (2.2-11.0)), aortic arch (3.3 (1.5-7.4)) and coronary artery calcification (3.1 (1.3-7.3)), independent of cardiovascular risk factors. Only the association of carotid artery calcification with presence of stroke was independent of calcification in the other two vessel beds.

**Conclusion:** In this population-based study, we found a strong and graded association of prevalent stroke with carotid artery, aortic arch and coronary artery calcification, independent of cardiovascular risk factors. After additional adjustment for calcification in the other vessel beds, prevalent stroke was still significantly related to carotid calcification, but no longer to aortic arch or coronary calcification.

## INTRODUCTION

Stroke is one of the leading causes of death in the Western world, and persons surviving a stroke often have permanent functional impairments leading to substantially reduced quality of life and high healthcare costs. Despite clear progress in therapeutic options, prevention remains the cornerstone for reducing the burden of stroke. Primary prevention is particularly important, since about 77% of strokes are first events.[1] To identify persons at high risk of stroke, risk assessment tools, like the Framingham Stroke Risk Score (FSRS), are widely used. These tools are based on clinical parameters and might benefit from the addition of non-invasive measures of atherosclerosis, assessed by imaging techniques, to improve stroke risk prediction.[2]

An important condition for this application is a strong, independent relation of the measure of atherosclerosis with cardiovascular events. Two established, ultrasound based measures of carotid atherosclerosis, intima-media thickness (cIMT) and carotid plaque score have been found to be independent risk factors for stroke.[3-7] Whereas plaques cannot be accurately quantified with ultrasound, elevated IMT reflects an early phase of the atherosclerotic process. Both measures appear to have only a limited additional value to predict cerebrovascular events beyond the FSRS.[8] Another independent risk factor for stroke is atherosclerosis of the thoracic aorta.[9-12] Ultrasound assessment of this risk factor, however, requires transesophageal echocardiography (TEE), an invasive method not suitable for use in population-based studies.

With the development of electron beam and multi-detector computer tomography (EBCT and MDCT) it has become possible to non-invasively quantify arterial calcifications. Although numerous studies using CT have focused on the relation between coronary calcium and coronary heart disease (CHD), less is known about the association between coronary artery, thoracic aorta, and carotid artery calcium and stroke. Until now, only few CT studies on carotid,[13, 14] aortic,[15, 16] and coronary[17, 18] calcification in relation to transient ischemic attack (TIA) and stroke have been performed.

We examined the association of carotid artery, aortic arch, and coronary artery calcification (CAC), as assessed by MDCT, with prevalent stroke within the large, population-based Rotterdam Study.

## METHODS

### Study population

The study is embedded in the Rotterdam Study, a population-based study that started in 1990. All inhabitants aged 55 years and older and living in a suburb of Rotterdam were invited and 7,983 agreed to participate (78%). In 2000, the cohort was extended

with 3,011 persons with the same inclusion criteria. The design and rationale of the Rotterdam Study have been described elsewhere.[19]

From September 2003 until February 2006, all participants who completed a center visit (the fourth for the original cohort and the second for the extended cohort) were invited to participate in the present study and undergo a MDCT scan of the coronary arteries and a second scan of the aortic arch and carotid arteries. A total of 2,521 participants (response rate 79%) were scanned. Six persons had a pacemaker and did not undergo a coronary artery scan. Fifty-three persons did not have a valid coronary calcium score because of previous coronary stent implantation. Furthermore, the coronary arteries of 21 persons and the aortic arch of 3 persons could not be evaluated due to severe artifacts in the image acquisition. Hence data on coronary artery calcification and aortic arch calcification were available for 2,441 and 2,518 persons, respectively. The median duration between the study center visit and the MDCT scan was 117 days.

The study was approved by the Medical Ethics Committee and the Radiation Protection Unit of the Erasmus Medical Center, Rotterdam, The Netherlands. All participants gave written informed consent.

### Scan protocol and analysis of calcification

Methods have been described in detail previously.[20] In short, imaging was performed with a 16-slice (n=785) or a 64-slice (n=1,736) MDCT scanner (SOMATOM Sensation 16 or 64, Siemens, Forcheim, Germany). Two scans were performed. The cardiac scan reached from the apex of the heart to the tracheal bifurcation. The second scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). The aortic arch comprised the origin of the aortic arch (defined as the image in which the ascending and descending aorta merge into the inner curvature of the aortic arch) to the first 1 cm of the common carotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. The carotid arteries comprised both right and left carotid artery within 3 cm proximal and distal of the bifurcation. Atherosclerotic calcification was identified based on a threshold of 130 Hounsfield Units (HU), using dedicated software (Syngo Calcium Scoring, Siemens, Forcheim, Germany). Calcification was quantified by calculating the Agatston score.[21] The total score per vessel bed (coronary, aortic arch and carotid arteries) was calculated by adding the scores of all lesions in that bed. Readings were blinded for knowledge about history of stroke. Inter- and intra-observer agreement on calcium scoring was excellent (intra-class correlation coefficients for inter- and intra-observation data for all three measures of calcification ranged from 0.96 to over 0.99 for readings of both scanner types).

At the time of study inclusion, coronary calcium was not considered as an indicator for treatment in the Netherlands. Nevertheless, participants in the highest 10% of the CAC score distribution for men and the highest 5% for women (with distributions based on

our previous coronary calcium study[22]), were informed about their high calcium score and advised to visit their primary care physician.

### Covariables

Information on medical history, medication use, and smoking behavior was collected using a computerized questionnaire. Anthropometric measures were obtained during the visit at the research center. Blood pressure was measured at the right brachial artery with a random-zero sphygmomanometer with the participant in sitting position. The mean of 2 consecutive measurements was used. After an overnight fast, blood samples were obtained at the research center. Serum total cholesterol was determined by an automated enzymatic procedure using the Roche CHOD-PAP reagent agent, and HDL was measured with the Roche HDL-cholesterol assay using PEG-modified enzymes and dextran sulfate. Hypercholesterolemia was defined as the use of cholesterol-lowering medication or a fasting total cholesterol level of ≥6.5 mmol/L. Glucose was determined enzymatically by the Hexokinase method. Diabetes was diagnosed based on a fasting plasma glucose level ≥7.0 mmol/l or use of anti-diabetic medication. Coronary heart disease comprised a history of myocardial infarction (MI), percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG). Information on prevalent CHD and atrial fibrillation at the time of the CT scan was collected at baseline in 1990 (original cohort) or at baseline in 2000 (extended cohort) and during follow-up as described previously.[23]

### History of stroke

A history of stroke prior to CT scanning was based on either a history of stroke reported at baseline, or the occurrence of stroke during follow-up but before the time of scanning. History of stroke at baseline for the original cohort was assessed by asking 'did you ever suffer from a stroke, diagnosed by a physician?'. Medical records of persons who answered 'yes' were verified.[24] For the extended cohort, all medical records were checked for previous diagnosis of stroke. After entrance into the Rotterdam Study, participants were continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. For reported events, additional clinical information and the results of brain imaging were obtained from hospital records. Research physicians discussed information on all potential strokes with an experienced stroke neurologist (P.J.K.) to verify all diagnoses. For the current study, case definition included a history of ischemic (n=77) or unspecified (n=19) stroke but not hemorrhagic stroke (n=7), because these strokes are primarily caused by other mechanisms than ischemic atherosclerotic disease, like hypertension and amyloid angiopathy.

## Statistical analysis

Because calcium scores had a highly skewed distribution, we used natural log-transformed values and added 1 in order to deal with persons who had a calcium score of zero (i.e. log(calcium+1)) when we analyzed calcium scores linearly. We used binary logistic regression to examine the association between carotid, aortic arch and coronary calcification and history of stroke. We examined effect modification by gender. Since the interaction terms gender x carotid artery calcium score, gender x aortic arch calcium score and gender x coronary calcium score were not statistically significant, we used gender as a covariable. Model 1 was adjusted for age and gender. Model 2 was additionally adjusted for systolic blood pressure, use of anti-hypertensive medication, current smoking, diabetes, total cholesterol, HDL-cholesterol, use of cholesterol-lowering medication, atrial fibrillation and history of CHD. In model 3, we additionally adjusted for calcification in the other two vessel beds. We modeled the calcium scores linearly (log(calcium+1)) and calculated standardized odds ratios (OR) to be able to compare the effect of carotid with aortic arch and coronary artery calcification on the presence of stroke. To facilitate interpretation of our results, we examined the association of quartiles of carotid, aortic arch and coronary artery calcium scores with prevalent stroke, using the lowest quartile of calcium as a reference. Since calcium scores were higher in men than in women, we used gender-specific quartiles.

**Table 1.** Baseline characteristics of the study population

| Variable | No stroke n=2,425 | Stroke n=96 |
|---|---|---|
| Age, y | 69.5±6.7 | 73.1±8.0 |
| Men | 48 | 63 |
| Systolic blood pressure | 147±20 | 146±24 |
| Use of anti-hypertensive medication | 31.0 | 47.1 |
| Current smoker | 12.5 | 12.6 |
| Diabetes | 11.1 | 19.5 |
| Hypercholesterolemia | 40.9 | 59.8 |
| Atrial fibrillation | 4.0 | 12.6 |
| History of CHD | 9.4 | 13.8 |
| Carotid artery Agatston score* | 24 (0-135) | 196 (42-496) |
| Aortic arch Agatston score*,† | 305 (46-1049) | 1125 (299-3332) |
| Coronary artery Agatston score*,‡ | 65 (3-350) | 255 (67-801) |
| CT scanner (64-slice MDCT) | 68.9 | 66.7 |

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation. CHD: coronary heart disease. *Value is expressed as median (inter-quartile range) because of its skewed distribution. † Based on n=2,518. ‡ Based on n=2,441.

Covariables were missing in less than 3% of persons. We used single imputation by the Expectation Maximization method. Missing calcium scores were only imputed when the calcium scores were used as covariables in model 3. All analyses were performed using SPSS 15.0 for Windows (SPSS, Inc, Chicago, Illinois, USA).

## RESULTS

Baseline characteristics of the study population, stratified by history of stroke, are displayed in Table 1. Of the 2,521 participants, 96 persons had a stroke prior to CT scanning. Compared to persons without stroke, the population with previous stroke was older, consisted of more men, had more cardiovascular risk factors and higher calcium scores.

**Table 2A.** Age and gender adjusted odds of having experienced stroke per standard deviation increase in calcification

| **Model 1** | **OR per 1-SD increase (95% CI)**** | | |
|---|---|---|---|
| Parameter | Carotid calcium | Aortic calcium | Carotid calcium |
| Log(calcium+1) | 2.3 (1.7-3.0)§ | 2.1 (1.5-2.9)§ | 1.8 (1.4-2.4)§ |
| Age | 1.2 (1.0-1.5)# | 1.2 (1.0-1.5) | 1.0 (1.0-1.0)# |
| Male gender | 1.1 (0.9-1.4) | 1.2 (1.0-1.5)# | 1.0 (0.9-1.2) |

**Table 2B.** Cardiovascular risk factor adjusted odds of having experienced stroke per standard deviation increase in calcification

| **Model 2** | **OR per 1-SD increase (95% CI)**** | | |
|---|---|---|---|
| Parameter | Carotid calcium | Aortic calcium | Coronary calcium |
| Log(calcium+1) | 2.0 (1.5-2.7)§ | 1.8 (1.3-2.5)§ | 1.6 (1.2-2.1)‖ |
| Age | 1.3 (1.1-1.6)# | 1.3 (1.0-1.6)# | 1.3 (1.1-1.7)# |
| Male gender | 1.1 (0.9-1.4) | 1.2 (1.0-1.5) | 1.1 (0.9-1.4) |
| Current smoking | 1.0 (0.8-1.3) | 1.1 (0.9-1.3) | 1.1 (0.9-1.3) |
| Systolic BP | 0.8 (0.7-1.0) | 0.8 (0.7-1.0) | 0.9 (0.7-1.1) |
| BP lowering med | 1.1 (0.9-1.4) | 1.1 (0.9-1.4) | 1.2 (1.0-1.5)# |
| Total cholesterol | 1.0 (0.8-1.3) | 1.0 (0.8-1.2) | 0.9 (0.7-1.2) |
| HDL-cholesterol | 0.9 (0.7-1.2) | 0.9 (0.7-1.2) | 1.0 (0.8-1.3) |
| Chol lowering med | 1.4 (1.1-1.7)‖ | 1.4 (1.2-1.8)§ | 1.4 (1.1-1.7)‖ |
| Diabetes | 1.1 (0.9-1.3) | 1.1 (0.9-1.3) | 1.1 (0.9-1.3) |
| Atrial fibrillation | 1.1 (1.0-1.3) | 1.1 (1.0-1.3) | 1.1 (1.0-1.3) |
| History of CHD | 0.9 (0.7-1.1) | 0.9 (0.8-1.1) | 0.9 (0.7-1.1) |

**Table 2C.** Odds of having experienced stroke per standard deviation increase in calcification, after additional adjustment for calcification in other vessel beds

| **Model 3** | **OR per 1-SD increase (95% C.I.)**** | | |
|---|---|---|---|
| Parameter | Carotid calcium | Aortic calcium | Coronary calcium |
| Log(calcium+1) | 1.7 (1.2-2.4)§ | 1.2 (0.9-1.8) | 1.2 (0.9-1.6) |

OR: odds ratio, SD: standard deviation. ** OR per SD increase of log(calcium+1) is a standardized odds ratio enabling comparison of the effects of the three measures of calcification on the history of stroke. § P<0.001. || P<0.01. # P<0.05.

Table 2A, B and C display the odds ratios for presence of stroke per standard deviation increase in the logarithm of carotid, aortic arch and coronary artery calcification and respectively represent logistic regression models 1, 2, and 3. We found a strong association of carotid, aortic arch and coronary artery calcification with presence of stroke. The OR (95% CI) for the log(calcium+1) scores were 2.0 (1.5-2.7) for carotid artery calcification, 1.8 (1.3-2.5) for aortic arch calcification and 1.6 (1.2-2.1) for coronary artery calcification after adjustment for cardiovascular risk factors (model 2, table 2B). After additional ad-

**Table 3.** Odds of having experienced stroke per quartile of calcium scores

| **Model 1** | | **OR (95% CI)** | |
|---|---|---|---|
| Quartile | Carotid calcium | Aortic calcium | Coronary calcium |
| 1 | reference | Reference | reference |
| 2 | 1.5 (0.6-3.8) | 1.4 (0.6-3.3) | 2.3 (1.0-5.7) |
| 3 | 3.3 (1.5-7.5)|| | 2.4 (1.1-5.3)# | 3.3 (1.4-7.6)|| |
| 4 | 6.4 (3.0-13.9)§ | 4.7 (2.2-10.1)§ | 4.3 (1.9-10.0)|| |
| **Model 2** | | **OR (95% CI)** | |
| Quartile | Carotid calcium | Aortic calcium | Coronary calcium |
| 1 | reference | Reference | reference |
| 2 | 1.4 (0.5-3.5) | 1.2 (0.5-2.9) | 2.1 (0.8-5.0) |
| 3 | 2.9 (1.3-6.5)# | 1.9 (0.9-4.3) | 2.6 (1.1-6.3)# |
| 4 | 5.0 (2.2-11.0)§ | 3.3 (1.5-7.4)|| | 3.1 (1.3-7.3)# |
| **Model 3** | | **OR (95% CI)** | |
| Quartile | Carotid calcium | Aortic calcium | Coronary calcium |
| 1 | reference | Reference | reference |
| 2 | 1.2 (0.5-3.1) | 0.9 (0.4-2.1) | 1.6 (0.7-4.1) |
| 3 | 2.2 (0.9-5.1) | 1.2 (0.5-2.7) | 1.7 (0.7-4.1) |
| 4 | 3.3 (1.4-7.8)|| | 1.4 (0.6-3.5) | 1.5 (0.6-3.7) |

OR: odds ratio. CI: confidence interval. Model 1: adjusted for age and gender. Model 2: additionally adjusted for systolic blood pressure, use of anti-hypertensive medication, current smoking, diabetes, total cholesterol, HDL-cholesterol, use of cholesterol-lowering medication, atrial fibrillation and history of coronary heart disease. Model 3: model 2 additionally adjusted for calcification in other vessel beds. § P < 0.001. || P < 0.01. # P < 0.05.

justment for calcification in the other vessel beds, this relation only slightly diminished for carotid artery calcification (OR 1.7 (1.2-2.4), but was no longer statistically significant for aortic arch and coronary artery calcification (OR 1.2 (0.9-1.8) and 1.2 (0.9-1.6), respectively (model 3, Table 2C). Odds ratios of measures of calcification are not directly comparable to odds ratios of cardiovascular risk factors in the models because of use of the logarithmic scale for calcium scores. The majority of odds ratios of cardiovascular risk factors were not statistically significant related to prevalent stroke. This might be due to change of lifestyle (e.g. smoking cessation) and start of risk reducing medication after occurrence of stroke.

Table 3 shows the odds ratios for the presence of stroke per quartile of carotid, aortic arch and coronary artery calcium scores. We found strong and graded associations of presence of stroke with carotid calcification (OR quartile 4 vs 1 (95% CI): 5.0 (2.2-11.0)), with aortic arch calcification (3.3 (1.5-7.4) and with coronary calcification (3.1 (1.3-7.3)).

After additional adjustment for calcification in the other vessel beds, relations were no longer statistically significant, except for carotid calcification (OR quartile 4 vs 1 (95% C.I.): 3.3 (1.4-7.8)).

## DISCUSSION

In this population-based study, we found a strong association between the amount of calcium in the carotid, aortic arch and coronary arteries and presence of stroke. The associations were independent of cardiovascular risk factors. After additional adjustment for calcification in the other vessel beds, presence of stroke was still significantly related to carotid calcification, but no longer to aortic arch or coronary calcification.

### Previous studies on calcification and stroke

Nandalur et al.[14] examined the relation between the amount of carotid calcification and stroke in a retrospective study among 49 patients (mean age 70 years, 57% males) referred for MDCT angiography. The volume of carotid artery calcification was significantly related to presence of TIA (n=9) and stroke (n=12), independently of cardiovascular risk factors. The results of our study are in line with their findings. Culebras et al.[13] measured the amount of calcium, assessed by CT, in the carotid arteries in 40 symptomatic persons. They did not find a significant difference in calcium scores between the symptomatic and asymptomatic sides of the carotid arteries and concluded that calcium does not contribute to the development of symptoms. However, this study was hampered by lack of participation of asymptomatic patients.

Until now, only two studies investigated the association between aortic calcification, as assessed by CT, and risk of ischemic cerebrovascular events. One community-based

study comprised 2,618 persons (mean age 53 years, 51% males) of which 28 persons had a history of cerebral infarction. Presence of calcification in the ascending, arch and/or descending aorta was related to history of stroke, independent of cardiovascular risk factors.[15] The other study comprised 455 hypertensive patients with at least one additional cardiovascular risk factor of whom 27 persons developed a TIA or stroke during 3-year follow-up. Severe calcification (defined by thickness and extension) of the descending, but surprisingly, not of the ascending aorta, was independently related to ischemic cerebrovasular events.[16] Iribarren et al.[25] examined aortic arch calcification based on the presence or absence of calcification on chest radiographs and risk of stroke in a large prospective study among volunteers. In women, an independent association of calcification in the aortic arch with ischemic stroke was found, but no association was present in men.

Scarce research has been performed on the relation of CAC and stroke. A previous cross-sectional study within the Rotterdam Calcification Study cohort also found a graded relation of CAC, measured with EBCT, with risk of stroke (OR (95% CI) up to 3.0 (1.3-6.8)) for CAC >500 compared to a CAC score of 0-100).[17] In contrast, the Multi-Ethnic Study of Atherosclerosis (MESA) followed 6,698 persons free of cardiovascular disease (CVD) for a median period of 3.9 years and did not find an association of CAC with future stroke risk (Hazard Ratio (95% CI): 1.1 (0.8-1.4) per standard deviation increase of log(calcium+1).[18]

We found that, independently of calcification in the other vessel beds, presence of stroke was still significantly related to carotid calcification, but no longer to aortic arch or coronary calcification. A recent population-based study showed that the relation of thoracic calcification with CHD and CVD was not independent of the amount of coronary calcification,[26] whereas another recent, large study by Santos et al. found that the association of thoracic aorta calcium with total mortality was independent of coronary calcification.[27] To our knowledge, no previous study on the relation between arterial calcification and stroke examined multiple vessel beds simultaneously. Additional research is needed to examine whether measuring the amount of carotid calcification is sufficient to improve stroke risk prediction.

### Non-invasive measures of atherosclerosis

MDCT measures calcification, not plaque per se. Coronary calcification determined by EBCT is correlated with the total area of coronary plaque.[28] Also the assessment of aortic calcification has been shown to be related to aortic atherosclerosis.[29] To the best of our knowledge, there are no data on the relation between carotid artery calcification and carotid plaque burden. As long as we do not have reasons to assume that the process of calcification differs across vessel beds, we believe that carotid artery calcification reflects carotid atherosclerosis.

Most studies on aortic arch atherosclerosis and risk of stroke used TEE. This method, however, is an invasive investigation and allows only subjective quantification of the plaque. In most studies, carotid atherosclerosis was assessed by ultrasound. The measurement of carotid plaques by ultrasound is based on the presence of plaques at different sites, and therefore involves an imprecise quantification of the extent of plaque. Carotid IMT is a standardized and quantitative measure of atherosclerosis. However, it is thought to only represent early stages of atherosclerosis.[30] The use of MDCT has the advantage that it is non-invasive, provides an accurate assessment of the amount of calcification, and different vessel beds can be measured during one session. However, the use of MDCT has also some drawbacks. MDCT costs amount to $200 to $600 per scan which might make screening at the population level expensive at this point in time.[31] Furthermore, MDCT generates images with the use of X-rays. The radiation dose for detecting calcified atherosclerosis is relatively low (effective dose of 0.7 milliSievert (mSv) with EBCT and 1.0–2.0 mSv with MDCT. In persons with an irregular heart rhythm, cardiac scans require somewhat higher dosages (up to 4.1 mSv).[32] The lifetime excess cancer risk due to radiation exposure from a single examination at age 40 years is estimated at 9 cancers per 100,000 men and 28 cancers per 100,000 women.[31] Costs, risks and benefits should be carefully analyzed and weighed to determine if and for whom calcium screening would be an adequate preventive measure. At this moment, there is insufficient evidence to decide which imaging modality and which vessel bed is best for risk stratification. Prospective data on the different measures in relation to risk of stroke are necessary for proper evaluation.

**Strengths and limitations**

The advantages of our study are the large population and the inclusion of three vessel beds measured by the same diagnostic tool. Since calcification was measured without knowledge of a history of stroke, information bias is not likely to have influenced our results. However, to appreciate our results, some limitations of our study should also be addressed. First, for assessment of calcification, we used two types of MDCT scanners (16-slice and 64-slice). However, we do not expect this to have influenced our results for the percentages of persons who got a 64-slice scan were similar for persons with and without stroke (66.7 versus 68.9%). When we repeated all analyses with additional adjustment for scanner type, our results indeed did not change substantially (data not shown). Second, the occurrence of stroke may have led to changes in life-style and medication use to reduce risk of a recurrent event. The change of life-style and intervention therapy could have reduced the difference in amount of arterial calcification between persons with and without stroke, and therefore the observed risk estimates may have been underestimated. On the other hand, we cannot exclude that arterial calcification progressed faster in persons with a history of stroke compared to persons without a

stroke, due to mutual cardiovascular risk factors, which would lead to an overestimation of the found association. The only prospective study performed thus far on the relation of CAC with stroke did not find a signification association.[18] Additional research should answer the question whether the associations of arterial calcifications with stroke found in cross-sectional studies can or cannot be confirmed prospectively.

## Conclusion

In this population-based study, we found a strong and graded association of previous stroke with carotid artery, aortic arch and coronary artery calcification, independent of cardiovascular risk factors. After additional adjustment for calcification in the other vessel beds, presence of stroke was still significantly related to carotid calcification, but no longer to aortic arch or coronary calcification.

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# CHAPTER 4

## Coronary calcification in cardiovascular risk prediction

**Manuscript based on this chapter**

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# CHAPTER 4.1

## Coronary calcium scoring in cardiovascular risk stratification


**SUMMARY**

**Background:** Although coronary calcium scoring has been found to improve coronary heart disease (CHD) risk prediction, there are limited data on its impact in clinical practice. The aim of this study was to examine the effect of coronary calcium on the classification of 10-year hard CHD risk and to empirically derive cut-off values of the calcium score for a general population of elderly.

**Methods:** The study comprised 2,028 asymptomatic participants (69.6±6.2 years) from the Rotterdam study. During a median follow-up of 9.2 years, 135 hard coronary events occurred. Persons were classified into low (<10%), intermediate (10-20%) and high (>20%) 10-year coronary risk categories based on a Framingham refitted risk model. In a second step, the model was extended by coronary calcium and reclassification percentages were calculated. Cut-off values of coronary calcium for persons in the intermediate risk category were empirically derived based on 10-year hard CHD risk.

**Results:** Reclassification by means of coronary calcium scoring was most substantial in persons initially classified as intermediate risk. In this group, 52% of men and women were reclassified, all into more accurate risk categories. Coronary calcium values above 615 or below 50 were found appropriate to reclassify persons into high or low risk, respectively.

**Conclusion:** In a general population of elderly at intermediate CHD risk, coronary calcium scoring is a powerful method to reclassify persons into more appropriate risk categories. Empirically derived coronary calcium cut-off values at which persons at intermediate risk reclassified to either high or low risk were 615 and 50 AU, respectively.

## INTRODUCTION

In primary prevention of CHD, clinical management is generally based on a person's 10-year CHD risk as estimated by risk scoring algorithms like the Framingham Risk Score. Individuals are typically classified into categories of low, intermediate and high 10-year CHD risk and treated accordingly. However, risk scoring algorithms appear to have limited accuracy to identify persons at high risk to develop CHD. Risk prediction may be improved by use of non-invasive tests of atherosclerosis such as assessment of coronary artery calcium (CAC) by computed tomography (CT) scan which is known to predict cardiovascular disease.[1-8]

To establish the clinical value of a new test, an important issue is to assess the reclassification of individuals into different risk categories when the new test is added to classical risk factors.[9] Additional testing for atherosclerosis is proposed to be most useful if applied to the intermediate risk category (10-20% CHD risk in 10 years), in which treatment decisions are uncertain.[10-12] However, reclassification percentages for coronary calcium and empirically derived coronary calcium cut-off values at which persons may be reclassified to a more appropriate risk category, based on 10-year CHD risk data, are lacking.

In a population-based cohort study among persons of ≥55 years of age with nearly 10-year follow-up data, we studied the usefulness of coronary calcium to reclassify individuals into more accurate risk categories and derived empirically based cut-off values of coronary calcium.

## METHODS

### Study population

The study was embedded in the Rotterdam Study, a prospective, population-based study among persons aged 55 years and older in a municipality of Rotterdam. The rationale and design of the Rotterdam Study have been described elsewhere.[13] The baseline examination was completed between 1990 and 1993, followed by a second round between 1993 and 1995. The third examination took place from 1997 to 1999. From 1997 onwards, participants through 85 years of age were invited to undergo electron beam CT (EBCT) scanning of the heart to perform coronary calcium scoring. This has been referred to as the Rotterdam Coronary Calcification Study.[8] The in- and exclusion of persons in the study (response rate, 61%) have been described in detail by Vliegenthart et al.[8] During the inclusion phase, the study population was supplemented with inhabitants who reached the age of 55 years after the baseline examination and persons aged 55 years and over who migrated into the research area. Data were available for a total of 2,292 participants. Of these, 252 participants had a history of CHD, defined as a his-

tory of clinically manifest myocardial infarction, coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI) at the time of EBCT scanning. Hence, the current analysis was carried out in 2,040 asymptomatic persons. The Medical Ethics Committee of Erasmus Medical Center approved the study, and all participants gave informed consent.

### Coronary calcium

We assessed coronary calcium in the epicardial coronary arteries detected on EBCT scans. Imaging was performed with a C-150 Imatron scanner (GE-Imatron). Before participants 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 millisecond scan time and 3 millimeter slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of coronary calcium was performed with AccuImage software (AccuImage Diagnostics Corporation) displaying all pixels with a density above 130 Hounsfield units (HU). A calcification was defined as a minimum of two adjacent pixels with a density over 130 HU. A coronary calcium score was calculated according to Agatston's method.[14] The trained scan readers were blinded to the clinical data of the participants. Conform the study protocol participants were not informed about the calcium score.

### Cardiovascular risk factors

At the third examination, information on medical history, drug use, and smoking behavior was collected using a computerized questionnaire. Anthropometric measures were obtained during the visit at the research center. Blood pressure was measured at the right brachial artery with a random-zero sphygmomanometer with the participant in sitting position. The mean of 2 consecutive measurements was used. After an overnight fast, blood samples were obtained at the research center. Serum total cholesterol was determined by an automated enzymatic procedure using the Roche CHOD-PAP reagent agent, and HDL was measured with the Roche HDL-cholesterol assay using PEG-modified enzymes and dextran sulfate. Glucose was determined enzymatically by the Hexokinase method. Diabetes was diagnosed based on a fasting plasma glucose level ≥7.0 mmol/L or use of anti-diabetic medication. C-reactive protein was measured in serum using a nephelometric method (Immage; Beckman Coulter). Median duration between measurement of cardiovascular risk factors and CAC scanning was 62 days.

### Clinical outcomes

Information on fatal and non-fatal cardiovascular endpoints was obtained from general practitioners and letters and discharge reports from medical specialists. All events were classified by study physicians according to the ICD-10. The follow-up procedures have

previously been described in detail.[8] Twelve participants were lost to follow-up, which left 2,028 participants for analyses. As an outcome we used hard CHD (non-fatal myocardial infarction and CHD mortality). If a non-fatal event occurred within 28 days prior to CHD death, the event was attributed to CHD mortality. Persons were followed for a median time (inter-quartile range) of 9.2 (8.3-10.0) years

**Statistical analysis**

We used a parametric Weibull proportional hazards regression model that allows computation of individual 10-year predicted risk of CHD from our available 9.2 years of median follow-up duration. Hazard ratio estimations from a Weibull model are very similar to those from a Cox model, both models being proportional hazards regression models.[15] We chose a Weibull model over the more frequently used Cox model because the latter can not estimate the 10-year cumulative incidence by extrapolation of the actual median follow-up time of 9.2 years. In contrast, the Weibull model can make this extrapolation because of its parametric nature. We fitted two Weibull prediction models to the Rotterdam Study data: model 1, referred to as the "Framingham refitted" model, was based on variables included in the Framingham risk function, namely age, systolic blood pressure, anti-hypertensive medication, total and HDL-cholesterol, diabetes and current smoking.[16] Gender was used as a covariate in this model. We chose to fit a model comprising Framingham risk factors on our data (Framingham refitted model) instead of using the Framingham risk function [16] because previous research showed that application of the Framingham risk function to the elderly Rotterdam Study population led to systematic overestimation of CHD risk in men. [17] In a second step, we extended the "Framingham refitted" model with the natural logarithm of CAC (log(CAC+1), model 2). We added 1 to all CAC values in order to deal with persons who had a CAC score of zero. We fitted this extended model to our data and compared the performance of these two models using the methods described in the next sections. In an extra analysis, we examined the importance of CRP to enhance predictive performance of the "Framingham refitted" and the "Framingham refitted" plus CAC model.

To determine the functional form used for each predictor, we examined restricted cubic spline transformations for continuous predictors [18] and used the likelihood ratio test to examine the linearity assumption. If appropriate, we chose the simplest form, usually a linear term. We assessed the appropriateness of the Weibull survival time distribution by plotting observed Kaplan-Meier survival curves based on hard CHD events (n=135) against estimates from the Weibull model in strata of low, intermediate and high Framingham risk categories and found good agreement.

We compared global model fit using the likelihood ratio chi-square test. To examine the discriminative ability each model, we calculated the optimism-corrected C-statistic using 150 bootstrap repetitions for each model by method of Harrell.[18, 19] Next, we com-

puted reclassification percentages to study the incremental ability of coronary calcium to classify persons in risk categories according to commonly used categories of 10-year CHD risk: low (<10%), intermediate (10-20%), and high risk (>20%).[20] Estimated 10-year risks were calculated for each cell of the reclassification table to show calibration of reclassified observations with observed risk. To evaluate true improvement in classification by addition of coronary calcium to the "Framingham refitted model" we calculated the net reclassification improvement (NRI). The original method for calculation of the NRI by Pencina was proposed for binary data.[21] In that case, the number of cases and non-cases is apparent. However, in survival data, the number of cases and non-cases at the time point of interest usually is not because not all persons have a complete follow-up. We calculated the NRI with the expected number of cases, as recently proposed by Steyerberg and Pencina.[22] For each of the three risk categories, we first estimated the cumulative incidence of hard CHD events at 10 years by use of Weibull proportional hazard analysis. We then estimated the absolute number of cases in each category by multiplying the cumulative incidence by the number of persons in that category. The number of non-cases per category was the total number of persons minus the number of cases. In accordance with the original NRI calculation method, any 'upward' movement in persons with an event implied correct reclassification, and any 'downward movement' indicated incorrect reclassification. The interpretation was opposite for people who did not develop events. The net improvement in reclassification was quantified as the proportion of correct minus the proportion of incorrect reclassification.

Since additional calcium scoring is proposed to be most useful if applied to the intermediate risk category,[10-12] we derived coronary calcium cut-off values at which individuals at intermediate risk were reclassified into the high or low risk category. For this purpose, we included the Framingham risk categories as categorical and log(CAC+1) as a linear term in the Weibull model. By means of a gender x CAC interaction we examined the need for gender-specific CAC cut-off values.

Information on covariables was missing in up to 3.5% of persons, except for antihypertensive medication use, which was missing in 24% of persons. Missing values were imputed using the mice library of R, as described previously.[17] All analyses were performed using SPSS 12.01 for Windows (SPSS, Inc., Chicago, Illinois, USA) and R version 2.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

Baseline characteristics of the study population, overall and by gender, are shown in Table 1. Mean age of the population at baseline was 69.6 years, 43% were male. During the median follow-up duration (inter-quartile range) of 9.2 (8.3-10.0) years, 503 persons

**Table 1.** Baseline characteristics of the study population

| Variable | All (n=2,028) | Men (n=864) | Women (n=1,164) |
|---|---|---|---|
| Age, years | 69.6±6.2 | 69.5±6.1 | 69.7±6.3 |
| Male, % | 42.6 | | |
| Body-mass-index, kg/m$^2$ | 27.1±4.0 | 26.5±3.2 | 27.5±4.5 |
| Systolic blood pressure, mmHg | 143±21 | 144±21 | 143±21 |
| Diastolic blood pressure, mmHg | 76±11 | 78±12 | 75±11 |
| Anti-hypertensive medication, % | 27.6 | 23.4 | 30.4 |
| Total cholesterol, mmol/L | 5.9±1.0 | 5.7±1.0 | 6.0±0.9 |
| HDL-cholesterol, mmol/L | 1.4±0.4 | 1.3±0.3 | 1.5±0.4 |
| Cholesterol-lowering medication, % | 14.0 | 12.0 | 15.2 |
| Smokers, % | | | |
| Current | 16.8 | 19.1 | 15.1 |
| Former | 52.0 | 68.9 | 39.6 |
| Serum glucose, mmol/L | 5.9±1.5 | 6.0±1.6 | 5.8±1.4 |
| Anti-diabetic medication, % | 6.5 | 8.0 | 5.4 |
| Family history of MI, % | 18.4 | 17.0 | 19.6 |
| Calcium score, Agatston | 84 (8-382) | 191 (35-623) | 37 (3-210) |
| Persons without coronary calcium, % | 10.5 | 3.8 | 15.5 |

Categorical variables are presented as percentage. Continuous values are expressed as mean ± standard deviation. Median (inter-quartile range) is presented in case of skewed distribution.
HDL: high-density lipoprotein, MI: myocardial infarction.

died, while 135 had their first hard coronary event (81 non-fatal myocardial infarctions and 54 CHD deaths). The median calcium score was 84 (inter-quartile range 8-382).

Regression parameters of the Weibull models are displayed in Table 2. Compared to the Framingham refitted model, addition of coronary calcium significantly improved model fit (LR $\chi^2$ increase: 36.4; P<0.001). The C-statistic improved significantly from 0.72 to 0.76, indicating better average discriminative ability of the model including coronary calcium.

In an extra analysis, we examined the importance of CRP to enhance predictive performance of the Framingham refitted model and found that addition of CRP did not result in a significant improvement of the C-statistic (C-statistic FRS+CRP model: 0.72 compared to 0.72 of the FRS model alone, P=0.31). Furthermore, CRP did not significantly improve the C-statistic of the FRS+CAC model (C-statistic FRS+CAC+CRP model: 0.76 compared to 0.76 of the FRS+CAC model, P= 0.61).

Persons stratified according to categories of estimated 10-year hard CHD risk based on the Framingham refitted model and after adding coronary calcium to that model are presented in Tables 3 and 4. In men, percentages in the low, intermediate and high risk categories were 54%, 33% and 13% respectively, for women these percentages were 83%, 15% and 2%. The largest proportions of reclassified persons were seen in

**Table 2.** Parameter estimates and performance measures of the Framingham refitted and the Framingham plus CAC models

| Model | Framingham | | Framingham + CAC | |
|---|---|---|---|---|
| Parameter | HR | 95% CI | HR | 95% CI |
| Age* | 2.64 | 1.97-3.52 | 2.09 | 1.54-2.83 |
| Male gender | 1.61 | 1.12-2.32 | 1.18 | 0.81-1.72 |
| Systolic blood pressure | 1.02 | 0.94-1.11 | 1.01 | 0.93-1.10 |
| Anti-HT medication use | 1.23 | 0.85-1.80 | 1.06 | 0.73-1.56 |
| Total cholesterol | 1.19 | 1.00-1.43 | 1.17 | 0.97-1.40 |
| HDL-cholesterol | 0.30 | 0.17-0.53 | 0.31 | 0.18-0.54 |
| Diabetes | 1.25 | 0.75-2.08 | 1.15 | 0.69-1.91 |
| Current smoking | 1.66 | 1.09-2.52 | 1.46 | 0.95-2.23 |
| Log(CAC+1) | | | 1.33 | 1.21-1.47 |
| **Performance measures** | | | | |
| Likelihood chi-square | 83.93 | | 120.32 | P < 0.001 |
| C-statistic | 0.72 | | 0.76 | P < 0.001 |

HR: hazard ratio, CI: confidence interval, HT: hypertension, HDL: high-density lipoprotein, CAC: coronary artery calcium. * HR per 10 years of increase in age instead of per one unit increase.

**Table 3.** Cardiovascular risk reclassification comparing the Framingham refitted model with the model additionally including coronary calcium

| Framingham refitted 10-year risk categories | Framingham refitted + coronary calcium 10-year risk categories | | | N (%) reclassified |
|---|---|---|---|---|
| | <10% | 10-20% | >20% | |
| **<10%** | | | | |
| N=1,433 | 1,273 (88%) | 156 (11%) | 4 (1%) | 160 (12%) |
| Observed risk (95%CI) | 0.03 (0.02,0.05) | 0.13 (0.08,0.20) | NA | |
| **10-20%** | | | | |
| N=451 | 134 (30%) | 216 (48%) | 101 (22%) | 235 (52%) |
| Observed risk (95%CI) | 0.09 (0.05,0.16) | 0.14 (0.10,0.20) | 0.29 (0.20,0.41) | |
| **>20%** | | | | |
| N=144 | 7 (5%) | 42 (29%) | 95 (66%) | 49 (34%) |
| Observed risk (95%CI) | 0.49 (0.15,0.94) | 0.13 (0.05,0.31) | 0.31 (0.21,0.44) | |

CI: confidence interval, NA: not applicable.

the intermediate Framingham risk group (n=451; 22% of the total population). Among men, 51% were reclassified, 30% moved to the low risk category and 21% to the high risk group. In women at intermediate risk, 53% were reclassified with 29% moving downward and 24% moving upward in risk. Reclassification percentages were generally smaller in persons initially classified as low or high with the Framingham refitted model. In men at low Framingham risk, 15% moved to the intermediate risk group but only 2

**Table 4.** Cardiovascular risk reclassification comparing the Framingham refitted model with the model additionally including coronary calcium, by gender

| Framingham refitted 10-year risk categories | Framingham refitted + coronary calcium 10-year risk categories | | | N (%) reclassified |
|---|---|---|---|---|
| | <10% | 10-20% | >20% | |
| **Men** | | | | |
| **<10%** | | | | |
| N=467 | 394 (85%) | 71 (15%) | 2 (0%) | 73 (15%) |
| Observed risk (95%CI) | 0.04 (0.02,0.06) | 0.12 (0.06,0.24) | NA | |
| **10-20%** | | | | |
| N=281 | 84 (30%) | 137 (49%) | 60 (21%) | 144 (51%) |
| Observed risk (95%CI) | 0.04 (0.01,0.12) | 0.15 (0.09,0.23) | 0.32 (0.20,0.48) | |
| **>20%** | | | | |
| N=116 | 6 (5%) | 32 (28%) | 78 (67%) | 38 (33%) |
| Observed risk (95%CI) | 0.57 (0.19,0.97) | 0.13 (0.05,0.36) | 0.33 (0.22,0.47) | |
| **Women** | | | | |
| **<10%** | | | | |
| N=966 | 879 (91%) | 85 (9%) | 2 (0%) | 87 (9%) |
| Observed risk (95%CI) | 0.03 (0.02,0.05) | 0.14 (0.08,0.24) | NA | |
| **10-20%** | | | | |
| N=170 | 50 (29%) | 79 (47%) | 41 (24%) | 91 (53%) |
| Observed risk (95%CI) | 0.17 (0.08,0.32) | 0.13 (0.08,0.32) | 0.26 (0.14,0.45) | |
| **>20%** | | | | |
| N=28 | 1 (3%) | 10 (36%) | 17 (61%) | 11 (39%) |
| Observed risk (95%CI) | NA | 0.12 (0.02,0.61) | 0.24 (0.09,0.58) | |

CI: confidence interval, NA: not applicable.

persons moved to the high risk group. In low risk women 9% moved to the intermediate group and 2 persons to the high risk group. In men at Framingham high risk, 28% moved to the intermediate risk group and 5% to the low risk group. In women these percentages were 36% and 3%. Generally, point estimates of the observed risks agreed with the corresponding categories of predicted risk indicating good calibration. However, in some groups calibration assessment was hampered by small numbers of reclassified persons. In all persons, addition of coronary calcium to the Framingham refitted model significantly improved risk classification as indicated by an NRI of 0.14 ($P< 0.01$).

Figure 1 displays the association of individual coronary calcium scores against 10-year predicted risk of CHD in persons at intermediate Framingham risk. Since there was no evidence of a gender-specific prognostic effects of CAC ($P=0.55$), an overall curve is presented. Empirically derived cut-off values correspond to the cross-section of the curve with the low and high risk demarcation. CAC scores above 615 and below 50 Agatston units suggest reclassification to the high or low risk stratum, respectively.

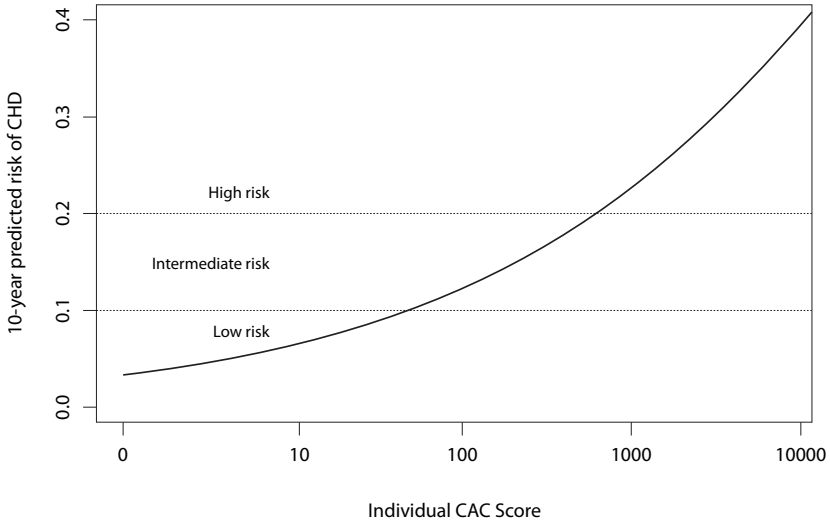


**Figure 1.** Association of individual coronary calcium scores with predicted 10-year risk of CHD. 10-year absolute coronary heart disease (CHD) risk estimation by coronary calcium score of the persons at intermediate risk (n=451). Empirically derived coronary calcium cut-off values at which persons at intermediate risk reclassified to either high or low risk were 615 and 50 Agatston units, respectively.

## DISCUSSION

This population-based study among persons ≥55 years of age, with almost 10 years of follow-up shows that adding coronary calcium to the Framingham risk model leads to substantial reclassification between CHD risk categories, especially in persons at intermediate Framingham risk. Over 50 percent of both men and women in the intermediate risk group were reclassified into the high or low risk category. The empirically derived cut-off values at which individuals moved from the intermediate to the high or the low risk group were 615 and 50 Agatston units, respectively.

### Coronary calcium in CHD risk assessment

Non-invasive assessment of atherosclerosis is regarded as most useful in persons classified as intermediate Framingham risk in which treatment decisions are uncertain.[10-12] In our study, reclassification by additional coronary calcium testing was particularly high in the intermediate risk group, where 52% of persons (men and women combined) were reclassified (30% to the low risk and 22% to high risk group). This is in accordance with the findings of a recent population-based cohort study by MESA among 5,878 individuals who were free of cardiovascular disease (CVD) at baseline (mean age 62 years, 46% men) that studied the additional value of CAC beyond traditional risk factors using a 5-year CHD risk prediction model.[23] In this study, the reclassification proportion in the

intermediate risk group was 55% (39% to the low risk and 16% to high risk group). Reclassification percentages by addition of CAC in the intermediate risk group are much higher than those reported for adding ankle-brachial-index (ABI) to classical risk factors in a recent meta-analysis study.[24] In this study, 4% of men and 10% of women at intermediate risk were reclassified after adding ABI to the risk model. The Women's Health Study previously reported 19% reclassification for C-reactive protein (CRP) in women at intermediate risk.[25] The Framingham offspring study reported 23% reclassification by addition of CRP in this group.[26] Reclassification percentages were smaller in the initial low and high risk groups. In men at low Framingham risk, 15% moved to the intermediate risk group but only 2 persons moved to the high risk group; in low risk women 9% moved to the intermediate group and 2 persons to the high risk group. In men at Framingham high risk, 28% moved to the intermediate risk group and 5% to the low risk group. In women these percentages were 36% and 3%. Overall, 12% of low risk persons and 34% of high risk persons reclassified. These percentages are comparable with the recent MESA study which reported 11% reclassification in the low risk group and 36% reclassification in the high risk group.[23] At present, it is uncertain whether the decline in absolute risk has treatment implications because there is no evidence that intensive preventive interventions can be safely mitigated in persons at high Framingham risk.[10, 11]

Other studies have shown an effect of coronary calcium testing on CHD risk assessment in Framingham risk categories, but did not present reclassification percentages.[1, 5] Two cross-sectional studies showed that the use of calcium testing enabled identification of a higher risk subset among intermediate risk individuals.[4, 6] Finally, a study among patients referred for calcium screening by primary care physicians showed that coronary calcium scoring is an effective risk stratification tool in both young and elderly persons.[7] Limitations of this study are the use of self-reported, categorical risk factors, the possibility of referral bias, and the use of total mortality rather than CHD events as outcome.

### Cut-off values

Coronary calcium cut-off values at which individuals reclassify to another risk category are a prerequisite for incorporation of coronary calcium testing in clinical practice. However, currently used cut-off values are not based on empirical data. A recent study by MESA found that using predefined absolute CAC cut-off values of 1, 100 and 400 AU performed better than age-, sex-, and race/ethnicity-specific percentiles in terms of model fit and discrimination. However, this study had a median of 3.75 years of follow-up and therefore no empirically derived absolute cut-off values for classification of 10-year CHD risk.[27] To our knowledge, we are the first to have estimated coronary calcium cut-off values based on nearly 10 years of follow-up. Our empirically derived upper cut-off value of 615 AU seems to be higher than the commonly used cut-off values of 400 AU, while our lower cut-off value of 50AU seems to be lower than the commonly use cut-off of 100 AU.

However, our cut-off values need to be validated in other, large population-based studies to further establish whether empirically based cut-offs are indeed different from the arbitrary cut-offs of 400 and 100 AU. Furthermore, one should realize that these cut-off values refer only to men and women in the intermediate risk group that comprised 33% of men and only 15% women. Using lower boundaries for intermediate risk category in women would lead to more women eligible to undergo calcium scoring. However, we based our definition of the intermediate risk group (10-20% 10-year CHD risk) on widely used guidelines.[20] The contention that coronary calcium testing may be useful for refining risk assessment in women at low to intermediate risk (5-20%) has been suggested based on cross-sectional data [28] and an analysis of the Multi-ethnic Study of Atherosclerosis (MESA) based on 24 soft and hard incident CHD events.[29]

**Strengths and limitations**

Strengths of the current study include a large, unselected sample of asymptomatic individuals and an almost 10-year follow-up. Our cohort provided the opportunity to study the impact of coronary calcium testing on risk classification for hard CHD events and to derive cut-off values at which persons were reclassified to other risk categories. Moreover, participants were unaware of their calcium score, therefore change in lifestyle or medication use, or additional cardiac testing on the basis of the calcium score was unlikely to occur. Limitations of our study also need to be addressed. Firstly, while previous research within the Rotterdam study showed that application of the Framingham risk function led to systematic overestimation of CHD risk in men,[17] we chose to fit a model based on Framingham risk factors to stratify our population in the well known risk categories. A potential drawback is over-fitting of the model, which could lead to underestimation of the additional value of CAC measurement. However, the C-statistics of both the Framingham and the Framingham plus CAC model were corrected for over-optimism using the bootstrap method, as described in the methods section. Secondly, the model extended with coronary calcium calibrated generally well with observed risks, except in categories with small numbers. We computed 95% confidence intervals to show plausible ranges of observed risk. Thirdly, in order to comply with current guidelines, we extrapolated CHD risk estimates to 10-year risk from an actual follow-up period of 9.2 years using a parametric survival modeling approach. Although the accuracy of the extrapolation cannot be verified, large deviations are very unlikely for the actual follow-up time is so close to 10 years. Fourthly, we estimated empirically based cut-off points for CAC suitable for our population. Of course, accruing data is needed to validate this finding before it can be applied in the clinical setting of primary prevention. Furthermore, the ultimate judgment about the selection of persons undergoing CAC scoring and subsequent implications regarding clinical management should also be based on randomized clinical trials and cost-effectiveness analyses. Finally, our study

was performed in older persons. The predictive power of traditional cardiovascular risk factors decreases with age while increased CAC can be seen as a cumulative measure of the effect of lifetime exposure to cardiovascular risk factors on the arterial vessel wall and may therefore improve risk stratification particularly at older age. This implies that our results should not automatically be generalized to a younger population.

**Conclusion**

In a general population of elderly at intermediate CHD risk, coronary calcium scoring is a powerful method to reclassify persons into more appropriate risk categories. Based on coronary calcium testing, over 50% of an asymptomatic older population at intermediate risk was reclassified as having either low or high risk of hard CHD events. Empirically derived coronary calcium cut off values at which persons at intermediate risk reclassified to high or low risk were 615 and 50 AU, respectively.

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**Manuscript based on this chapter**

Vliegenthart-Proença R, Elias-Smale SE, van Rooij F, Hofman A, Witteman JCM, Oudkerk M. Coronary calcification has a very high negative predictive value in older adults: the Rotterdam Study. Submitted.

# CHAPTER 4.2

## The value of a low coronary calcium score


**SUMMARY**

**Background:** Absent or minimal coronary calcification is associated with very low rates of coronary heart disease (CHD) in general, middle-aged populations. Whether this high negative predictive value persists at older age has not been studied.

**Methods:** Coronary calcification was assessed by electron beam computed tomography (EBCT) scanning in a general population of 2,038 asymptomatic older adults with a mean age (± standard deviation) of 69.6 (6.2) years. The current study focused on the subgroup with absent (calcium score 0) or minimal coronary calcification (calcium score 1-10) in comparison to the remainder of the population (calcium score >10). The population was followed for the occurrence of coronary events for a median of 6.8 (inter-quartile range: 6.1-7.5) years. Characteristics of the subgroup, association with CHD and negative predictive value were determined.

**Results:** Coronary calcium was absent or minimal in 553 persons (28% of the elderly population), with 214 persons (11%) having a zero calcium score. During follow-up, 5 coronary events occurred (3 myocardial infarctions, 2 CHD deaths), of which 1 occurred in the group without coronary calcification. The incidence rate of CHD for calcium score 0-10 was 0.14 per 100 person-years. The negative predictive value was 99.1%. No significant difference in negative predictive value was found between the group with a zero calcium score and the group with a very low calcium score.

**Conclusion:** In elderly, absent and minimal coronary calcification virtually rules out the risk of coronary events, irrespective of the presence of risk factors.

## INTRODUCTION

Risk stratification using risk assessment algorithms based on cardiovascular risk factor levels and/or summation of the number of present cardiovascular risk factors[1-3] is the foundation of the primary prevention of coronary heart disease (CHD). Treatment goals are based on the results of risk stratification, with intensified therapy being recommended at increasing levels of risk.[4, 5] In risk assessment algorithms, age is an important factor, with growing point scores at higher ages. Many elderly may therefore classify for intensive treatment of cardiovascular risk factors, primarily based on their age. However, a substantial part of the older population never develops CHD and may therefore be unnecessarily treated with medication such as blood pressure and cholesterol-lowering drugs against considerable costs and with potential side effects. Corroborative evidence has shown that cardiovascular risk stratification can be improved by including the computed tomography (CT)-detected amount of coronary calcification, expressed in the calcium score.[6-14] The calcium score has already been shown to have a very high negative predictive value for mortality and cardiovascular disease in mainly middle-aged populations.[15, 16] In the only study focusing on the predictive value of coronary calcification at advancing age, a zero or low calcium score was associated with a low risk of all-cause mortality in elderly.[17] However, this study did not examine CHD as an outcome. The aim of this population-based study was to investigate the negative predictive value of coronary calcification for development of CHD in older adults.

## METHODS

### Study population

The study was embedded in the Rotterdam Study, a prospective, population-based study among persons aged 55 years and older in a municipality of Rotterdam, which started in 1990. The rationale and design of the Rotterdam Study have been described elsewhere.[18, 19] From 1997 onwards, participants through 85 years of age were invited to undergo electron beam CT scanning of the heart to determine the amount of coronary calcium. The in- and exclusion of participants of the study (response rate 61%) has been described in detail by Vliegenthart et al.[13] During this phase the study population was supplemented with inhabitants who reached the age of 55 years after the baseline examination and persons aged 55 years and over who migrated into the research area. The Medical Ethics Committee of Erasmus Medical Center approved the study, and all participants gave informed consent.

### Coronary calcium

Imaging of coronary calcium was performed with a C-150 Imatron scanner (GE-Imatron). Before the persons 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 milliseconds scan time and 3 millimeter (mm) slice thickness. We acquired images at 80% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of coronary calcium was performed with AccuImage software (AccuImage Diagnostics Corporation) displaying all pixels with a density above 130 Hounsfield units (HU). A calcification was defined as a minimum of 2 adjacent pixels (area = 0.65 $mm^2$) with a density over 130 HU. A coronary calcium score was calculated according to Agatston's method.[20] The trained scan readers were blinded to the clinical data of the participants. Conform the study protocol, participants were not informed about the calcium score.

### Cardiovascular risk factors

At the third examination, information on medical history, drug use, and smoking behavior was collected using a computerized questionnaire. During a visit at the research center, body mass index and blood pressure were measured. Additionally, fasting blood samples were obtained to measure total and HDL-cholesterol, glucose and serum C-reactive protein were measured during a visit at the research center as described previously.[13, 21] Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher, or use of medication for the indication hypertension. Hypercholesterolemia was considered present in case of a total cholesterol level of at least 6.2 mmol/L or use of cholesterol-lowering drugs. The HDL-cholesterol was considered low in case of a blood level below 0.9 mmol/L. Diabetes was diagnosed based on a fasting plasma glucose level of at least 7.0 mmol/L or use of anti-diabetic medication. Family history was defined as a family history of myocardial infarction occurring before 65 years of age in first-degree relatives.

### Clinical outcomes

Information on vital status was obtained regularly from municipal health authorities in Rotterdam. Information on fatal and non-fatal cardiovascular endpoints was obtained from general practitioners and letters and discharge reports from medical specialists. All events were classified by study physicians according to the International Classification of Diseases, version 10. The follow-up procedures have previously been described in detail.[18] We used total CHD (non-fatal myocardial infarction, coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI) and CHD mortality) as outcome. Follow-up was available until January 1, 2007.

### Statistical analyses

Calcium scores were available for a total of 2,292 participants. Of these, 248 participants had a history of CHD, defined as a history of clinically manifest myocardial infarction, CABG or PCI at the time of CT scanning. Six participants were lost during follow-up, leaving 2,038 individuals. The in- and exclusion of persons in the current analysis can be appreciated in Figure 1. Analyses were performed separately in the group without coronary calcification (calcium score 0) and the group with minimal coronary calcification (calcium score 1-10). Characteristics were determined in both groups, and compared to assess statistically significant differences. For categorical variables, this was assessed by chi-square analysis. In case of continuous variables, a general linear model was used, with age and sex as covariates (except in case of age: only sex included as covariate). Also, the characteristics for the combined group with zero and minimal calcification were compared to those of the remainder of the study population that had a calcium score over 10. The number of established cardiovascular risk factors was calculated by counting the following risk factors: age at least 70 years, hypertension, hypercholesterolemia, low HDL-cholesterol, current smoking, diabetes mellitus, and family history, based on ATP III criteria.[4] The incidence of CHD events was calculated. Furthermore, we calculated the negative predictive value of a zero calcium score and of a very low calcium score. All analyses were performed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, Illinois, USA). Missing values for the study population were imputed using the mice library of R version 2.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

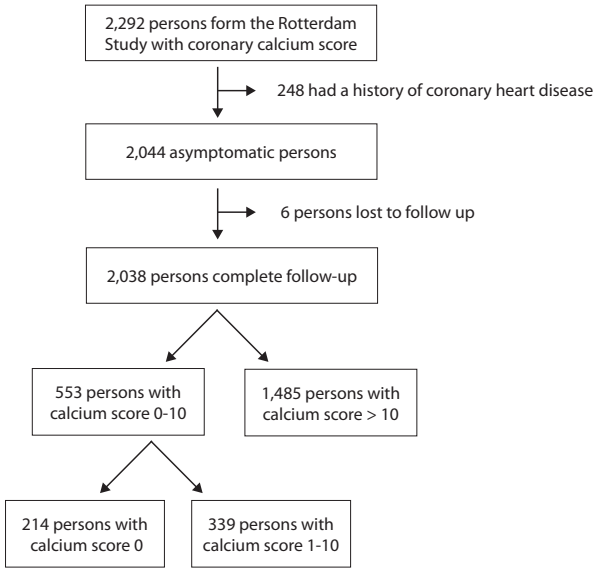


**Figure 1.** Diagram of the in- and exclusion of participants.

## RESULTS

Coronary calcium was absent in 214 participants, comprising 11% of the asymptomatic elderly population. A very low calcium score (calcium score of 1-10) was present in 339 perons (17%). Characteristics of the two groups are provided in Table 1. Compared to those with a positive calcium score up to 10, persons without coronary calcium were more likely to be female, had a lower level of C-reactive protein, had on average a lower body-mass-index and were less likely to have diabetes (all: $P<0.05$). The follow-up period was slightly but significantly longer for those with a negative calcium score compared to those with a very low calcium score: 6.8 (standard deviation: 1.4) years versus 6.5 (1.7) years, $P<0.05$. Compared to those with a calcium score up to 10, the remainder of the population, with a calcium score higher than 10, included significantly more men,

**Table 1.** Baseline characteristics of the population for analysis, by calcium score

| Variable | Calcium score 0 | Calcium score 1-10 | Calcium score >10 |
|---|---|---|---|
| Age, years | 66.8±5.5 | 67.4±6.1 | 70.6±6.1** |
| Male (%) | 15.4 | 30.4* | 49.2** |
| Body-mass-index, kg/m$^2$ | 25.5±3.1 | 27.4±3.9* | 27.3±4.1** |
| Systolic blood pressure, mmHg | 138±21 | 139±20 | 145±21** |
| Diastolic blood pressure, mmHg | 75±10 | 76±10 | 77±11** |
| Anti-hypertensive medication (%) | 19.6 | 18.0 | 30.0** |
| Hypertension (%) | 50.5 | 49.3 | 67.8** |
| Total cholesterol, mmol/L | 5.9±0.9 | 5.8±1.0 | 5.9±0.9** |
| HDL-cholesterol, mmol/L | 1.6±0.4 | 1.5±0.4* | 1.4±0.4** |
| Cholesterol-lowering medication (%) | 11.2 | 8.8 | 15.0** |
| Hypercholesterolemia (%) | 45.8 | 37.8 | 46.4** |
| Low HDL-cholesterol (%) | 3.3 | 5.0 | 5.9 |
| Smokers (%) | | | |
| Current | 9.3 | 12.7 | 18.8** |
| Former | 43.0 | 49.0 | 54.2 |
| Serum glucose, mmol/L | 5.5±0.8 | 5.8±1.1* | 6.0±1.6** |
| Anti-diabetic medication (%) | 1.4 | 2.7 | 7.0** |
| Diabetes mellitus (%) | 3.3 | 7.4* | 12.7** |
| Family history (%) | 16.4 | 14.5 | 19.7** |
| C-reactive protein (mg/L) | 2.7±3.0 | 3.4±3.3* | 3.9±6.4** |
| Number of risk factors | 1.5±1.0 | 1.6±1.1 | 2.2±1.1** |
| At least 2 risk factors (%) | 48.6 | 52.2 | 75.1** |

Categorical variables are presented as percentage. Continuous values are expressed as mean ± standard deviation. HDL: high-density lipoprotein. * Statistically significant difference between calcium score 1-10 and calcium score 0. ** Statistically significant difference between calcium score >10 and calcium score up to 10.

and had significantly higher levels of all cardiovascular risk factors, except for low HDL-cholesterol. About 50% of the individuals with a calcium score up to 10 had at least 2 cardiovascular risk factors, and are thereby regarded as being at increased cardiovascular risk. [4] This was 75% in case of a calcium score above 10.

An overview of the outcomes is provided in Table 2. In the group without any coronary calcification, 1 case of CHD mortality occurred during the follow-up period, giving rise to an incidence rate of coronary events of 0.07 per 100 person-years. The negative predictive value for coronary events was 99.5%. The case, a male, had as only risk factor hypertension. During follow-up, 4 persons with a very low calcium score experienced a coronary event (2 myocardial infarctions, 2 CHD deaths) leading to an incidence rate of 0.18 per 100 person-years. The calculated negative predictive value was 98.8%. Of the 4 cases, which included 2 men, 1 had no risk factors, 1 had a single risk factor (hypercholesterolemia), 1 had 2 risk factors (current smoking and positive family history) and 1 had 3 risk factors (hypercholesterolemia and hypertension and positive family history). Three of these 4 cases had an elevated C-reactive protein level.

**Table 2.** Overview of different coronary heart disease (CHD) outcomes by calcium score

| Calcium score | N | Person-years | MI | CABG | PCI | CHD mortality |
|---|---|---|---|---|---|---|
| **0** | 214 | 1451 | 0 | 0 | 0 | 1 |
| 1-10 | 339 | 2205 | 3 | 0 | 0 | 1 |
| >10 | 1485 | 9192 | 44 | 13 | 19 | 48 |

MI: myocardial infarction. CABG: coronary artery bypass grafting, PCI: percutaneous coronary intervention.

Combining the two groups into one group with a calcium score of 0-10, the incidence rate was 0.14 per 100 person-years and the negative predictive value 99.1%. In comparison, the incidence rate in the population with a calcium score above 10 was 1.35 per 100 person-years.

## DISCUSSION

The current study shows that in older adults, absent and minimal coronary calcification is associated with a negligible risk of coronary heart disease, even in persons regarded as being at increased risk by presence of two or more cardiovascular risk factors. After more than 6 years of follow-up, the negative predictive value for hard coronary events was 99%, both in case of a negative calcium score and in case of a positive but very low calcium score.

Coronary calcification is known to be a very strong predictor of cardiovascular events.[6, 7, 10, 11, 13] Apart from a high calcium score, a clinically relevant finding may be the absence of coronary calcification. Recently, two studies were published specifically on the negative predictive value of the calcium score.[15, 16] The authors of a systematic review concluded that the absence of coronary calcification was associated with very low cardiovascular risk, with 0.56% of the pooled individuals having an event during more than four years.[16] The cumulative incidence found in our study for a longer follow-up period and an older study population was only slightly higher than in the systematic review (0.9%). The percentage of individuals without coronary calcium was lower in our study than reported in the systematic review (11 vs 41%), which we contribute to the higher average age of our population. Secondly, a prospective study by Blaha et al. among more than 44,000 asymptomatic persons (mean age 54±10 years, 54% men) concluded that zero or low coronary calcification was associated with excellent survival, with annualized all-cause mortality rates for CAC 0, CAC 1-10, and CAC >10 of 0.87, 1.92, and 7.48 deaths/1,000 person-years, respectively.[15] The all-cause mortality rates between individuals with a negative calcium score and those with a calcium score of 1-10 were statistically significant in this study. We did not find a significant difference in the coronary event rate between these two calcium score groups. However, due to the much smaller size of our population with absent or minimal coronary calcification, we can not exclude the possibility that a positive calcium score is associated with a small but significantly higher risk than in case of a truly negative calcium score. This is a limitation of our study. In the only study focusing on the predictive value of coronary calcification at advancing age, a zero or low calcium score was also associated with a low risk of all-cause mortality in elderly.[17] However, this study did not investigate cardiovascular outcomes, nor did it make the distinction between absent and very low amount of coronary calcification. As far as we are aware, the Rotterdam Study is the first to focus on the negative predictive value of coronary calcium for CHD in the elderly population. Our results suggest that a negative or very low calcium score has similar predictive value in older individuals as in middle-aged persons.

The high negative predictive value of the calcium score in elderly is particularly important because in the well-know risk assessment algorithms age itself becomes the predominant factor.[1-3] Consequently, the individual variation in plaque burden at similar age is not adequately taken into account. Furthermore, the predictive value of cardiovascular risk factors at older age is known to deteriorate because of, amongst others, selective survival and the influence of co-morbidity on risk factor levels.[22-24]For example, decreasing cholesterol levels may be an indicator of underlying morbidity and impending death. Due to these issues, risk scoring algorithms based on risk factor levels are hampered to accurately distinguish elderly at low or high cardiovascular risk. It is widely known that although the average risk of CHD is higher at older age, not all elderly

have the propensity to suffer a coronary event. And many elderly are categorized as being at increased risk, mainly due to the risk points attributed to age. The current study shows that a substantial part of the older population can in fact, regardless of present risk factors, be regarded as being at extremely low risk of CHD, namely when the calcium score is 0 or very low.

Strengths of the current study include the population-based sample, standardized measurement of risk factors, near complete follow-up of participants and strict definition of coronary events by qualified physicians. Furthermore, participants' unawareness of the calcium scoring result prevented change in lifestyle or medication use.

## Conclusion

A negative or very low calcium score virtually rules out the risk of coronary events in older individuals, despite presence of risk factors by which persons would be regarded as being at increased cardiovascular risk. The extremely low risk in elderly with a negative calcium score may have important implications with regards to preventive measures in daily clinical practice and on a population level. Further studies should investigate whether asymptomatic individuals without coronary calcium are at such low risk, even in the presence of cardiovascular risk factors, that prescription of medication can be mitigated.

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**Manuscript based on this chapter**

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# CHAPTER 4.3

## Carotid intima-media thickness in cardiovascular risk stratification


**SUMMARY**

**Background:** Non-invasive measures of atherosclerosis, such as carotid intima-media thickness (cIMT), may improve global cardiovascular risk prediction. The aim of this study was to determine whether common carotid IMT in addition to traditional risk factors improves risk classification in a general population of older persons.

**Methods:** A group of 3,580 non-diabetic persons aged 55-75 years and free of cardiovascular disease (CVD) at baseline were followed for a median time of 12.2 years. Compared to models based on Framingham risk factors, we studied the ability of common cIMT measurement to better classify persons into categories of low (<10%), intermediate (10-20%) and high (>20%) 10-year risk of hard coronary heart disease (CHD) and stroke.

**Results**: In older men, addition of cIMT to Framingham risk factors did not improve prediction of hard CHD or stroke. In older women, addition of cIMT to Framingham risk factors significantly improved risk classification. CIMT improved the C-statistic of the model for hard CHD from 0.711 to 0.719 and for stroke from 0.712 to 0.721, at good calibration. Reclassification was least in the majority of women classified as low risk (4% (n=76) for hard CHD and 3% (n=62) for stroke) and most substantial in women at intermediate risk (43% (n=70) for hard CHD and 28% (n=76) for stroke). The net reclassification improvement (NRI) in women was 8.2% (p=0.03) for hard CHD and 8.0% (p=0.06) for stroke.

**Conclusion:** CIMT had some additional value beyond traditional risk factors in the cardiovascular risk stratification of older women, but not of older men.

## INTRODUCTION

Cardiovascular disease (CVD) is the major cause of morbidity and premature death in the Western world. The underlying atherosclerosis develops over many years and symptoms usually do not occur until the disease is already at an advanced stage. The majority of CVD is related to modifiable risk factors and modification of these factors has been shown to reduce CVD morbidity and mortality.[1]

The current, clinically accepted concept of matching treatment intensity to the degree of cardiovascular risk implicates that it is important to identify the most accurate approach to risk stratification as a solid base for the best treatment strategies in persons at risk of CVD. In accordance with current guidelines, global risk factor assessment using algorithms like the SCORE risk chart[2] or Framingham Risk Score[3] are increasingly used to stratify persons in categories of low, intermediate and high risk, based on 10-year absolute risk of CVD or coronary heart disease (CHD). However, these risk factor algorithms are of limited accuracy**,** especially in elderly and women.[4, 5] To improve risk stratification, expert panels have proposed non-invasive measures predicting atherosclerotic disease, like carotid intima-media measurement (cIMT), as a second step after risk stratification based on traditional risk factors.[1, 3, 6, 7] Research has shown that addition of a cIMT measurement to established risk factors led to small and sometimes significant improvements in cardiovascular risk prediction by means of increases in the C-statistic.[8] However, in the evaluation of the clinical value of a new test, it is important to assess the reclassification of individuals into different risk categories when the new test is added to traditional risk factors[9] and to date, limited research has addressed this issue.[10, 11]

In the population-based Rotterdam study, we stratified non-diabetic men and women of 55-75 years and free of CVD at baseline into 3 widely used clinical risk categories of low (<10%), intermediate (10-20%) and high (>20%) 10-year risk of hard CHD and stroke based on Framingham risk factors and studied the ability of common carotid IMT to reclassify persons to a more accurate risk category.

## METHODS

### Study population

The study was embedded in the Rotterdam Study, a prospective, population-based study among subjects aged 55 years and older in a municipality of Rotterdam, which started in 1990. The rationale and design of the Rotterdam Study have been described elsewhere.[12, 13] The baseline examination took place from 1990 to 1993. Of the 7,983 participants (response rate 78%), 5,643 persons underwent a common carotid IMT measurement. Missing cIMT measurements were due to restricted availability of ultra-

sonographers. In line with current guidelines for cardiovascular screening,[3] we excluded persons of 75 years and older (n=1,302) and persons already defined as being at high cardiovascular risk based on having diabetes (n=431) or established cardiovascular disease (CVD) at baseline (n=330). Prevalent CVD was defined as a history of clinically manifest myocardial infarction, coronary artery bypass grafting, percutaneous coronary intervention, stroke, symptomatic peripheral artery disease (PAD), aortic or carotid surgery. Hence, the current analysis was carried out in 3,580 asymptomatic individuals. The Medical Ethics Committee of Erasmus Medical Center approved the study, and all participants gave informed consent.

### Common carotid IMT measurement

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). Measurements of the common carotid artery (CCA) intima-media thickness involved a length of 10 mm distal of the bulb. CIMT was determined as the average of mean near- and far-wall IMT, providing the average of left and right common carotid IMT. The procedure has been described in detail previously.[14]

### Cardiovascular risk factors

Information on cardiovascular risk factors was acquired during the baseline examination as described previously.[15] Diabetes was defined as a non-fasting glucose >11 mmol/L and/or use of anti-diabetic medication.

### Clinical outcomes

The follow-up procedures of the Rotterdam Study have been reported formerly.[15] None of the persons considered for analysis were lost to follow-up. As outcome we used hard CHD, consisting of incident myocardial infarction (MI) and coronary heart disease mortality and stroke which included ischemic stroke (mortality). If persons died within 4 weeks after an MI or ischemic stroke, events were coded as fatal. Follow-up was completed by January 1, 2005.

### Statistical analysis

*Common carotid IMT and risk of CVD*

We used Cox proportional hazard regression analysis to estimate the hazard ratio (HR) of a 1-standard deviation increase of cIMT to predict hard CHD and stroke for the total population and as well as stratified by gender. In the analysis, we corrected for age and, if appropriate, gender. We additionally adjusted for current smoking, total cholesterol, HDL-cholesterol, systolic blood pressure and use of anti-hypertensive medication and

for atrial fibrillation for the outcome stroke. We used log minus log plots to check for proportionality of hazards over time and found no indications of violation of the proportionality assumption.

*Risk prediction models*

We used Cox proportional hazard regression analysis to derive estimates of individual 10-year hard CHD and stroke risk. Analyses were performed by gender. For the outcome hard CHD, we used the same predictors as defined in the Framingham risk score, in accordance with the ATP III guidelines.[3] The model thus included: age, gender, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL-cholesterol and current smoking (model 1). For the outcome stroke, model 1 also included atrial fibrillation, in line with the Framingham stroke risk score.[16] In a second step, we extended model 1 with common carotid IMT (model 2). Both models were fitted to the gender-specific subsets of the Rotterdam Study data. We used restricted cubic spline transformations with 4 knots for continuous variables in the models, 4 knots being a good compromise between flexibility and loss of precision caused by over-fitting of the model.[17] We used the likelihood ratio-test to study the null-hypothesis of no effect of common carotid IMT to predict hard CHD and stroke beyond Framingham risk predictors. To examine the discriminative ability of the two models, we calculated the bootstrap corrected C- statistic using 150 repetitions for both gender-specific models. The purpose of using the bootstrap method is to correct for over-optimism of the fitted models.[18] Next, we computed reclassification percentages to study the incremental ability of common carotid IMT to classify subjects in risk categories according to commonly used categories of 10-year hard CHD and stroke risk: low (<10%), intermediate (10-20%), and high risk (>20%).[3] Observed 10-year risk of CVD was estimated by Kaplan-Meier survival analysis for each cell of the reclassification table to show calibration of reclassified persons with observed risk. To evaluate true improvement in classification by addition of common carotid IMT to the Framingham model we calculated the net reclassification improvement (NRI).[19]

In secondary analyses, we repeated the prediction analyses using the mean of the maximum of IMT values of near and far walls of both common carotid arteries as a determinant instead of the mean of the mean IMT values.

Covariables were missing in less than 2% of persons. We used single imputation by the Expectation Maximization method**.** All analyses were performed using SPSS for Windows (SPSS, Inc., Chicago, Illinois, USA) and R version 2.7.2. (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

Baseline characteristics of men and women are shown in Table 1. The total population of 3,580 persons consisted of 1,398 men and 2,182 women (61%). Median follow-up duration (inter-quartile range) was 12.2 (11.7-13.1) years. During this period, 530 events occurred of which 267 in men and 263 in women. One hundred and six men and 68 women had a myocardial infarction, 89 men and 118 women had an ischemic stroke whereas 60 men and 59 women died of CHD and 12 men and 18 women had a fatal ischemic stroke.

**Table 1.** Baseline characteristics of the population by gender

| Variable | Men n=1,398 | Women n=2,182 | P value |
|---|---|---|---|
| Age, y | 64.5±5.3 | 64.8±5.6 | 0.068 |
| Body-mass-index, kg/m$^2$ | 25.7±2.9 | 26.6±3.9 | <0.001 |
| Current smoking, % | 30.3 | 22.5 | <0.001 |
| Systolic blood pressure, mmHg | 137±22 | 136±22 | 0.072 |
| Diastolic blood pressure, mmHg | 76±12 | 73±11 | <0.001 |
| Anti-hypertensive medication, % | 10.2 | 15.7 | <0.001 |
| Total cholesterol, mmol/L | 6.4±1.1 | 6.9±1.2 | <0.001 |
| HDL-cholesterol, mmol/L | 1.2±0.3 | 1.5±0.4 | <0.001 |
| Cholesterol-lowering medication, % | 1.5 | 2.2 | 0.137 |
| Anti-thrombotic medication, % | 3.6 | 1.4 | <0.001 |
| Atrial fibrillation, % | 2.9 | 2.0 | 0.113 |
| Common carotid IMT, mm | 0.82±0.14 | 0.77±0.12 | <0.001 |

Values are mean (standard deviation) for continuous variables and percentages for dichotomous variables. HDL: High-density lipoprotein. IMT: intima-media thickness.

For the risk of hard CHD, the age- and gender adjusted hazard ratios (95% CI) for a 1-standard deviation increase in common carotid IMT in the total population was 1.26 (1.13-1.41) and decreased to 1.16 (1.03-1.30) after additional adjustment for the remaining cardiovascular risk factors. Corresponding risks of stroke were: 1.33 (1.18-1.50) and 1.25 (1.10-1.42). In gender-specific subsets, the analogous hazard ratios for the outcome hard CHD were 1.08 (0.92-1.27) and 1.02 (0.86-1.20) in men and 1.56 (1.34-1.81) and 1.40 (1.19-1.65) in women. For the outcome stroke, corresponding hazard ratios were 1.28 (1.07-1.54) and 1.18 (0.97-1.43) in men and 1.38 (1.18-1.62) and 1.32 (1.12-1.56) in women.

In hard CHD risk prediction in men, addition of common carotid IMT did not improve model performance of the refitted Framingham risk model. The likelihood chi-square of model 2 did not significantly increase compared to model 1 (P=0.84). Hence, the C-

statistic did not improve by addition of cIMT: 0.611 (model 1) and 0.610 (model 2). In contrast, addition of common carotid IMT significantly improved model performance in women. The likelihood chi-square increased by 15.2 points (P<0.001). The C-statistic improved from 0.711 to 0.719, indicating slightly better average discriminative ability of the model including cIMT.

In stroke risk prediction in men, addition of common carotid IMT did not improve model performance of the refitted Framingham risk model. The likelihood chi-square did not significantly increase (P=0.11), the C-statistic slightly improved by addition of cIMT: from 0.692 (model 1) to 0.698 (model 2). In women, the likelihood chi-square increased by 10.0 points (P=0.002). The C-statistic improved from 0.712 to 0.721.

**Table 2.** Risk reclassification comparing the Framingham risk model and Framingham risk model plus common carotid IMT by gender in the prediction of hard CHD

| Framingham 10-year risk categories, % | 10-year risk categories for Framingham risk covariates + cIMT | | | N (%) reclassified |
|---|---|---|---|---|
| | <10% | 10-20% | >20% | |
| **Men** | | | | |
| **<10%** | | | | |
| N=742 (53%) | 740 (100%) | 2 (0%) | 0 (0%) | 2 (0%) |
| Observed risk (95%CI) | 0.07 (0.06,0.09) | NA | NA | |
| **10-20%** | | | | |
| N=598 (43%) | 7 (1%) | 598 (99%) | 2 (0%) | 9 (1%) |
| Observed risk (95%CI) | NA | 0.13 (0.11,0.16) | NA | |
| **>20%** | | | | |
| N=530 (4%) | 0 (0%) | 0 (0%) | 58 (100%) | 0 (0%) |
| Observed risk (95%CI) | NA | NA | 0.24 (0.16,0.37) | |
| NRI: 0.21%; P=NS | | | | |
| **Women** | | | | |
| **<10%** | | | | |
| N=2005 (92%) | 1929 (96%) | 73 (4%) | 3 (0%) | 76 (4%) |
| Observed risk (95%CI) | 0.04 (0.03,0.05) | 0.11 (0.06,0.20) | 0.58 (0.19,0.97) | |
| **10-20%** | | | | |
| N=163 (7%) | 57 (35%) | 93 (57%) | 13 (8%) | 70 (43%) |
| Observed risk (95%CI) | 0.05 (0.02,0.13) | 0.13 (0.08,0.21) | 0.28 (0.12,0.59) | |
| **>20%** | | | | |
| N=14 (1%) | 0 (0%) | 4 (29%) | 10 (71%) | 4 (29%) |
| Observed risk (95%CI) | NA | 0.18 (0.03-0.76) | 0.22 (0.06,0.62) | |
| NRI: 8.2%; P=0.03 | | | | |

cIMT: carotid intima-media thickness, CHD: coronary heart disease, CI: confidence interval, NA: not applicable, NRI: net reclassification improvement, NS: not significant.

Table 2 displays categories of estimated 10-year hard CHD risk based on the Framingham risk model before and after adding common carotid IMT in men and women. In men, percentages in the low, intermediate and high risk categories were 53%, 43% and 4%, respectively. For women these percentages were 92%, 7% and 1%. In accordance with the finding that addition of cIMT did not improve the C-statistic of the Framingham risk model, additional cIMT measurement hardly led to any reclassification. Correspondingly, the net reclassification improvement (NRI) was 0.21% (P=0.50)). In women, additional cIMT measurement led to more reclassification. Of women at low risk, 4% (n=73) of persons reclassified to the intermediate risk category and 3 persons to high risk. Of women at intermediate risk, 35% (n=57) were downgraded to low risk and 8%

**Table 3.** Risk reclassification comparing the Framingham risk model and Framingham risk model plus common carotid IMT by gender in the prediction of stroke

| Framingham 10-year risk categories, % | 10-year risk categories for Framingham risk covariates + cIMT | | | N (%) reclassified |
|---|---|---|---|---|
| | <10% | 10-20% | >20% | |
| **Men** | | | | |
| **<10%** | | | | |
| N=1188 (85%) | 1152 (97%) | 36 (3%) | 0 (0%) | 36 (3%) |
| Observed risk (95%CI) | 0.09 (0.08,0.11) | 0.19 (0.10,0.36) | NA | |
| **10-20%** | | | | |
| N=181 (13%) | 29 (16%) | 145 (80%) | 7 (4%) | 36 (20%) |
| Observed risk (95%CI) | 0.12 (0.05,0.29) | 0.12 (0.08,0.18) | 0.55 (0.26,0.88) | |
| **>20%** | | | | |
| N=29 (2%) | 0 (0%) | 4 (1%) | 25 (86%) | 4 (1%) |
| Observed risk (95%CI) | NA | NA | 0.22 (0.10,0.45) | |
| NRI: 3.9%; P=0.16 | | | | |
| **women** | | | | |
| **<10%** | | | | |
| N=1886 (87%) | 1824 (97%) | 59 (3%) | 3 (0%) | 62 (3%) |
| Observed risk (95%CI) | 0.03 (0.02,0.04) | 0.11 (0.06,0.21) | 0.29 (0.15,0.51) | |
| **10-20%** | | | | |
| N=269 (12%) | 51 (19%) | 193 (72%) | 25 (9%) | 195 (28%) |
| Observed risk (95%CI) | 0.08 (0.05,0.13) | 0.12 (0.06,0.23) | 0.29 (0.15,0.51) | |
| **>20%** | | | | |
| N=27 (1%) | 0 (0%) | 10 (37%) | 17 (63%) | 10 (37%) |
| Observed risk (95%CI) | NA | 0.09 (0.01-0.49) | 0.10 (0.03,0.36) | |
| NRI: 8.0%; P=0.06 | | | | |

cIMT: carotid intima-media thickness, CI: confidence interval, NA: not applicable, NRI: net reclassification improvement.

(n=13) upgraded to high risk. Of women at high risk, 29% (n=4) were downgraded to the intermediate risk category. Addition of common cIMT in women led to a net gain in reclassification of 9.1% in persons with an event and a net decline in reclassification of 0.9% in persons without event, resulting in a NRI of 9.1-0.9=8.2% (P=0.03).

Table 3 displays categories of estimated 10-year stroke risk based on the Framingham risk model before and after adding common carotid IMT in men and women for the outcome stroke. In men, percentages in the low, intermediate and high risk categories were 85%, 13% and 2%, respectively. For women corresponding percentages were 87%, 12% and 1%. In men, more reclassification was observed compared with the outcome stroke. However, this was not clinically significant, by means of the NRI (3.9%; P=0.16). Of women at low risk, 3% (n=59) of persons reclassified to the intermediate risk category and 3 persons to high risk. Of women at intermediate risk, 19% (n=51) were downgraded to low risk and 9% (n=25) upgraded to high risk. Of women at high risk, 37% (n=10) were downgraded to the intermediate risk category. Addition of common cIMT in women led to a net gain in reclassification of 9.0% in persons with an event and a net decline in reclassification of 1.0% in persons without event, resulting in a NRI of 9.0-1.0=8.0% (P=0.06). Repeating the analyses using the mean of the maximal cIMT values of near and far walls did not lead to substantial changes in reclassification percentages and NRI values (data not shown).

Generally, point estimates of the observed risks agreed with the corresponding categories of predicted risk indicating good calibration, except for cells containing small numbers.

## DISCUSSION

This population-based study shows that addition of common carotid IMT measurement to the Framingham risk model does not significantly improve hard CHD and stroke risk prediction in older men and only modestly improves risk classification for these outcomes in older women. Most substantial reclassification was observed in women classified as intermediate risk based on traditional risk factors. Of the women classified as being at intermediate risk for hard CHD (7% of all women), cIMT measurement reclassified 35% of persons to low and 8% to high risk categories. The net reclassification improvement was 8.2% (P=0.03). Of the women classified as being at intermediate risk for stroke (12% of all women), cIMT measurement reclassified 19% of persons to low and 9% to high risk categories. The net reclassification improvement was 8.0% (P=0.06).

### CIMT and future cardiovascular disease

An essential prerequisite for the use of cIMT in cardiovascular risk stratification is its ability to predict future cardiovascular events. A meta-analysis by Lorenz et al.[20] comprising 8 large, longitudinal population-based cohort studies showed that common carotid IMT is a strong predictor of MI and stroke in the total population. The overall age- and gender-adjusted estimates of relative risk per a 1-standard deviation increase of cIMT reported in this meta-analyses were 1.26 (1.21-1.30) for MI and 1.18 (1.16-1.21) for stroke. In line with these results, the corresponding estimates we found for hard CHD and stroke risk in the total population were 1.26 (1.13-1.41) and 1.33 (1.18-1.50). In extent, we found that associations were stronger in women than in men. All associations were attenuated by additional adjustment for cardiovascular risk factors.

### Additional value of cIMT in cardiovascular risk prediction

The Atherosclerosis Risk in Communities (ARIC) study[10] recently reported on the additional value of carotid IMT and plaque beyond traditional risk factors in the 10-year risk prediction of CHD. In contrast to our results, this study found a higher additional value of cIMT in men than in women. Addition of cIMT beyond traditional risk factors resulted in a significant increase in C-statistic from 0.742 to 0.750 in men and a non-significant increase from 0.759 to 0.762 for women. The NRI (95% confidence interval) was 8.9 (3.4-15.1) for men and 6.1 (-2.3-9.4). Unfortunately, reclassification percentages for addition of cIMT were not presented. The discrepancy in results between the ARIC and our study might at least partly be explained by the fact that their study population was younger (mean age 54 versus 65 years). Since IMT is thought to represent an early stage of atherosclerosis,[21] it could be possible that the additional value of IMT diminishes with advancing stages of atherosclerosis, which would particularly affect older men. Another possible explanation for not finding an additional value of IMT in older men might be that persons prone to the effects of atherosclerosis already died before study inclusion or developed a cardiovascular event which was one of the exclusion criteria of our study. Since men develop atherosclerosis at an earlier age than women, this selection would have greater impact on our male than on our female study population. Differences in results might also be related to the fact that the ARIC study used total CHD as an outcome, which included revascularizations and silent MI whilst we studied hard CHD only. Both studies calculated the mean cIMT of near and far wall and measured IMT in the distal common carotid artery (CCA). However, in extent, the ARIC study also took the IMT in the carotid artery bifurcation and the proximal internal carotid arteries into account. Despite the differences in results between the two studies, it is of notice that in both the ARIC and our study, the found additional value of IMT seems to be modest. A recent publication by the Carotid Atherosclerosis Progression Study (CAPS) group reported that in 4,904 individuals free of CVD at baseline (mean age 50

years, 48% male), carotid intima media thickness was significantly and independently predictive for cardiovascular events. However, for the outcome myocardial infarction, addition of common carotid IMT to a model using the Framingham risk factors led to a small increase in C-statistic from 0.732 to 0.741 and only 25 persons (0.6%) were reclassified. The net reclassification improvement was 0.14% (P>0.05). Analyses including other endpoint definitions (angina/MI/ all-cause mortality), other carotid segments (carotid bifurcation/internal carotid artery), and the SCORE risk model for baseline prediction did not result in consistently better risk prediction with carotid IMT. The fact that the mean age and thereby the mean cIMT (0.72 mm) was relatively low might have played a role in the negative findings. Furthermore, the assessment of clinical events was based on health insurance data and questionnaires which could have led to misclassification of the outcome and underestimation of the additional effect of cIMT.[11] A systematic review of studies in persons free from CVD reported that addition of a cIMT measurement to established risk factors led to small and sometimes significant improvements in cardiovascular risk prediction by means of increases in the C-statistic.[8]

The amount of women that were upgraded in risk exceeded that of women downgraded in risk (4.0% (n=89) versus 2.8% (n=61) for hard CHD and 2.0 % (n=43) versus 1.4% (n=31) for stroke (Tables 2 and 3)). This implicates that addition of cIMT measurement would lead to more women with an indication to receive medical therapy according to current prevention guidelines.[1, 3]

In conclusion, despite the well-established association between cIMT and risk of future CHD and stroke, accruing evidence indicates at best a modest benefit of additional cIMT in the prediction of cardiovascular disease.

### Strengths and limitations

In order to appreciate the results, strengths and limitations of the present study need to be considered. Strengths of our study include a large population-based cohort, in whom standardized measurements of risk factors and common cIMT measurement were performed. Furthermore, the complete and long follow-up generated a large number of events enabling us to use hard events as an outcome and separate the analyses by gender. However, some limitations also need to be addressed. First, while previous research within the Rotterdam study[22] showed that application of the Framingham Risk Score led to systematic overestimation of CHD risk in men, we chose to fit a model based on Framingham risk factors to stratify our population in the well known risk categories. A potential drawback is over-fitting of the model, which could lead to underestimation of the additional value of cIMT measurement. However, all C-statistics were corrected for over-fitting using the bootstrap method. Second, we used the mean of mean IMT values of near and far walls of both common carotid arteries in our analyses. However, when we used the mean of the maximal cIMT values of near and far walls, reclassification percent-

ages and NRI values did not change substantially (data not shown). Third, since usage of risk-modifying therapy other than anti-hypertensive medication is not accounted for in the Framingham risk model, the cardiovascular risk of persons on this treatment might have been overestimated. However, at baseline few people used cholesterol-lowering medication or anti-thrombotic therapy (Table 1). Therefore, it is not likely that usage of these agents have biased our results

Fourth, our study was performed in older persons. The predictive power of traditional cardiovascular risk factors decreases with age while increased cIMT can be seen as a cumulative measure of the effect of lifetime exposure to cardiovascular risk factors on the arterial vessel wall and may therefore improve risk stratification particularly at older age. This implies that our results should not automatically be generalized to a younger population.

## Conclusion

Despite a well-established association between carotid intima-media-thickness and risk of future CVD events, the additional predictive value of a cIMT measurement within clinical risk categories based on traditional risk factors seems to be limited. In older men, common carotid IMT measurement did not significantly improve risk stratification based on traditional risk factors. In older women, common carotid IMT showed a modest ability to reclassify persons to a more accurate cardiovascular risk category.

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**Manuscript based on this chapter**

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# CHAPTER 4.4

## Calcium scoring in prediction of coronary heart disease and cerebrovascular events


### SUMMARY

**Background**: Since atherosclerosis is a systemic process, risk prediction would benefit from targeting multiple components of cardiovascular disease (CVD) simultaneously. To this end, it is useful to examine the predictive value of non-invasive measures of atherosclerosis in various vascular beds for both coronary heart disease (CHD) and cerebrovascular disease.

**Methods:** Between September 2003 and February 2006, 2,153 asymptomatic participants (69.6±6.6 years) from the Rotterdam Study underwent a multi-detector computed tomography (MDCT) scan. During a median follow-up of 3.5 years, 58 CHD events (MI and CHD deaths) and 52 cerebrovascular events (TIA and stroke) occurred. Participants were classified into low (<5%), intermediate (5-10%) and high (>10%) 5-year risk categories based on a refitted Framingham risk model. The model was extended by coronary, aortic arch or carotid calcium and reclassification percentages were calculated.

**Results:** For the outcome CHD, the C-statistic improved from 0.693 for the Framingham refitted model to 0.743, 0.740 and 0.749 by addition of coronary, aortic arch and carotid calcium, respectively. Reclassification was most substantial in the intermediate risk group where addition of coronary calcium reclassified 56% of persons (net reclassification improvement (NRI): 15%; P<0.01)). Adding aortic arch calcium led to a reclassification of 32% of persons (NRI: 8%; P=0.01) and adding carotid calcium reclassified 51% (NRI: 9%; P=0.02). In contrast, calcification in any of the three vascular beds did not improve cerebrovascular risk prediction.

**Conclusion:** Coronary, aortic arch and carotid artery calcification significantly improved risk prediction of CHD but not of cerebrovascular events.

## INTRODUCTION

Non-invasive measures of atherosclerosis are useful in cardiovascular risk prediction, especially in persons classified as intermediate risk based on risk scoring algorithms like the Framingham Risk Score.[1-4] As atherosclerosis is a systemic process, with a substantial number of persons afflicted with disease in more than one vascular bed, risk prediction would benefit from targeting multiple components of cardiovascular disease (CVD) simultaneously.[5, 6] To date, most studies focused on one component of CVD, like coronary heart disease (CHD) or stroke, in relation to one -or a limited number- of measures of atherosclerosis. In light of the general aspect of cardiovascular disease, it is useful to relate various non-invasive measures of atherosclerosis to different components of CVD simultaneously.

One current non-invasive measures of atherosclerosis is assessment of the amount of arterial calcification with computed tomography (CT). An advantage of this modality is that it can measure atherosclerosis simultaneously in multiple vascular beds. Until now, research primarily focused on measurement of coronary artery calcification (CAC). CAC has been established as a strong and independent risk factor for CHD and appears to improve CHD risk prediction beyond traditional risk factors by means of enhancement of the C-statistic.[7-10] However, currently only one prospective study investigated CAC in relation to stroke,[11] whilst the predictive value of CAC in cerebrovascular risk prediction remains unclear.

Recently, interest in arterial calcification has been extended to other parts of the vascular tree. Two studies on thoracic aorta calcification (TAC) found that TAC had a less strong relation with CHD[12] and CVD[12, 13] compared to CAC. Furthermore, thoracic aorta calcium did not improve risk prediction over coronary calcium.[12] However, cerebrovascular disease was not examined as a separate outcome. Prospective CT studies on the association of carotid calcification and risk of cardiovascular events are still lacking.

Therefore, within the prospective Rotterdam Study, we examined the association of coronary artery, aortic arch and carotid artery calcification, assessed by CT, with risk of CHD and cerebrovascular events. Additionally we investigated the incremental value of calcification in these vascular beds over the Framingham risk factors.

## METHODS

### Study population

This study is embedded in the Rotterdam Study, a population-based cohort study that started in 1990. All inhabitants aged 55 years and older, living in a suburb of Rotterdam were invited and 7,983 agreed to participate (78%). This group is referred to as

Rotterdam Study cohort I (RS-I). In 2000 a second study cohort was introduced (RS-II) comprising 3,011 persons.[14]

From September 2003 until February 2006, participants completing a center visit (visit 4 for RS-I and visit 2 for RS-II) were invited to undergo an MDCT scan. Overall 2,524 participants (79%) were scanned. We excluded 328 persons with prevalent CVD at baseline, defined as percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), recognized myocardial infarction (MI) or stroke. A further 43 participants were lost to follow-up providing 2,153 persons for analyses. Due to severe artefacts in image acquisition, respectively 19, 4, and 3 persons did not have a valid coronary, aortic arch or carotid calcium score. Hence, the study population available for analyses for these measures were 2,134, 2,149, and 2,150, respectively. The median duration between the study center visit and the MDCT scan was 117 days. At the time of study inclusion, coronary calcium was not considered as an indicator for treatment in the Netherlands. Nevertheless, participants in the highest 10% of the CAC score distribution for men and the highest 5% for women (with distributions based on our previous coronary calcium study[10]), were informed about their score and advised to visit their physician.

This study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. All participants gave written informed consent.

### Scan protocol and analysis of calcification

Methods have been described in detail previously.[15] In short, persons were scanned with a 16-slice (n=636; 30%) or 64-slice (n=1,517; 70%) MDCT scanner (SOMATOM Sensation 16 or 64, Siemens, Forcheim, Germany). Two scans were performed. The cardiac scan reached from the apex of the heart to the tracheal bifurcation. The second scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). The aortic arch comprised the origin of the aortic arch (defined as the image in which the ascending and descending aorta merge into the inner curvature of the aortic arch) to the first 1 cm of the common carotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. The carotid arteries comprised both right and left carotid artery within 3 cm proximal and distal of the bifurcation. Atherosclerotic calcification was identified based on a threshold of 130 Hounsfield Units (HU), using dedicated software (Syngo Calcium Scoring, Siemens, Forcheim, Germany). Calcification was quantified by using the Agatston score.[16] The total score per vascular bed was calculated by adding the scores of all lesions in that bed. The estimated radiation dose was up to 2.1 millisievert (mSv) for the cardiac scan and 2.8 ms for the extra-cardiac scan. In the minority of persons with a heart rhythm disorder, cardiac scans required somewhat higher dosages (up to 4.1 mSv). Inter- and intra-observer variability for scores of all three vascular beds was excellent. (Intra-class correlation coefficients ranging from 0.96 for TAC to >0.99 for CAC).

## Covariables

Data on medical history, medication use, and smoking behavior was collected using a computerized questionnaire. Established cardiovascular risk factors were measured during a visit to the research center.[10] Diabetes was diagnosed based on a fasting plasma glucose level ≥7.0 mmol/L and/or use of anti-diabetic medication. Information on atrial fibrillation at the time of the CT scan was collected at baseline and during follow-up[10]

## Clinical outcomes

Information on cardiovascular endpoints were obtained from general practitioners and from letters and discharge reports from medical specialists.[10] Events were classified by experienced physicians according to the International Classification of Diseases, 10th edition (ICD-10). As an outcome we used CHD (MI and CHD mortality; ICD-10 codes: I21, I 46, I50, R96) and cerebrovascular events (transient ischemic attack (TIA) and ischemic stroke; ICD-10 codes: G45, I63). If a non-fatal event occurred within 28 days of death, mortality was attributed to that event. Participants were followed for a median time (inter-quartile range) of 3.5 (2.5-4.3) years.

## Statistical analysis

*Association of arterial calcification with CHD and cerebrovascular events*

Using Cox proportional hazard models we investigated the relation of coronary, aortic arch and carotid calcium with CHD and cerebrovascular events. Three models were pre-specified: Model 1 was adjusted for age and gender. Model 2 was adjusted for Framingham risk factors, namely: age, gender, systolic blood pressure, anti-hypertensive medication, total and HDL-cholesterol, diabetes, current smoking and prevalent atrial fibrillation (for the cerebrovascular outcome only).[1, 17] Model 3 comprised the variables of Model 2 with additional adjustment for calcification in other vascular beds.

We tested the linearity assumption of continuous variables by using restricted cubic spline transformations and natural log-transformed the three measures of calcification and added 1 to all values to deal with calcium scores of zero (i.e. log(CAC+1). Gender was evaluated as an effect modifier by adding a gender x calcification term to models 1 and 2. None of the interaction terms were significant (P>0.05).

All three measures of calcification were categorized in the same manner. Although there are established categories for absolute coronary calcification scores, no such categories exist for aortic arch or carotid calcification scores. As about one third of men and women of our population had a carotid calcium score of zero, we utilized tertiles. As arterial calcification scores are higher in men gender-specific tertiles were used.

*Additional value of calcium scores in CHD and cerebrovascular risk prediction*

As previous research showed that application of the Framingham risk function[1] to the older Rotterdam Study population led to systematic overestimation of CHD risk in men,[18] we fitted a model comprising Framingham risk factors on our data instead of using the Framingham risk function. We used a parametric Weibull proportional hazards regression model, instead of a Cox model, to compute individual 5-year predicted risks from our available 3.5 years of median follow-up duration (half the time horizon normally applied in risk prediction). This Weibull model was based on the variables of the Framingham risk function (equivalent to Cox model 2) and will be referred to as the "Framingham refitted" model. In a second step, we extended this model with coronary, aortic arch or carotid artery calcium as continuous variable (log(calcium+1)). We compared the performance of Framingham refitted with each extended model by the following methods. Global model performance was checked by comparing the likelihood chi-square values of the models with and without arterial calcification. A significantly higher likelihood chi-square value by means of the likelihood ratio test indicates better model performance. To examine the discriminative ability of the models, we calculated the bootstrap corrected C-statistic using 150 repetitions for both models. The bootstrap method was used to correct for over-optimism of the fitted model.[19] Next, reclassification percentages were computed to study the incremental ability of calcification measures to classify subjects in 5-year risk categories of low (<5%), intermediate (5-10%), and high risk (>10%).[20] Extrapolated ("observed") 5-year risk was calculated for each cell of the reclassification table to show calibration of reclassified observations with observed risk. To evaluate true improvement in classification by addition of arterial calcium to the Framingham model we calculated the net reclassification improvement (NRI).[21]

Covariables were missing in less than 3% of persons. We used single imputation by the Expectation Maximization method**.** All analyses were performed using SPSS 15.0 for Windows (SPSS, Inc, Chicago, Illinois, USA) and R version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

Baseline characteristics of the total population and within gender-specific tertiles are shown in Table 1. During the median follow-up time of 3.5 (2.5-4.3) years, 58 persons had a first CHD event (42 MI and 16 CHD mortality), whilst 52 persons had a first cerebrovascular event (26 TIA; 26 ischemic stroke of which 2 were fatal).

Table 2 displays the hazard ratios (95% C.I.) for the risk of CHD per tertile of calcification. Compared to persons within the lowest tertile, participants in the second and third tertiles of coronary calcification had a respectively 2.1 (0.7-5.9) and 5.3-fold (2.0-13.8)

**Table 1.** Baseline characteristics of the total study population and by gender-specific tertile of calcification

| Variable | Total | By gender-specific tertile of calcification | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Coronary arteries | | | Aortic Arch | | | Carotid arteries | | |
| | Overall n=2,153 | 1 n=713 | 2 n=710 | 3 n=711 | 1 n=717 | 2 n=719 | 3 n=713 | 1 n=718 | 2 n=713 | 3 n=719 |
| Age, y | 69.6±6.6 | 67.4±5.9 | 68.8±6.3 | 71.3±7.0 | 66.0±7.0 | 68.8±6.0 | 72.7±7.1 | 67.3±5.6 | 68.7±6.4 | 71.6±7.1 |
| Men, % | 45.2 | 45.0 | 45.2 | 45.1 | 45.6 | 44.9 | 45.2 | 45.4 | 45.3 | 45.0 |
| SBP, mm Hg | 147±20 | 143±19 | 147±19 | 150±21 | 142±17 | 147±19 | 151±22 | 143±18 | 146±19 | 152±21 |
| Anti-HT use, % | 30.0 | 22.0 | 28.3 | 39.5 | 20.2 | 30.2 | 29.5 | 23.4 | 26.4 | 40.1 |
| Current smoking, % | 12.7 | 9.7 | 13.2 | 15.3 | 8.6 | 11.4 | 17.9 | 8.1 | 11.2 | 18.6 |
| Diabetes, % | 10.5 | 5.0 | 13.0 | 13.6 | 7.4 | 10.7 | 13.4 | 7.7 | 9.4 | 14.4 |
| Total chol, mmol/dL | 5.6±0.9 | 5.7±0.9 | 5.8±0.9 | 5.7±0.9 | 5.8±0.8 | 5.8±0.9 | 5.7±1.0 | 5.8±0.9 | 5.8±0.9 | 5.7±1.0 |
| HDL chol, mmol/dL | 1.4±0.4 | 1.5±0.4 | 1.5±0.4 | 1.4±0.4 | 1.5±0.4 | 1.4±0.4 | 1.4±0.4 | 1.5±0.4 | 1.4±0.4 | 1.4±0.4 |
| BMI, kg/m2 | 27.7±4.0 | 27.4±3.8 | 27.7±4.1 | 27.9±4.0 | 27.5±3.8 | 27.8±3.9 | 27.7±4.3 | 27.7±4.1 | 27.7±4.0 | 27.6±4.1 |
| Atrial fibrillation, % | 3.7 | 3.1 | 4.1 | 3.7 | 1.8 | 3.8 | 5.5 | 2.3 | 3.5 | 5.1 |
| Calcium score men, Agatston | | 3 (0-17) | 115 (72-201) | 721 (476-1358) | 10 (0-40) | 287 (176-433) | 1499 (964-2582) | 0 (0-0) | 33 (15-60) | 262 (159-445) |
| Calcium score women, Agatston | | 0 (0-0) | 16 (6-41) | 240 (135-495) | 6 (0-38) | 246 (164-389) | 1401 (921-2466) | 0 (0-0) | 10 (2-24) | 153 (80-310) |
| CHD events, n | 58 | 5 | 13 | 38 | 8 | 15 | 35 | 5 | 18 | 35 |
| TIA and stroke, n | 52 | 19 | 18 | 14 | 13 | 24 | 15 | 12 | 20 | 20 |

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation or as median (inter-quartile range) in case of skewed distribution. SBP: systolic blood pressure, Anti-HT: anti-hypertensive medication, chol: cholesterol, HDL: high density lipoprotein, BMI: body-mass-index, CHD: coronary heart disease, TIA: transient ischemic attack.

**Table 2.** Hazard ratios (95% CI) for risk of CHD events per tertile of coronary, aortic arch and carotid calcification

| Calcification | Tertile | N/events | Model | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| **Coronary** | | | | | |
| | 1 | 713/5 | Reference | Reference | Reference |
| | 2 | 710/13 | 2.3 (0.8-6.6) | 2.1 (0.7-5.9) | 1.8 (0.6-5.1) |
| | 3 | 711/38 | 6.0 (2.3-15.5)‡ | 5.3 (2.0-13.8)† | 3.6 (1.3-10.0)* |
| **Aortic arch** | | | | | |
| | 1 | 717/8 | Reference | Reference | Reference |
| | 2 | 719/15 | 1.5 (0.6-3.6) | 1.4 (0.6-3.2) | 0.9 (0.4-2.2) |
| | 3 | 713/35 | 2.9 (1.3-6.7)* | 2.5 (1.1-5.6)* | 1.0 (0.4-2.4) |
| **Carotid** | | | | | |
| | 1 | 718/5 | Reference | Reference | Reference |
| | 2 | 713/18 | 3.2 (1.2-8.8)* | 3.0 (1.1-8.0)* | 2.9 (1.0-8.9) |
| | 3 | 719/35 | 5.2 (2.0-13.4)† | 4.6 (1.7-11.9)† | 3.4 (1.1-10.6)* |

CI: confidence interval, CHD: coronary heart disease. Model 1 is adjusted for age and gender. Model 2 is adjusted for Framingham risk factors. Model 3 is additionally adjusted for calcification in the other vessel beds. * P<0.05. † P< 0.01. ‡ P<0.001.

higher risk of CHD, independent of the Framingham risk factors (model 2). Corresponding hazard ratios (HR) were: 1.4 (0.6-3.2) and 2.5 (1.1-5.6) for aortic arch calcification and 3.0 (1.1-8.0) and 4.6 (1.7-11.9) for carotid artery calcification.

Table 3 displays the hazard ratios for cerebrovascular event risk per tertile of calcification in the coronary arteries, aortic arch and carotid arteries. In contrast to our findings regarding CHD, no clear associations were found between the three measures of calcification and risk of cerebrovascular events. Carotid artery calcification seemed to be related to cerebrovascular event risk (HR around 1.4), although clearly less strong than its relation to CHD. However, this association was not significant. No clear, significant associations were found between coronary and aortic arch calcification and cerebrovascular events.

In CHD risk prediction, addition of all three calcium measures significantly improved model performance**.** The likelihood chi-square of the Framingham refitted model was 38.0 and improved to 54.8 (P<0.001) by addition of CAC, to 45.1 (P<0.01) by addition of aortic arch calcium and to 51.3 (P<0.001) by addition of carotid calcium. Discrimination was most substantially improved by CAC (C-statistic: 0.743 compared to 0.693 for the Framingham refitted model). Addition of aortic arch and carotid calcium improved the C-statistic from 0.693 to 0.740 and 0.749, respectively. Adding aortic arch calcium to the model comprising the Framingham risk factors plus coronary calcium did not significantly improve risk prediction. Addition of carotid calcium to the model including coronary calcium led to slight improvement of the likelihood chi-square (increase 5.8; P=0.02) and

**Table 3.** Hazard ratios (95% CI) for risk of cerebrovascular events per tertile of coronary, aortic arch and carotid calcification

| Calcification | Tertile | N/events | Model | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| **Coronary** | | | | | |
| | 1 | 713/19 | Reference | Reference | Reference |
| | 2 | 710/18 | 0.9 (0.5-1.7) | 0.8 (0.4-1.5) | 0.8 (0.4-1.5) |
| | 3 | 711/14 | 0.6 (0.3-1.2) | 0.5 (0.2-1.0) | 0.5 (0.2-1.0) |
| **Aortic arch** | | | | | |
| | 1 | 717/13 | Reference | Reference | Reference |
| | 2 | 719/24 | 1.5 (0.8-3.1) | 1.3 (0.7-2.6) | 1.4 (0.7-2.9) |
| | 3 | 713/15 | 0.8 (0.4-1.8) | 0.6 (0.3-1.4) | 0.7 (0.3-1.7) |
| **Carotid** | | | | | |
| | 1 | 718/12 | Reference | Reference | Reference |
| | 2 | 713/20 | 1.5 (0.8-3.2) | 1.4 (0.7-2.9) | 1.6 (0.8-3.4) |
| | 3 | 719/20 | 1.4 (0.6-2.9) | 1.1 (0.5-2.3) | 1.4 (0.6-3.2) |

CI: confidence interval. Model 1 is adjusted for age and gender. Model 2 is adjusted for Framingham risk factors and atrial fibrillation. Model 3 is additionally adjusted for calcification in the other vessel beds.

C-statistic (0.764 versus 0.743). Risk prediction of cerebrovascular events was not significantly improved by any measures of calcification. The C-statistic of the Framingham refitted model for this outcome was 0.611. This relatively low value is probably due to the fact that the Framingham risk model was not developed for cerebrovascular disease. The C-statistic did not improve after addition of any of the measures of calcification.

Categories of estimated 5-year CHD risk based on the Framingham risk model before and after adding coronary, aortic arch and carotid calcium are presented in Tables 4 to 6. Because arterial calcification measures did not improve cerebrovascular event risk prediction, reclassification tables were only constructed for CHD risk. The largest proportions of reclassified persons were seen in the intermediate Framingham risk group. Addition of coronary calcium reclassified 56% of persons, 36% moved to low risk while 20% moved to high risk. Addition of coronary calcium led to a net gain in reclassification of 18% in persons with an event and a net decline in reclassification of 3% in persons without event, resulting in a net reclassification improvement of 18-3=15% ($P<0.01$). Aortic arch calcium reclassified 32% (22% to low and 10% to high risk). The net gain in reclassification for events was 10%, the net decline for non-events was 2%, resulting in a NRI of 10-2=8% ($P=0.01$). Carotid calcium reclassified 51% of persons, of whom 34% to low and 17% to high risk. The corresponding NRI was 11-2=9% ($P=0.02$). In most intermediate Framingham risk categories, point estimates of the observed risks agreed with the corresponding categories of predicted risk, indicating good calibration.

**Table 4.** CHD risk reclassification comparing the Framingham risk model and the model additionally including coronary calcium

| **Framingham 5-year risk categories** | **5-year risk categories for Framingham risk covariates + coronary calcium** | | | | | |
|---|---|---|---|---|---|---|
| <5% | <5% | 5-10% | >10% | Overall | Reclassified as higher risk | Reclassified as lower risk |
| No. of participants | 1,463 | 170 | 8 | 1,641 | 178 (11) | NA |
| No. with event | 38 | 17 | 4 | 59 | 21 (36) | NA |
| No. without event | 1,425 | 153 | 4 | 1,582 | 157 (10) | NA |
| Observed risk (95%CI) | 0.03 (0.02,0.04) | 0.10 (0.06,0.17) | 0.47 (0.14,0.92) | | | |
| **5-10%** | | | | | | |
| No. of participants | 129 | 163 | 71 | 363 | 71 (20) | 129 (36) |
| No. with event | 7 | 20 | 8 | 35 | 8 (23) | 7 (20) |
| No. without event | 122 | 143 | 63 | 328 | 63 (19) | 122 (37) |
| Observed risk (95%CI) | 0.06 (0.03,0.13) | 0.12 (0.07,0.20) | 0.11 (0.05,0.24) | | | |
| **>10%** | | | | | | |
| No. of participants | 9 | 38 | 83 | 130 | NA | 47 (36) |
| No. with event | 0 | 1 | 16 | 17 | NA | 1 (6) |
| No. without event | 9 | 37 | 67 | 113 | NA | 46 (41) |
| Observed risk (95%CI) | NA | 0.04 (0.01,0.24) | 0.20 (0.12,0.32) | | | |
| **Overall** | | | | | | |
| No. of participants | 1,601 | 371 | 162 | 2,134 | 249 (12) | 176 (8) |
| No. with event | 45 | 38 | 28 | 111 | 29 (26) | 8 (7) |
| No. without event | 1,556 | 333 | 134 | 2,023 | 220 (11) | 168 (8) |
| NRI: 18-3=15%; P<0.01 (net gain in reclassification for events of 18% and a net decline for non-events of 3%) | | | | | | |

CI: confidence interval; NRI: net reclassification improvement.

**Table 5.** CHD risk reclassification comparing the Framingham risk model and the model additionally including aortic arch calcium

| **Framingham 5-year risk categories** | **5-year risk categories for Framingham risk covariates + aortic arch calcium** | | | | | |
|---|---|---|---|---|---|---|
| <5% | <5% | 5-10% | >10% | Overall | Reclassified as higher risk (%) | Reclassified as lower risk (%) |
| No. of participants | 1,518 | 115 | 0 | 1,633 | 115 (7) | NA |
| No. with event | 52 | 7 | 0 | 59 | 7 (12) | NA |
| No. without event | 1,466 | 108 | 0 | 1,574 | 108 (7) | NA |
| Observed risk (95%CI) | 0.03 (0.02,0.05) | 0.06 (0.03,0.14) | NA | | | |
| **5-10%** | | | | | | |
| No. of participants | 86 | 261 | 40 | 387 | 40 (10) | 86 (22) |
| No. with event | 3 | 25 | 10 | 38 | 10 (26) | 3 (8) |
| No. without event | 83 | 236 | 30 | 349 | 30 (9) | 83 (24) |
| Observed risk (95%CI) | 0.04 (0.01,0.14) | 0.10 (0.06,0.15) | 0.26 (0.13,0.47) | | | |
| **>10%** | | | | | | |
| No. of participants | 3 | 21 | 105 | 129 | NA | 24 (19) |
| No. with event | 0 | 3 | 15 | 18 | NA | 3 (17) |
| No. without event | 3 | 18 | 90 | 111 | NA | 21 (19) |
| Observed risk (95%CI) | NA | 0.14 (0.04,0.46) | 0.15 (0.08,0.25) | | | |
| **Overall** | | | | | | |
| No. of participants | 1,607 | 397 | 145 | 2,149 | 155 (7) | 110 (5) |
| No. with event | 55 | 35 | 25 | 110 | 17 (15) | 6 (5) |
| No. without event | 1,552 | 362 | 120 | 2,039 | 138 (7) | 104 (5) |
| NRI: 10-2=8%; P=0.01 (net gain in reclassification for events of 10% and a net decline for non-events of 2%) | | | | | | |

CI: confidence interval; NRI: net reclassification improvement.

**Table 6.** CHD risk reclassification comparing the Framingham risk model and the model additionally including carotid calcium

| **Framingham 5-year risk categories** | **5-year risk categories for Framingham risk covariates + carotid calcium** | | | | | |
|---|---|---|---|---|---|---|
| <5% | <5% | 5-10% | >10% | Overall | Reclassified as higher risk (%) | Reclassified as lower risk (%) |
| No. of participants | 1,471 | 162 | 1 | 1,634 | 163 (10) | NA |
| No. with event | 47 | 11 | 0 | 58 | 11 (19) | NA |
| No. without event | 1,424 | 151 | 1 | 1,576 | 151 (10) | NA |
| Observed risk (95%CI) | 0.03 (0.02,0.05) | 0.07 (0.03,0.13) | NA | | | |
| **5-10%** | | | | | | |
| No. of participants | 132 | 189 | 67 | 388 | 67 (17) | 132 (34) |
| No. with event | 6 | 17 | 12 | 35 | 12 (34) | 6 (17) |
| No. without event | 126 | 172 | 55 | 353 | 55 (16) | 126 (36) |
| Observed risk (95%CI) | 0.05 (0.03,0.13) | 0.09 (0.05,0.15) | 0.18 (0.10,0.32) | | | |
| **>10%** | | | | | | |
| No. of participants | 11 | 24 | 93 | 128 | NA | 35 (27) |
| No. with event | 0 | 4 | 14 | 18 | NA | 4 (22) |
| No. without event | 11 | 20 | 79 | 110 | NA | 31 (28) |
| Observed risk (95%CI) | NA | 0.17 (0.06,0.43) | 0.16 (0.09,0.27) | | | |
| **Overall** | | | | | | |
| No. of participants | 1,614 | 375 | 161 | 2,150 | 230 (11) | 167 (8) |
| No. with event | 53 | 32 | 26 | 111 | 23 (21) | 10 (9) |
| No. without event | 1,561 | 343 | 135 | 2,039 | 206 (10) | 157 (8) |
| NRI: 11-2=9%; P=0.02 (net gain in reclassification for events of 11% and a net decline for non-events of 2%) | | | | | | |

CI: confidence interval; NRI: net reclassification improvement.

## DISCUSSION

In this large, prospective, population-based study, calcification of the coronary arteries, aortic arch and carotid arteries was associated with a substantially increased risk of CHD, independent of the Framingham risk factors. Furthermore, all three measures of calcification improved risk prediction of CHD beyond Framingham risk factors. In contrast, there was no significant association between calcium in any of the three vascular beds with cerebrovascular events and none of the measures of calcification improved cerebrovascular risk prediction.

### Pathophysiological considerations

We found a strong and graded association of coronary, aortic arch and carotid calcification with risk of coronary heart disease but not with risk of cerebrovascular events. This discrepancy may be partially explained as atherosclerosis is the main underlying mechanism of CHD, whilst cerebrovascular events are related to a broader array of causes.[22] Although atherosclerosis is considered an important cause of ischemic stroke in older persons, a substantial proportion of strokes are based on other mechanisms such as embolisms resulting from cardiac arrhythmias and/or valvular disease or hypertension giving rise to small vessel disease.[23] Another important issue is that we measured arterial calcification. Whilst arterial calcification is an accepted measure of atherosclerotic burden,[3] its pathophysiological relation to the development of cardiovascular events is still not elucidated.

### Previous studies on arterial calcification and CHD risk

Coronary artery calcification is an established, strong and independent risk factor for CHD and has been shown to improve CHD risk prediction beyond traditional risk factors by means of enhancement of the C-statistic.[7-10] The only prospective study on CAC examining reclassification percentages used a 5-year CHD risk model based on 209 CHD events (of which 122 hard events), among almost 6,000 asymptomatic persons and found reclassification percentages to be most substantial in the intermediate risk group. In this group, 55% were reclassified, 16% to high risk, 39% to low risk. The NRI was 0.55 ($P<0.001$).[24] Two recent studies in older persons free of CVD found that thoracic aorta calcium had a less strong relation with CHD and CVD compared to coronary calcium[12, 13] and did not significantly improve CHD and CVD risk prediction over Framingham risk factors or coronary calcium, as judged by change in C-statistic.[12] To our knowledge, no previous studies evaluated the relation between carotid artery calcification and risk of CHD.

### Previous studies on arterial calcification and cerebrovascular risk

Minimal research has evaluated CAC and stroke risk. The Multi-Ethnic Study of Atherosclerosis (MESA) followed 6,698 persons free of CVD for a median period of 3.9 years and did not find an association of CAC with future stroke (HR (95% CI): 1.1 (0.8-1.4) per standard deviation increase of log(calcium+1).[11] In contrast to the negative results of the current prospective study and MESA, a previous cross-sectional study within the Rotterdam Calcification Study cohort found a graded relation of CAC with history of stroke (HR (95% CI) up to 3.0 (1.3-6.8)) for CAC >500 compared to a CAC score of 0-100).[25] However, CAC measurement was performed on average 8.8 years after the stroke. During this period, arterial calcification may have progressed faster in persons with a history of stroke compared to persons without a stroke, due to mutual cardiovascular risk factors. This may have led to an overestimation of the observed association. One small prospective study investigated the association between aortic calcification, as assessed by CT, and risk of cerebrovascular events. The study comprised 455 hypertensive patients of whom 27 persons developed a TIA or stroke during 3-year follow-up. Severe calcification (defined by thickness and extension) of the descending, but not of the ascending aorta, was independently related to ischemic cerebrovascular events.[26] To date, no prospective studies investigated the association of carotid calcification and future cerebrovascular risk.

### Strengths and Limitations

Our study comprised a large cohort with almost complete follow-up in which standardized measurements were performed. However, some limitations should be addressed. First, according to our study protocol, participants with a very high calcium score were informed of their score. Thus, awareness of a high calcium score may have led to lifestyle changes, initiation of medication to reduce cardiovascular risk or even an increased rate of revascularisations. Because we only used hard CHD events as an outcome, we do not expect that this information influenced our results significantly. Second, for assessment of calcification, we used two types of MDCT scanners (16-slice and 64-slice). However, when analyses were repeated adjusting for scanner type, our results did not change substantially (data not shown). Third, in the risk prediction analyses we fitted a model based on Framingham risk factors instead of applying the Framingham risk function. This potentially let to over-fitting, which could lead to an underestimation of the additional CAC value. However, C-statistics were corrected using bootstrapping for over-fitting. Finally, our study was performed in older persons. The predictive power of traditional cardiovascular risk factors decreases with age while arterial calcification might improve risk stratification particularly at older age. Thus our results may not be generalizable to younger populations.

### Screening implications

In accordance with prior research coronary calcium scoring presents as clinically useful in risk prediction of asymptomatic persons at intermediate CHD risk. Additional screening by scoring of aortic calcium did not have extra value, while additional carotid calcium measurement had a small incremental value beyond coronary calcium scoring. However, arterial calcium scores may not be clinically useful in cerebrovascular risk prediction. Although atherosclerosis is considered a generalized process, our results suggest that it is more accurate to use risk prediction algorithms for CHD and cerebrovascular disease separately than using one risk prediction algorithm to assess the risk of major CVD events.

### Conclusion

Calcification of the coronary arteries, aortic arch and carotid arteries was associated with a substantially increased risk of CHD. Moreover, all three measures of calcification significantly improved risk prediction of CHD beyond Framingham risk factors. In contrast, there was no significant association between calcium in any of the three vascular beds with cerebrovascular events and none of the measures of calcification improved cerebrovascular risk prediction.

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# CHAPTER 5

General discussion

# CHAPTER 5.1

## Coronary calcium screening; Ready to be put into practice?

## INTRODUCTION

Accurate risk prediction is vital for cardiovascular disease (CVD) prevention. According to current CVD prevention guidelines, algorithms based on traditional cardiovascular risk factors, like the European SCORE risk chart[1] or American Framingham risk score[2, 3] are increasingly used to stratify persons in categories of low, intermediate, or high risk, based on 10-year absolute risk of CVD or coronary heart disease (CHD). In current prevention guidelines, these risk categories are matched to the intensity of recommended preventive measures.[4, 5] In general, persons at low risk will receive healthy lifestyle advice, whereas persons at high cardiovascular risk will additionally receive drug treatment such as blood pressure and cholesterol-lowering medication. For persons at intermediate risk, treatment decisions are less clear. Part of this group will only receive lifestyle advice, while persons with persistent hypertension and/or hypercholesterolemia will need additional drug therapy.

Although very practical tools, risk assessment algorithms appear to be of limited accuracy.[6, 7] To improve risk stratification, expert panels have proposed non-invasive measurements of atherosclerosis, such as coronary artery calcium (CAC) scoring, as a second step after risk stratification by a risk assessment algorithm, especially for persons at intermediate risk.[8]

## EVALUATION OF CAC AS A NOVEL MARKER IN CARDIOVASCULAR RISK PREDICTION

### Current stage

Abundant research indicates that coronary artery calcium, assessed by computed tomography (CT), is a very promising non-invasive measure to improve cardiovascular risk prediction. It is a strong and independent predictor for CHD and CVD [9-12] that has been shown to add predictive information to traditional risk factors.[9-17] Furthermore, two large population-based studies have indicated that CAC screening in adjunct to traditional risk factors leads to substantially improved risk classification, especially in persons classified as having intermediate CHD risk by traditional risk factors. In this group, CAC reclassified about 55% of persons to either the low (~35%) or high (~20%) risk category. In both studies the net reclassification improvement (NRI) was significant. These studies indicate that addition of CAC screening would lead to a change in recommended therapy in a substantial proportion of persons.[15, 18]

Recently, cost-effectiveness of CAC screening was studied with a Markov model in the Rotterdam Study population (men and women of ≥55 years). In persons of intermediate CHD risk (10-20% 10-year risk), 4 strategies were evaluated: 1) current practice; 2)

current American prevention guidelines for cardiovascular disease;[19] 3) CT screening for coronary calcium; and 4) statin therapy for all individuals. In men, CAC screening was more effective and more costly than the other 3 strategies. In women, CAC screening was more effective and more costly than current practice and statin therapy. However, implementing current guidelines was more effective compared to CAC screening, at a little higher expense.

Yet, the 2011 update of the American Heart Association effectiveness-based CVD prevention guidelines for women recommend use of a new cut point of 10% 10-year cardiovascular risk to define high risk in women.[20] This new cut-off level is based on evidence that women's risks for stroke and heart failure through middle and older age exceed their risk for CHD, in contrast to the pattern observed in men, for whom CHD risk increases earliest.[21, 22] Therefore, current guidelines on 10-year CHD risk may substantially underestimate cardiovascular risk in women.[23] With this new threshold, CAC screening might become more effective compared to current guidelines in intermediate risk women as well.

## Future steps

Randomized clinical trial (RCT) evidence on the additional value of CAC screening in cardiovascular prevention is much awaited to show true clinical benefit owing to CAC screening. The importance to perform an RCT is endorsed by the fact that CT generates images with the use of radiation, which is associated with increased cancer risk. The lifetime excess cancer risk due to radiation exposure from a single cardiac CT examination at age 40 years is estimated at 9 cancers per 100,000 men and 28 cancers per 100,000 women.[24] Furthermore, CT is currently an expensive tool, costing $200 to $600 per scan which might make screening at the population level expensive.[24]

## Evaluation of CAC screening by use of an RCT

Although the conduction of an RCT seems warranted, it will be no easy feat.

In the following paragraphs some issues will be addressed that need to be considered when designing an RCT on the efficacy of CAC screening in adjunct to risk stratification using a risk assessment algorithm. A European setting was chosen to illustrate the issues. However, the considerations could easily be translated to the American situation.

### *Study population*

Reclassification by CAC screening in adjunct to traditional risk factors was found to be most substantial in persons at intermediate risk.[15, 18] Furthermore, it is clinically desirable to reclassify persons from the intermediate to either the low or high risk category because current treatment recommendations are least clear in this group.[8] Thus, a reasonable study population for an RCT would consist of asymptomatic persons at intermediate

CHD risk. The thresholds defining intermediate risk might be chosen in accordance with the age and gender of the study population.[20]

*Aim of the trial*

Compliant with the proposal to use CAC screening as a second step after risk stratification based on traditional risk factors, a plausible aim of a trial conducted in a European setting would be to examine whether CAC screening reduces the number of CHD events in a population classified as being at intermediate cardiovascular risk based on a risk assessment algorithm, such as for example the SCORE risk chart. The reduction of the number of CHD events by CAC screening is expected to mainly take place in the group of persons reclassified from the intermediate to the high risk group who will thereby receive more drug treatment. It is assumed that the persons reclassified to low risk by CAC screening that will be withheld from drug therapy will be properly withheld implicating that the number of events due to this movement will not increase.

*Trial arms*

After being classified as intermediate risk by the risk assessment algorithm, participants will be randomized. Persons in the control arm will not receive additional risk assessment. Participants in the intervention arm will undergo CAC screening by use of a cardiac CT scan. Based on the acquired calcium score, persons will stay in the intermediate risk group or will be reclassified to either the low or high risk group. To strictly investigate the additional value of CAC screening, physicians will treat participants irrespective of the method of risk assessment (algorithm only or algorithm + CT scan) conform their risk level (low, intermediate or high risk), according to the latest European cardiovascular prevention guidelines.[5]

*Contrast between trial arms*

With regard to the expected CHD risk reduction by additional CAC screening, and thereby the required number of RCT participants, two important issues need to be addressed:

1. Reclassification by CAC screening.

As mentioned above, a reduction of the number of CHD events by addition of CAC screening is expected to be mainly caused by extra drug treatment in persons reclassified from intermediate to high risk. This implicates that the expected CHD risk reduction will primarily be generated by the minority of the population that is reclassified from intermediate to high risk by CAC screening. Thus, the contrast between the trial arms will only be part of the contrast that would have been obtained if all persons undergoing CAC screening would have had a reduction in CHD risk.

2. Difference in treatment intensity in the intermediate and high risk groups.

According to the latest European prevention guidelines,[5] drug treatment will primarily consist of anti-hypertensive therapy and statins to lower cholesterol levels. Drug treatment is advised if blood pressure exceeds 160/100 mm Hg in the intermediate risk group and 140/90 mm Hg in the high risk group. Treatment goals are <140/90 mm Hg for persons at intermediate risk and <130/80 mm Hg for persons at high risk. In the intermediate risk group, total cholesterol should be <5 mmol/L and/or LDL-cholesterol <3 mmol/L. Corresponding cholesterol levels for the high risk group are 4.5 mmol/L and 2.5 mmol/L. In persons at intermediate risk, drug treatment is advised if treatment goals can not be reached by life-style interventions, while in the high risk group immediate drug treatment is the therapy of choice. Thus, if current guidelines are stringently applied in both risk groups, the differences in drug treatment between the intermediate and high risk groups are not very large. However, in clinical practice drug therapy is expected to be more strictly applied in the high risk group.

## CONCLUSION

Abundant research indicates that coronary calcium screening in addition to risk assessment by traditional risk factors will improve risk prediction of coronary heart disease. Recent cost-effectiveness analyses suggest that CAC screening could be cost-effective, at least in men. But before CAC screening can be put into practice, its true clinical benefit still needs to be established, preferably through conduction of a randomized clinical trial. Yet, a clinical trial which is aimed to merely examine the additional value of coronary calcium screening beyond a risk factor algorithm in a population at intermediate risk is different from a two-arm trial in which all persons within a trial arm receive the same treatment. The power of such a reclassification trial is highly dependent on the group selected (thresholds for defining the intermediate risk group) because this determines the number that will move from the intermediate to the high risk group, and of the difference in intensity of pharmacological treatment between the intermediate and high risk group. Since the contrast between arms in a reclassification trial will probably be smaller than that of more traditional trials, higher numbers of participants are likely to be needed.

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# CHAPTER 6

## Summary / Samenvatting

## SUMMARY

Cardiovascular disease (CVD) is the major cause of morbidity and premature death in the Western world. The underlying atherosclerosis usually develops over many years before symptoms occur. The majority of CVD is related to modifiable risk factors and modification of these factors has been shown to reduce CVD morbidity and mortality. Hence, cardiovascular disease prevention is vital. The keystone of adequate disease prevention is accurate risk prediction. In accordance with current prevention guidelines, risk assessment algorithms based on traditional risk factors, such as the U.S. Framingham Risk Score and its European counterpart SCORE (Systemic Coronary Risk Evaluation), are widely used to stratify persons in categories of low (<10%), intermediate (10-20%) and high (>20%) risk, based on 10-year absolute risk of CVD or coronary heart disease (CHD). Since these risk factor algorithms appear to be of limited accuracy**,** it is essential to identify novel risk markers that enhance cardiovascular risk prediction beyond traditional risk factors. Measuring the amount of atherosclerosis, representing the end result of risk exposures, might be useful to improve cardiovascular risk prediction. An adequate proxy for the amount of atherosclerosis is measuring the quantity of arterial calcification by a computed tomography (CT) scan.

The purpose of the research described in this thesis was to expand our knowledge of coronary calcification and explore the value of calcification of the thoracic aorta and carotid arteries for their ability to improve of cardiovascular risk prediction beyond traditional risk factors in the general population. Most studies were conducted within the Rotterdam Study, a population-based cohort study among more than 10,000 men and women aged 55 years and older, living in a well-defined suburb of Rotterdam, The Netherlands. We focused on two subgroups, namely 2,292 participants in whom coronary calcification was assessed in 1999-2000 and 2,524 participants in whom coronary, aortic arch and carotid calcium was assessed in 2003-2006. Persons were followed-up for occurrence of coronary heart disease and cerebrovascular disease events.

**Chapter 2** of this thesis focuses on the association of arterial calcification with other novel risk markers of cardiovascular disease. In **chapter 2.1** we evaluate the relation of C-reactive protein (CRP), an established marker of inflammation, with arterial calcification measures and non-calcification measures of atherosclerosis. Although we found an independent, graded relation between CRP and abdominal aortic calcification, no independent association was found with progression of abdominal aortic calcification over a mean period of 6.4 years, or with the amount of coronary calcification. We did find an independent, graded association of CRP with extent and progression of carotid plaques and ankle-brachial-index. Furthermore, CRP was independently related to the highest level of carotid intima-media thickness (cIMT), while the association with change

in cIMT was not significant. Thus, associations of CRP with extent and progression of atherosclerosis were present but did depend on the applied measure of atherosclerosis.

In **chapter 2.2** we examined the relation of resting heart rate with the amount of coronary calcification. This Study was performed within the ADVANCE study, a cohort study among healthy elderly. Persons with a high resting heart rate (highest quintile, ≥ 74 beats/min) had a 62% higher chance of having relatively severe coronary calcification after adjustment for cardiovascular risk factors and heart rate variability, a measure of autonomic nervous system dysfunction. However, resting heart rate did not appear to be related to the rate of progression of coronary calcification over a mean time period of two years.

**Chapter 3** presents results on the relation of arterial calcification with cardiovascular disease risk. In **chapter 3.1**, the relation of coronary calcification and the risk of heart failure is described. We found a clear and graded association between the extent of coronary calcification and the risk of heart failure. Participants in the highest coronary calcium score category (above 400 Agatston units (AU)) were over four times more likely to develop heart failure compared to persons with a coronary calcium score of 0-10 AU, independent of cardiovascular risk factors. After censoring participants at the occurrence of a non-fatal coronary heart disease (CHD), a 3-fold increased risk of heart failure persisted for this calcium score category. This indicates a clear association between coronary calcification and risk of heart failure apart from overt CHD.

**Chapter 3.2** focuses on coronary, aortic arch and carotid calcification in association with history of stroke. Strong associations were present, independent of cardiovascular risk factors. After additional adjustment for calcification in the other vessel beds, presence of stroke was still significantly related to carotid calcification, but no longer to aortic arch or coronary calcification.

**Chapter 4** is dedicated to the additional value of arterial calcification beyond traditional risk factors for cardiovascular risk prediction. **Chapter 4.1** evaluates the additional value of coronary calcium scoring to improve classification of coronary heart disease risk beyond traditional risk factors. This study with almost 10 years of follow-up showed that adding coronary calcium to the Framingham risk model leads to substantial reclassification between CHD risk categories, especially in persons at intermediate Framingham risk (absolute 10-year CHD risk of 10-20%). Over 50 percent of both men and women in the intermediate risk group were reclassified into the high (>20%) or low risk (<10%) category. The net reclassification improvement was 14% ($p<0.01$). The empirically derived cut-off values at which individuals moved from the intermediate to the high or the low risk group were 615 and 50 AU, respectively. **Chapter 4.2** describes the value of a very low coronary calcium score for the development of coronary heart disease. Absent and minimal (1-10 AU) coronary calcification was associated with a negligible risk of CHD, even in persons regarded as being at increased risk by presence of two or more

cardiovascular risk factors. After more than 6 years of follow-up, the negative predictive value for hard coronary events was 99%, both in case of a negative calcium score or a positive but very low calcium score. **Chapter 4.3** answers the question whether carotid intima-media thickness (cIMT), a non-calcification measure of atherosclerosis, improves risk prediction of CHD and stroke. In men, addition of cIMT to Framingham risk factors did not improve prediction of hard CHD or stroke. In women, addition of cIMT to Framingham risk factors significantly improved risk classification. Reclassification was least in the majority of women classified as low risk (4% for hard CHD and 3% for stroke) and most substantial in women at intermediate risk (43% for hard CHD and 28% for stroke). The net reclassification improvement (NRI) in women was 8.2% (p=0.03) for hard CHD and 8.0% (p=0.06) for stroke. Thus, cIMT had some additional value beyond traditional risk factors in the risk stratification of women, but not of men. **Chapter 4.4** describes the value of coronary, aortic arch and carotid artery calcification in the risk prediction of coronary heart disease and cerebrovascular disease events. Calcification of the coronary arteries, aortic arch and carotid arteries was associated with a substantially increased risk of CHD, independent of the Framingham risk factors. Furthermore, all three measures of calcification improved risk prediction of CHD beyond Framingham risk factors. In contrast, calcification of the three vascular beds was not associated with the risk of cerebrovascular events. Accordingly, cerebrovascular risk prediction was not improved by addition of any of the three calcification measures.

The general discussion of this thesis **(Chapter 5)** elaborates on the question whether coronary calcium screening is ready to be integrated in cardiovascular primary prevention guidelines. Abundant research indicates that coronary calcium screening in addition to risk assessment by traditional risk factors will improve risk prediction of coronary heart disease. Recent cost-effectiveness analyses suggest that CAC screening could be cost-effective, at least in men. But before CAC screening can be put into practice, its true clinical benefit still needs to be established, preferably through conduction of a randomized clinical trial. In this chapter some issues will be addressed that need to be considered when designing an RCT on the efficacy of CAC screening in adjunct to a risk assessment algorithm.

## SAMENVATTING

Hart- en vaatziekten zijn de belangrijkste oorzaak van ziekte en vroegtijdige sterfte in de Westerse wereld. De onderliggende atherosclerose ontwikkelt zich meestal over vele jaren voordat symptomen van hart- en vaatziekten optreden. De meerderheid van deze ziekten is gerelateerd aan beïnvloedbare risicofactoren en het is gebleken dat verlaging van deze factoren leidt tot vermindering van ziekte en sterfte aan deze ziekten. Om die reden is preventie van hart- en vaatziekten van vitaal belang. De hoeksteen van adequate preventie is een nauwkeurige risico inschatting. In overeenstemming met de huidige preventie richtlijnen, worden risico scores die gebaseerd zijn op traditionele risicofactoren, zoals de Amerikaanse Framingham Risico Score and zijn Europese tegenhanger SCORE (Systemic Coronary Risk Evaluation), frequent gebruikt om personen in te delen in categorieën van laag (<10%), intermediair (10-20%) en hoog (>20%) risico, gebaseerd op het 10-jaars absoluut risico op (ischemische) hart- en vaatziekten. Omdat is gebleken dat de nauwkeurigheid van deze risico scores te wensen overlaat, is het van essentieel belang om nieuwe risicofactoren te identificeren die in staat zijn de risico inschatting op basis van traditionele factoren te verbeteren. Het meten van de hoeveelheid atherosclerose, het eindresultaat van blootstelling aan risicofactoren, lijkt hiervoor een geschikte kandidaat. Een adequate maat voor de hoeveelheid atherosclerose is het meten van de hoeveelheid verkalking in slagaders met behulp van een computed tomography (CT) scan.

Het doel van het onderzoek beschreven in dit proefschrift was het uitbreiden van onze kennis over verkalking van de kransslagaders en het verkennen van de toegevoegde waarde van verkalking in de grote levensslagader en de halsslagaders bovenop traditionele risicofactoren voor het voorspellen van de kans op hart- en vaatziekten in de algemene bevolking. De meeste studies werden uitgevoerd binnen het Erasmus Rotterdam Gezondheid Onderzoek (ERGO), een bevolkingsonderzoek onder meer dan 10.000 mannen en vrouwen van 55 jaar en ouder, woonachtig in de wijk Ommoord in Rotterdam. Ons onderzoek richtte zich voornamelijk op twee subgroepen: 2.292 deelnemers bij wie de mate van verkalking in de kransslagaders was gemeten tussen 1999-2000 en 2.524 deelnemers bij wie de verkalking in de kransslagaders, grote levensslagader en de halsslagaders was bepaald tussen 2003-2006. Deelnemers werden vervolgd voor het optreden van ischemische hartziekten en cerebrovasculaire ziekten.

**Hoofdstuk 2** van dit proefschrift gaat in op de relatie tussen verkalking in de slagaders en andere nieuwe risico markers van hart- en vaatziekten. In **hoofdstuk 2.1** evalueren we de relatie van C-reactive protein (CRP), een bekende ontstekingsmarker, met de mate van verkalking in slagaders en andere maten van atherosclerose. Hoewel we een onafhankelijke, graduele associatie vonden tussen CRP en verkalking van de buikslagader,

vonden we geen onafhankelijke relatie met de progressie van verkalking in deze slagader over een periode van 6.4 jaar, noch met de mate van verkalking in de kransslagaders. Wel vonden we een onafhankelijke en graduele relatie tussen CRP en de hoeveelheid en toename van plaques in de halsslagaders en met de enkel-arm-index. Daarnaast was CRP onafhankelijk geassocieerd met de hoogste mate van intima-media-verdikking in de halsslagaders, maar niet significant met de progressie van die verdikking. Kortgezegd vonden we een relatie tussen CRP en de hoeveelheid en toename van atherosclerose, maar de sterkte van deze relatie bleek afhankelijk van de gebruikte methode om atherosclerose te meten.

In **hoofdstuk 2.2** onderzochten we de relatie tussen de hartslag in rust en de hoeveelheid verkalking in de kransslagaders. Deze studie was uitgevoerd binnen de ADVANCE studie, een cohort studie onder gezonde ouderen. Personen met een hoge hartslag in rust (hoogste quintiel, ≥ 74 slagen/min), hadden een 62% hogere kans op het hebben van een ernstige verkalking in de kransslagaders, onafhankelijk van cardiovasculaire risicofactoren en variatie in hartslag, een maat voor disfunctie van het autonome zenuwstelsel. Desondanks bleek de hartslag in rust niet gerelateerd aan de toename van verkalking in de kransslagaders over een gemiddelde periode van twee jaar.

**Hoofdstuk 3** presenteert resultaten over de relatie tussen slagaderverkalking en het risico op hart- en vaatziekten. In **hoofdstuk 3.1** wordt de associatie tussen de mate van verkalking in de kransslagaders en het risico op hartfalen beschreven. We vonden een duidelijke en graduele relatie tussen de mate van verkalking en het risico op hartfalen. Deelnemers in de categorie met de hoogste kalkscore (> 400 Agatston eenheden (AE)) hadden een meer dan 4 maal hoger risico op het krijgen van hartfalen vergeleken met deelnemers die een kalkscore van 0-10 AE hadden, onafhankelijk van cardiovasculaire risicofactoren. Na censureren van participanten ten tijde van het optreden van een ischemische hartziekte, zagen we nog steeds een drie maal hoger risico voor de mensen met de hoogste kalkscores. Dit geeft aan dat er een duidelijke relatie bestaat tussen de mate van kransslagaderverkalking en het risico op hartfalen, onafhankelijk van apert ischemisch hartlijden. **Hoofdstuk 3.2** richt zich op de relatie tussen verkalking in de kransslagaders, grote levensslagader en halsslagaders en het hebben doorgemaakt van een herseninfarct. We vonden een sterke relatie hiertussen, onafhankelijk van cardiovasculaire risicofactoren. Na verdere correctie voor verkalking in de andere vaatbedden, was een doorgemaakt herseninfarct nog steeds gerelateerd aan de mate van verkalking in de halsslagaders, maar niet meer aan verkalking in de grote levensslagader en de kransslagaders.

**Hoofdstuk 4** is gewijd aan de toegevoegde waarde van het meten van slagaderverkalking bovenop traditionele risicofactoren voor de voorspelling van hart- en vaatziekten. **Hoofdstuk 4.1** evalueert de toegevoegde waarde van het scoren van kalk in de kransslagaders bovenop traditionele risicofactoren voor het classificeren van het risico

op ischemische hartziekten. Deze studie, met bijna 10 jaar follow-up, liet zien dat het toevoegen van kransslagaderverkalking aan het Framingham risicomodel leidt tot een substantiële reclassificatie tussen de verschillende risico categorieën, met name in personen met een intermediair Framingham risico (absoluut risico van 10-20%). Meer dan 50% van zowel mannen als vrouwen in de intermediaire groep werd gereclassificeerd naar de hoog risico (>20%) of de laag risico (<10%) groep. De netto verbetering in classificatie bedroeg 14% (p<0.01). De empirisch vastgestelde afkapwaarden waarop personen verschoven van de intermediare naar de hoog of de laag risico groep waren respectievelijk 615 en 50 Agatston eenheden. **Hoofdstuk 4.2** beschrijft de waarde van een bijzonder lage kalk score in de kransslagaders voor het risico op het ontwikkelen van ischemische hartziekten. Afwezige of minimale (1-10 AE) kransslagaderverkalking was geassocieerd met een verwaarloosbaar klein risico op ischemische hartziekten, zelfs in personen die beschouwd werden als hebbende een hoog risico op basis van het bezitten van twee of meer cardiovasculaire risicofactoren. Na meer dan 6 jaar follow-up was de negatief voorspellende waarde voor het ontwikkelen van een hartinfarct of overlijden aan een ischemische hartziekte 99%, zowel voor mensen zonder verkalking als voor mensen met een zeer lage kalkscore van de kransslagaders. **Hoofdstuk 4.3** beantwoordt de vraag of intima-media dikte meting in de halsslagaders, een niet-verkalkings maat van atherosclerose, de voorspelling van het risico op het krijgen van ischemische hartziekten of een herseninfarct verbetert. Bij mannen leidde toevoeging van intima-media dikte meting aan het Framingham risico model niet tot een significante verbetering in de risico classificatie. Bij vrouwen wel. Reclassificatie was het minst in de vrouwen geclassificeerd als laag risico (4% voor ischemische hartziekten en 3% voor herseninfarct) en was meest substantieel in de vrouwen geclassificeerd als intermediair risico (43% voor ischemische hartziekten en 28% voor herseninfarct). De netto verbetering in classificatie bij vrouwen bedroeg 8.2% (p=0.03) voor de uitkomst ischemische hartziekten en 8.0% (p=0.06) voor de uitkomst herseninfarct. Dus, het meten van intima-media dikte bleek van matige additionele waarde bovenop het meten van traditionele risicofactoren voor de risicostratificatie van vrouwen, maar niet van mannen. **Hoofdstuk 4.4** beschrijft de waarde van verkalking in de kransslagaders, grote levensslagader en halsslagaders voor het voorspellen van het risico op ischemische hartziekten en cerebrovasculaire ziekten. Verkalking in de kransslagaders, grote levensslagader en halsslagaders bleek geassocieerd te zijn met een significant verhoogd risico op ischemische hartziekten. Daarnaast hadden alle drie de kalkmaten een toegevoegde waarde bovenop traditionele risicofactoren voor het voorspellen van ischemische hartziekten. In tegenstelling hiermee, bleek verkalking in de drie vaatbedden niet geassocieerd te zijn met het risico op cerebrovasculaire ziekten. Dienovereenkomstig werd de risicovoorspelling op cerebrovasculaire ziekten door geen van de drie kalkmaten verbeterd.

De algemene discussie van dit proefschrift **(hoofdstuk 5)** gaat in op de vraag of de tijd rijp is om screening op verkalking in de kransslagaders op te nemen in cardiovasculaire primaire preventie richtlijnen. Talrijk onderzoek heeft aangetoond dat het meten van de hoeveelheid verkalking in de kransslagaders een toegevoegde waarde heeft boven op bepaling van traditionele risicofactoren voor het voorspellen van ischemische hartziekten. Recente kosten-effectiviteits analyses suggereren dat screening op verkalking kosten-effectief zou kunnen zijn, in ieder geval in mannen. Maar voordat deze screening toegepast kan worden in de klinische praktijk, dient de gezondheidswinst hiervan aangetoond te worden, bij voorkeur middels gerandomiseerd klinisch onderzoek. In dit hoofdstuk wordt een aantal zaken besproken waarmee rekening gehouden zou moeten worden tijdens het ontwerpen van zo'n onderzoek naar de meerwaarde van screening op verkalking bovenop traditionele risicofactoren.

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## LIST OP PUBLICATIONS

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## ABOUT THE AUTHOR

Suzette Smale was born on October, 18, 1973 in Ede, The Netherlands. After graduating secondary school at the Stedelijk Gymnasium in Nijmegen, she studied one year of chemistry and then went to Medical School at the University of Utrecht. In 2001 she started her residency in Cardiology at the Sint Antonius Hospital in Nieuwegein. From 2006 on, she combined her clinical work with the work presented in this thesis. The research was performed at the department of Epidemiology (chair: Prof.dr. A. Hofman), and the department of Radiology (chair: prof.dr. G.P. Krestin), Erasmus MC, Rotterdam. In 2009 she worked a year as a visiting scholar at the Stanford Research Prevention Center, Stanford University, Stanford, California, USA (chair: prof.dr. S.P. Fortmann).

In the same year she obtained her Master of Science degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences. In February 2012 she will finish her residency in Cardiology and will thereafter start her fellowship cardiovascular imaging at the University Hospital Sint Radboud in Nijmegen. Suzette is married to Sjoerd Elias and they have two children, Josefien and Quirijn.

## PHD PORTFOLIO SUMMARY

| | |
|---|---|
| Name PhD student: | Suzette Elias-Smale |
| Erasmus MC Department: | Epidemiology and Radiology |
| PhD period: | 2005-2007 and 2008-2010 |
| Promotors: | Prof.dr. J.C.M. Witteman, prof.dr. A. vd Lugt |

| | Year | Workload Hours/ECTS |
|---|---|---|
| **1. PhD training** | | |
| **General academic skills** | Self taught | n.a. |
| Biomedical English Writing and Communication | | |
| **Research skills** | | |
| MSc in Clinical Epidemiology, Nihes, The Netherlands | 2005-2009 | |
| **In-depth courses** | | |
| Principles of research in medicine, Nihes | 2005 | 0.7 |
| Methods of public health research, Nihes | 2006 | 0.7 |
| Clinical trials, Nihes | 2006 | 0.7 |
| Topics in evidence-based medicine, Nihes | 2006 | 0.7 |
| Case-control studies, Nihes | 2006 | 0.7 |
| Decision making in medicine, Nihes | 2005 | 0.7 |
| Study design, Nihes | 2005 | 4.3 |
| Classical methods for data-analysis, Nihes | 2005 | 5.7 |
| Clinical epidemiology, Nihes | 2006 | 5.7 |
| Methodological topics in epidemiologic research, Nihes | 2008 | 1.4 |
| Modern statistical methods, Nihes | 2008 | 4.3 |
| Advanced diagnostic research, Nihes | 2006 | 1.4 |
| Prognostic research, Nihes | 2006 | 1.4 |
| Decision making in medicine II, Nihes | 2006 | 1.4 |
| Repeated measurements in clinical studies, Nihes | 2006 | 1.4 |
| Analysis of time-varying exposure, Nihes | 2006 | 0.7 |
| **Presentations at (inter)national conferences** | | |
| 5th Scientific research meeting of the Dutch Heart Association (NHS), Amsterdam, The Netherlands | 2005 | 30 |
| European Congress of Epidemiology, Utrecht, The Netherlands | 2006 | 30 |
| Joint Conference - 49th Cardiovascular Disease Epidemiology and Prevention - and - Nutrition, Physical Activity, and Metabolism, Palm Harbor, Fl, USA | 2009 | 30 |
| American Heart Association Scientific Sessions, Orlando, Fl, USA | 2009 | 30 |
| American Heart Association Scientific Sessions, Chicago, Il, USA | 2010 | 30 |
| **Long term research visits** | | |
| Visiting Scholar, Stanford Prevention Research Center, Stanford University, Stanford, CA, USA | 2009-2010 | 1 year |

| | Year | Workload Hours/ ECTS |
|---|---|---|
| **2. Teaching activities** | | |
| **Supervising Master students** | | |
| Maarten Leening: Unrecognized myocardial infarction and long-term risk of heart failure in the elderly; The Rotterdam Study | 2008-2009 | 50 |
| Maarten Leening: Coronary calcification and the risk of heart failure in the elderly; The Rotterdam Study | 2009-2010 | 50 |
| Abdelilah el Barzouhi: Renal function is related to severity of coronary artery calcification in elderly persons; The Rotterdam Study | 2009-2010 | 50 |

Prevention of cardiovascular disease starts with adequate risk prediction. Traditional risk factors like age, gender, blood pressure and cholesterol are clearly related to atherosclerosis, the underlying mechanism of cardiovascular disease. However, at every level of risk factor exposure, there is a substantial variation in the quantity of atherosclerosis. Therefore, measuring the extent of atherosclerosis might be useful to improve cardiovascular risk prediction. The amount of arterial calcification, assessed by computed tomography (CT) scan, has been shown to adequately reflect a person's burden of atherosclerosis. The research described in this thesis shows that assessing calcification in the coronary arteries, aortic arch and carotid arteries indeed has a significant additional value beyond traditional risk factors for the prediction of myocardial infarction, but not of cerebrovascular disease.

Preventie van hart- en vaatziekten begint bij een goede risico inschatting. Traditionele risicofactoren als leeftijd, geslacht, bloeddruk en cholesterolspiegel zijn duidelijk gerelateerd aan atherosclerose, de onderliggende oorzaak van hart- en vaatziekten. Niettemin blijkt er bij eenzelfde mate van blootstelling aan risicofactoren een behoorlijke variatie te bestaan in de ernst van atherosclerose. Het is daarom aannemelijk dat bepaling van de hoeveelheid atherosclerose de risico inschatting op hart- en vaatziekten kan verbeteren. Het meten van de hoeveelheid kalk in slagaders met behulp van een computed tomography (CT) scan is een goede afspiegeling gebleken van de hoeveelheid atherosclerose in deze vaten. Uit het onderzoek beschreven in dit proefschrift blijkt dat het meten van verkalking van de kransslagaders, de grote levensslagader en de halsslagaders inderdaad een duidelijke meerwaarde heeft boven het meten van traditionele risicofactoren voor het inschatten van het risico op een hartaanval, maar niet van het risico op een herseninfarct.