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Epidemiologic Studies on Arterial Calcification

The Rotterdam Study

Epidemiologische studies naar arteriële calcificaties

Het ERGO onderzoek

Proefschrift

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Erasmus Universiteit Rotterdam
op gezag van de
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Manuscripts based on studies described in this thesis

Chapter 2.1
Odink AE, van der Lugt A, Dehghan A, Hofman A, Hunink MGM, Breteler MMB, Krestin GP, Witteman JCM. *Association between carotid calcification and carotid plaques. The Rotterdam Study. Submitted.*

Chapter 3.1
Odink AE, van der Lugt A, Hofman A, Hunink MGM, Breteler MMB, Krestin GP, Witteman JCM. *Association between calcification in the coronary arteries, aortic arch and carotid arteries. The Rotterdam Study. Atherosclerosis 2006.Aug 17.*

Chapter 4.1
Odink AE, van der Lugt A, Hofman A, Hunink MGM, Breteler MMB, Krestin GP, Witteman JCM. *Risk factors for coronary, aortic arch and carotid calcification. The Rotterdam Study. Submitted.*

Chapter 4.2
Odink AE, van der Lugt A, Bos MJ, Hofman A, Hunink MGM, Koudstaal P, Breteler MMB, Krestin GP, Witteman JCM. *Alcohol consumption and carotid calcification assessed by multislice CT. The Rotterdam Study. Submitted.*

Chapter 4.3
Odink AE, Mattace-Raso F, van der Lugt A, Hofman A, Hunink MGM, Breteler MMB, Krestin GP, Witteman JCM. *Arterial stiffness and aortic arch and carotid calcification. Submitted.*

Chapter 5.1
Odink AE, van der Lugt A, Bos MJ, Hofman A, Hunink MGM, Koudstaal P, Breteler MMB, Krestin GP, Witteman JCM. *Aortic arch and carotid calcification in subjects with a history of stroke. The Rotterdam Study. Submitted.*

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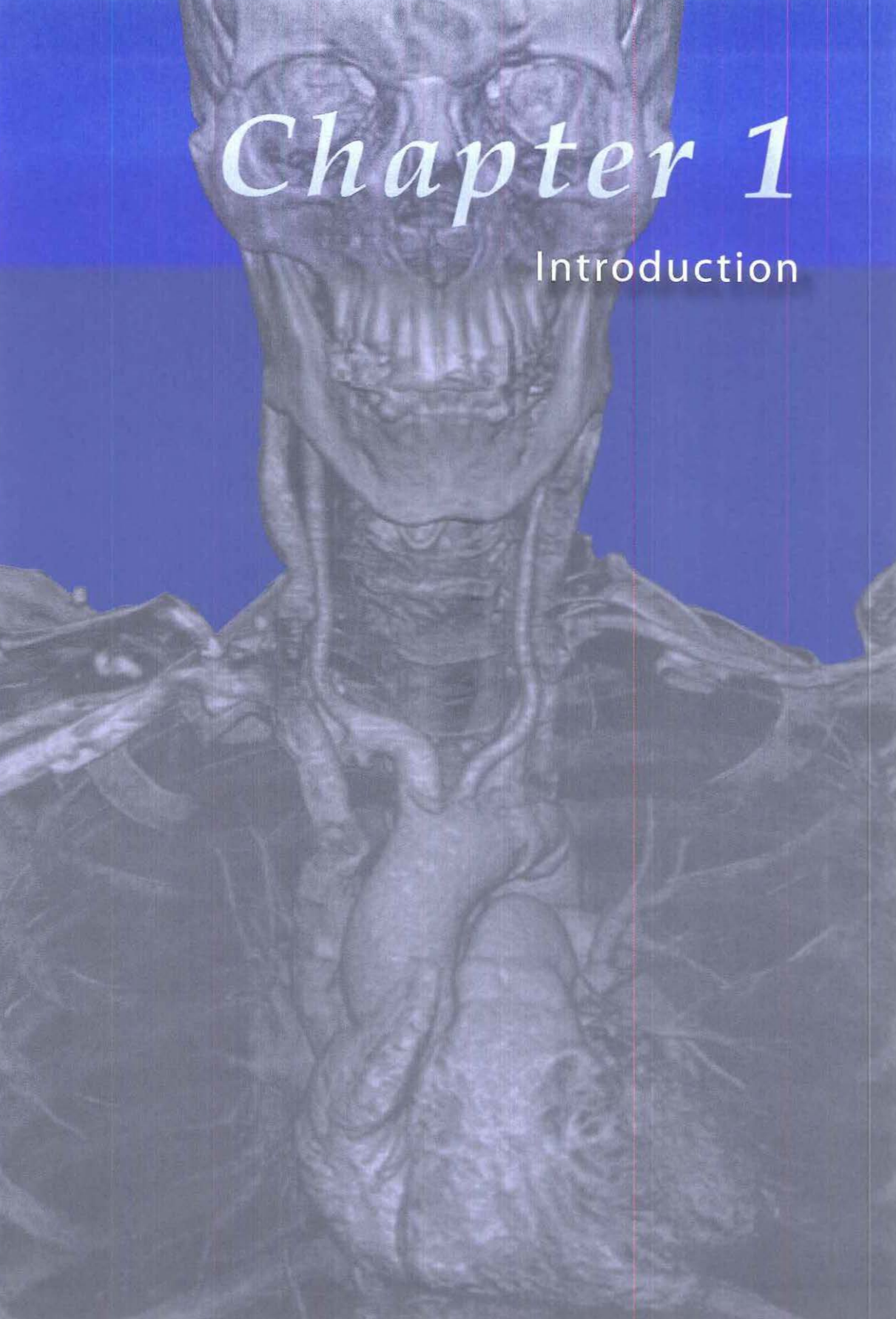


Chapter 1

Introduction

Introduction

Cardiovascular disease leads to a high morbidity and mortality and consequently puts a large burden on the health care system. Therefore, early identification of high-risk subjects is needed. Several non-invasive measures of atherosclerosis exist and may be useful in identifying subjects at high risk. Existing non-invasive measures of atherosclerosis include intima media thickness (IMT) and number of plaques of the carotid arteries by ultrasound and the ankle brachial index, measuring peripheral atherosclerosis. The introduction of electron beam computed tomography (EBCT) and multislice computed tomography (MSCT) has enabled the non-invasive visualization and accurate quantification of calcification in different arteries.

Coronary calcification has been found to be a predictor of coronary heart disease [1, 2]. However, calcification can also be measured in other vessel beds like the aorta [3] and the carotid arteries [4]. Quantification of calcification in these vessel beds might further improve cardiovascular risk assessment. Few data are available on the prevalence, risk factors for and predictive value of arterial calcification in different vessel beds. The focus of this thesis is to investigate determinants of coronary, aortic arch and carotid calcification, assessed by MSCT and to examine the association between arterial calcification in different vessel beds. In addition, the association of aortic arch and carotid calcification with history of stroke was examined. The study was carried out in the population-based Rotterdam Study.

The Rotterdam Study is a population-based study, which started with a baseline visit in 1990-1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited and 7,983 agreed to participate (78% response). In 2000-2001, the cohort was extended with 3,011 subjects (67% response) also aged 55 years and over. The design and rationale of the Rotterdam Study have been described elsewhere [5]. Study center visits took place approximately every three years. From September 2003 onwards, all participants who completed a regular visit to the research center were invited to participate in the present study and to undergo a MSCT scan of coronary arteries, the aortic arch and the carotid arteries. Between September 2003 and January 2005, 1,003 subjects were scanned.

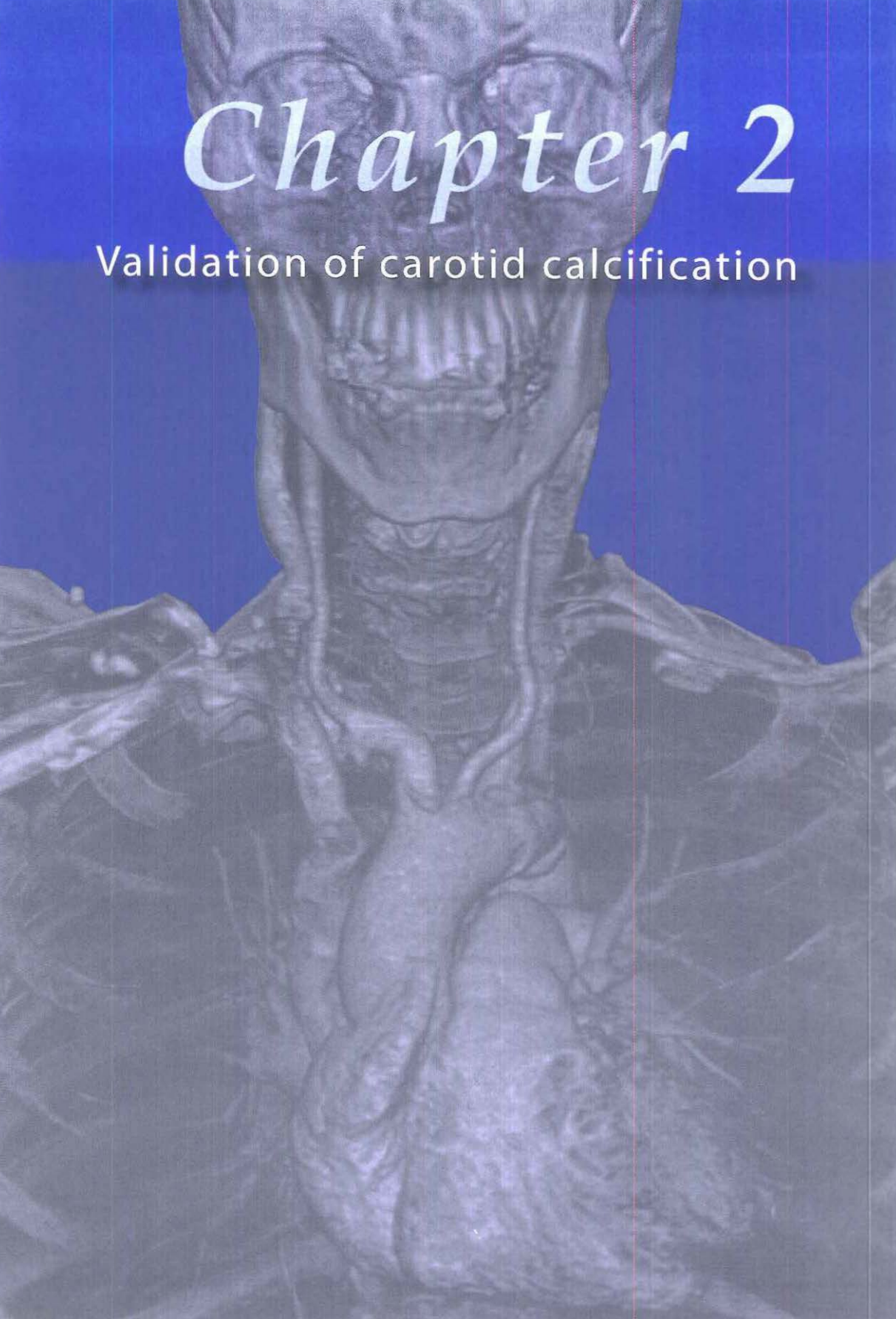
In **chapter 2**, the association of carotid calcification with carotid plaques was examined. Although a clear correlation has been demonstrated between coronary calcification and coronary plaque burden[6], we do not know the strength of the association between carotid calcification and carotid plaque burden. Therefore, this study examined the strength of the association between carotid calcification assessed by MSCT and carotid plaques assessed by ultrasound among 987 subjects.

In **chapter 3**, associations between calcification in the coronary arteries, aortic arch and carotid arteries were investigated. The use of MSCT enables non-invasive quantification of arterial calcification in various vessels and allows examination of intervessel correlations. This may give insight in whether the assessment of calcification in one vessel bed predicts calcification in other vessels and allows examination of the concept of generalized atherosclerosis. Therefore, in this chapter we examined among 600 subjects the prevalence of and associations between calcification in the coronary arteries, aortic arch and carotid arteries, assessed by MSCT.

Chapter 4 focuses on the determinants of arterial calcification. Firstly, we examined the association of cardiovascular risk factors with calcification in the coronary arteries, aortic arch, and the carotid arteries in a population-based study. Additionally, we investigated the association between alcohol consumption and carotid calcification. Finally, we studied the association between arterial stiffness and arterial calcification to examine whether arterial stiffness and arterial calcification are concurrent processes. **Chapter 5** describes a study on the association of aortic arch and carotid calcification with history of stroke. The predictive value of aortic arch and carotid calcification for stroke is unknown, we set on to examine this association among 1,003 subjects.

References

1. LaMonte MJ, FitzGerald SJ, Church TS, Barlow CE, Radford NB, Levine BD, Pippin JJ, Gibbons LW, Blair SN, Nichaman MZ. Coronary artery calcium score and coronary heart disease events in a large cohort of asymptomatic men and women. Am J Epidemiol. 2005;162:421-429.
2. Arad Y, Spadaro LA, Goodman K, Newstein D, Guerci AD. Prediction of coronary events with electron beam computed tomography. J Am Coll Cardiol. 2000;36:1253-1260.
3. Kuller LH, Matthews KA, Sutton-Tyrrell K, Edmundowicz D, Bunker CH. Coronary and aortic calcification among women 8 years after menopause and their premenopausal risk factors : the healthy women study. Arterioscler Thromb Vasc Biol. 1999;19:2189-2198.
4. Nandalur KR, Baskurt E, Hagspiel KD, Finch M, Phillips CD, Bollampally SR, Kramer CM. Carotid artery calcification on CT may independently predict stroke risk. AJR Am J Roentgenol. 2006;186:547-552.
5. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
6. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.



Chapter 2

Validation of carotid calcification

2.1

Association between carotid calcification and carotid plaques

Abstract

Background and purpose

This study examined the strength of the association between carotid calcification assessed by multislice computed tomography (MSCT) and carotid plaques assessed by ultrasound.

Methods

The study was part of the Rotterdam Study, a population-based study in subjects aged 55 years and over. From October 2003 until December 2004, subjects were invited to undergo an MSCT scan. Calcification in the carotid arteries was quantified according to the Agatston score. Carotid plaques and calcified carotid plaques were measured in the common carotid arteries, carotid bifurcations and the internal carotid arteries using ultrasound. Receiver operating characteristic (ROC) curves were used to examine whether MSCT calcium score can accurately estimate the presence of plaques, for different cut-points.

Results

The correlation coefficients between MSCT carotid calcification and carotid plaques were equal in men and women ($r = 0.67$). The corresponding coefficients between MSCT calcification and calcified carotid plaques were $r = 0.57$ and $r = 0.60$, respectively, after additional adjustment for total carotid plaques, (for all coefficients $p < 0.001$). The MSCT calcium score was an accurate predictor of the number of carotid plaques (area under the curve > 0.8) for different cut-points.

Conclusions

We observed a strong association between carotid calcification assessed by MSCT and carotid plaques assessed by ultrasound. The amount of carotid plaques could be accurately estimated by the calcium score. This suggests that calcification assessed by MSCT is a good measure of the presence of plaques in the carotid arteries.

Introduction

The use of multislice computed tomography (MSCT) enables accurate assessment and quantification of arterial calcification. Although a clear correlation has been demonstrated between coronary calcification and coronary plaque burden [1], we do not know the strength of the association between carotid calcification and carotid plaques. A recent study among asymptomatic subjects found a strong correlation between carotid calcification and carotid intima-media thickness (IMT) [2]. This study was performed using a small group of subjects and until now no data has been published on the correlation between carotid calcification and carotid plaques.

Ultrasound can distinguish between calcified and non-calcified plaques. However, to this date, no studies have compared the ultrasound assessment of carotid calcified plaques with the amount of carotid calcification measured by MSCT.

Therefore, we undertook this study to investigate the magnitude of the correlation between carotid calcification by MSCT and carotid plaques by ultrasound. Additionally, we examined the validity of calcified plaques measured by ultrasound by comparison with the amount of calcification assessed by MSCT.

Material and methods

Study population

The study was embedded in the Rotterdam Study, a population-based study, which started with a baseline visit in 1990-1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited and 7,983 agreed to participate (78% response). In 2000-2001, the cohort was extended with 3,011 subjects (67% response), also aged 55 years and over. The design and rationale of the Rotterdam Study have been described elsewhere [3]. Study center visits took place approximately every three years.

From September 2003 on all participants who completed the center visit (the fourth of the original cohort and the second center visit of the extended cohort) were invited to participate in the present study and to undergo a MSCT scan of the carotid arteries. We restricted the present analyses to participants who were scanned until December 2004. We scanned 1,003 subjects. Data on calcification and plaques in the carotid arteries were available in 987 subjects. The median duration between the study center visit and the MSCT scan was 96 days.

This 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

In the first 702 subjects, imaging was performed with a 16-slice MSCT scanner (SOMATOM Sensation 16, Siemens, Forcheim, Germany). The scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Scan parameters were: 16 × 0.75 mm collimation, 120 kVp, 100 effective mAs, 0.5 s rotation time, and normalized pitch of 1. Images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5 mm, 120 mm FOV and medium sharp convolution kernel ("B35f"). In the other 285 subjects image acquisition was performed with a 64-slice MSCT scanner (SOMATOM Sensation 64, Siemens, Forcheim, Germany). Scan parameters were similar for both MSCT scanners, except for collimation and effective mAs. With the use of the 64-slice MSCT scanner the collimation was 32 × 0.6 mm and the mAs value was real time adapted to body weight (CARE DOSE, Siemens, Forcheim, Germany) with a reference value of 100 mAs.

Analysis of calcification

Two reviewers, with a medical background, were trained by an experienced radiologist and scored arterial calcification using a standardized protocol. They were blinded to the clinical data of the participants. The examination of 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) and were scored using dedicated software (syngo Calcium Scoring, Siemens, Forcheim, Germany). Calcification was quantified by calculating the Agatston score. For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion (the number of voxels with an attenuation value ≥ 130 HU times the volume of one voxel) and a factor assigned according to the maximum attenuation value of the lesion [4]. For image data with an overlapping reconstruction increment, Agatston scores were normalized with the ratio of increment and slice width. The total score was calculated by adding up the scores of all lesions.

Carotid plaques

During the research center visit, ultrasound of both carotid arteries was performed. In the first 505 subjects imaging was performed with a 7.0-MHz linear array transducer (ACUSON 128, Siemens AG, Erlangen, Germany). In the other 482 subjects image acquisition was performed with a 7.5-MHz linear array transducer (AU3 Partner, Esaote, Florence, Italy). Plaque assessment was performed online.

A plaque score was derived by counting the number of sites with a plaque, leading to a maximum score of six per carotid artery (anterior and posterior wall of the common carotid artery, of the carotid bifurcation and of the internal carotid artery) and to a total plaque score with a maximum of twelve. The presence of a plaque was defined as a focal widening of at least 1.5 times the average IMT relative to the adjacent segments, with protrusion into the lumen and composed of calcified and/or non-calcified components. Plaque characteristics (calcifications and acoustic shadowing) were obtained during the measurement. A calcified plaque score was computed in a similar way as the total plaque score.

Cardiovascular risk factors

Information on smoking, blood pressure, and lipid lowering medication use was obtained during a home interview of the Rotterdam Study. Subjects were categorized as current, past, and never smokers. Clinical measures were obtained during a visit at the study center. Height and weight were measured and the body mass index was calculated (weight[kg]/ height [m]^2). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were obtained using an automatic enzymatic procedure (Hitachi 911, Roche CHOD PAP). Diabetes was defined as the use of anti-diabetic medication or a fasting glucose level ≥ 7 mmol/l. Information on a history of myocardial infarction and stroke was collected at baseline and during follow-up before CT scanning [5,6].

Statistical analysis

Baseline characteristics were computed for men and women separately. Since MSCT calcium scores were much higher in men than in women, all analyses were stratified by gender. Age-adjusted correlation coefficients were computed using Spearman's correlation coefficients for the association between MSCT calcium scores and carotid (calcified) plaques, for the right and left carotid artery separately and for both carotid arteries together.

The ROC curve is a plot of all the sensitivity and specificity pairs derived from continuously varying the threshold level [7]. To estimate the number of carotid plaques from the calcium score by MSCT, we categorized the plaques score. Firstly, we categorized the number of plaques into no plaques versus more than zero plaques. Secondly, we categorized the numbers of plaques, with the median (in men: 4 plaques, in women: 2 plaques) as the cut point.

Analysis of covariance was used to compute geometric mean MSCT calcium scores for categories of number of plaques and number of calcified plaques in the carotid arteries and for different age strata.

All analyses were adjusted for the type of MSCT and for ultrasound system; the analyses concerning calcified plaques were additionally adjusted for total number of plaques. SPSS 11.0 for Windows (SPSS, Inc, Chicago, Illinois) was used for data analysis.

Results

Table 1 describes the characteristics of the 987 study participants. The mean age (± sd) of the study population was 72 years (6.4) and 48% were male. Overall, MSCT calcium scores were higher in men than in women. The median MSCT calcium score (interquartile range) in the carotid arteries was 63.3 (1.8-234) in men and 25.8 (0-128) in women. The median amount of carotid plaques (interquartile range) in men was 4 (2-6) and in women 2 (1-4). Corresponding numbers for calcified carotid plaques were 2 (1-5) in men and 1 (0-3) in women. Prior to scanning myocardial infarction was reported in 12% of men and in 5% of women, stroke in 5% of men and 4% of women.

Table 2 shows the age-adjusted correlation coefficients of MSCT calcium scores with carotid plaques and calcified carotid plaques, for men and women separately. The correlation coefficients between MSCT calcium scores and carotid plaques were equal in men and women (r=0.67). The corresponding coefficients between MSCT calcium scores and carotid calcified plaques, were r=0.57 and r=0.60, respectively, after additional adjustment for total carotid plaques.

Figure 1 shows the ROC curves for two cut points (no plaques versus any plaque and below versus equal or above the median), for men and women separately. For men and women the Area under the Curve (AUC) was in both models over 0.80, which indicates a high accuracy of the MSCT calcium score in estimating the number of plaques.

Figure 2 shows the geometric mean MSCT calcium scores for categories of number of plaques in both carotid arteries, stratified by age. The geometric mean MSCT calcium score in subjects without carotid plaques was below 2. A steady increase in geometric mean calcium was found up to the category of 7 to 8 plaques, but the calcium score was remarkably higher in the upper two categories.

Table 1 Characteristics of the study population

Variable	Men (n=478)	Women (n=509)
Age (years)	72.0 ± 6.2	71.2 ± 6.6
Body mass index (kg/m²)	27.2 ± 3.4	27.8 ± 4.5
Systolic blood pressure (mm Hg)	148.8 ± 20.3	150.5 ± 21.3
Diastolic blood pressure (mm Hg)	81.3 ± 11.0	79.7 ± 11.0
Total cholesterol (mmol/l)	5.3 ± 0.9	5.9 ± 0.9
HDL-cholesterol (mmol/l)	1.3 ± 0.3	1.5 ± 0.4
Serum glucose (mmol/l)	5.8 ± 1.3	5.7 ± 1.3
DM (%)	12	11
Smokers (%)		
Current	12	13
Past	72	44
Never	16	43
Use of blood pressure lowering medication (%)	45	47
Use of lipid lowering medication (%)	26	23
History of myocardial infarction (%)	12	5
History of stroke (%)	5	4
Carotid artery Agatston score[1]	63.3 (1.8-234)	25.8 (0-128)
Plaques assessed by ultrasound[1]	4 (2-6)	2 (1-4)

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation. [1] Value is expressed as median (interquartile range) because of its skewed distribution.

Table 2 Correlation coefficients between carotid calcification and carotid plaques

	Calcification measured by MSCT					
	Right carotid artery		Left carotid artery		Both carotid arteries	
	Men	Women	Men	Women	Men	Women
Total plaque score						
Right carotid artery †	0.60	0.62				
Left carotid artery †			0.65	0.62		
Both carotid arteries †					0.67	0.67
Calcified plaques score						
Right carotid artery ‡	0.50	0.57				
Left carotid artery ‡			0.59	0.52		
Both carotid arteries ‡					0.57	0.60

† Adjusted for age, scanner type and ultrasound system

‡ Adjusted for age, scanner type, ultrasound system and total number of plaques

MSCT: multislice CT

For all correlations: p <0.001

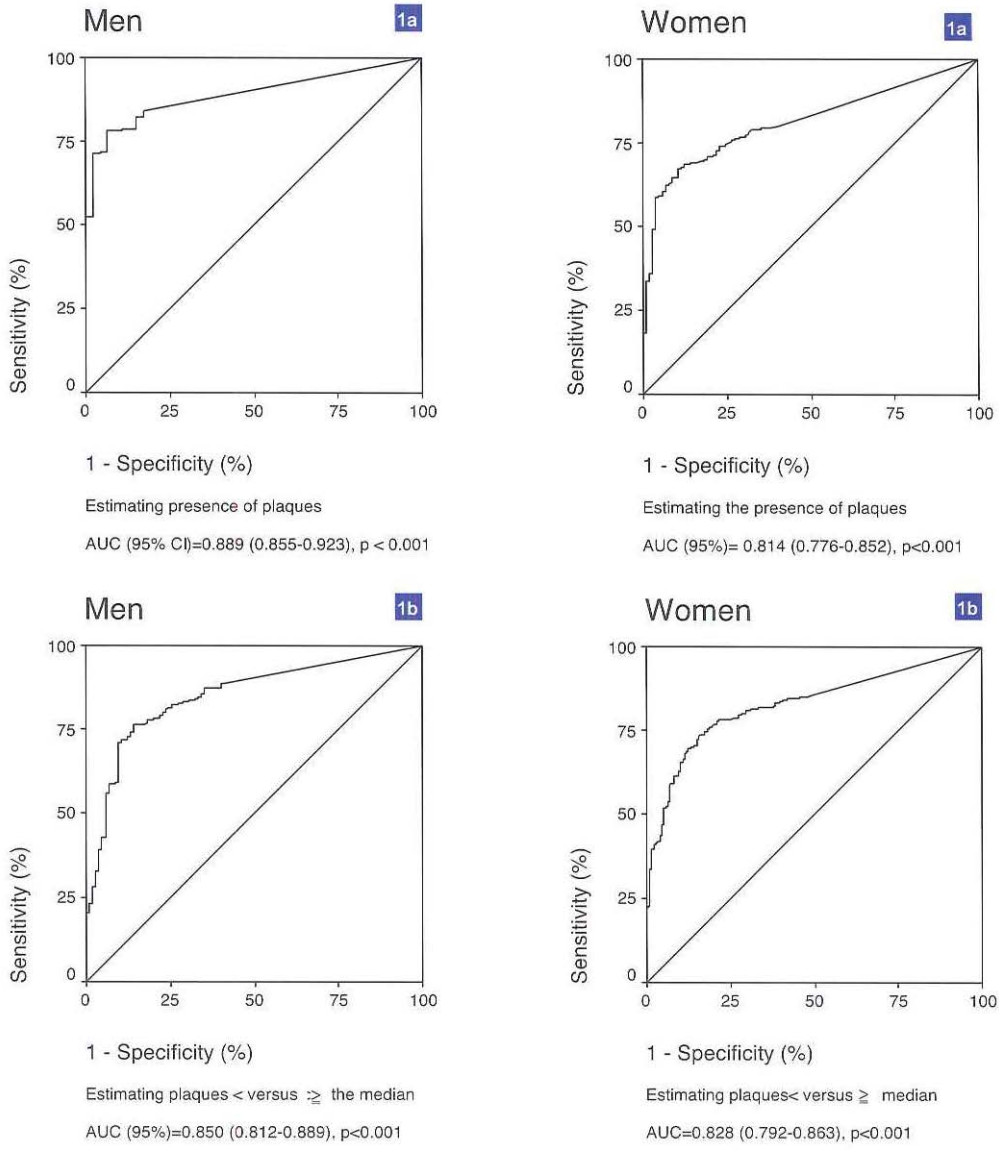


Figure 1 ROC curves for estimating plaques, with different cut points (fig 1a: no plaques versus any plaques; fig 1b: below the median versus equal or above the median)

Figure 3 shows the geometric mean MSCT calcium scores in categories of number of calcified plaques in both carotid arteries, stratified by age, and with additional adjustment for total number of plaques. The geometric mean MSCT calcium score in subjects without calcified carotid plaques was below 3. With increasing number of calcified plaques, the MSCT calcium score sharply increased.

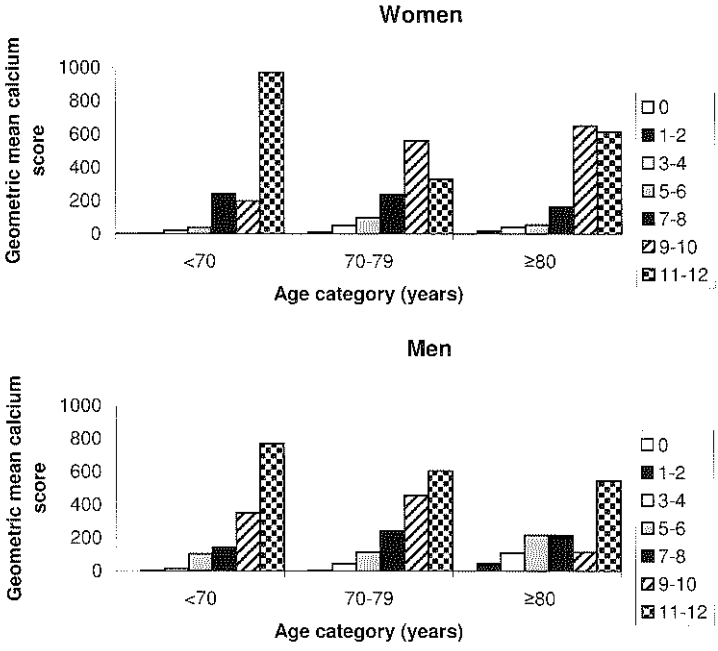


Figure 2 Geometric mean MSCT calcium score in the carotid arteries by number of carotid plaques, adjusted for age, gender, MSCT type and ultrasound system.

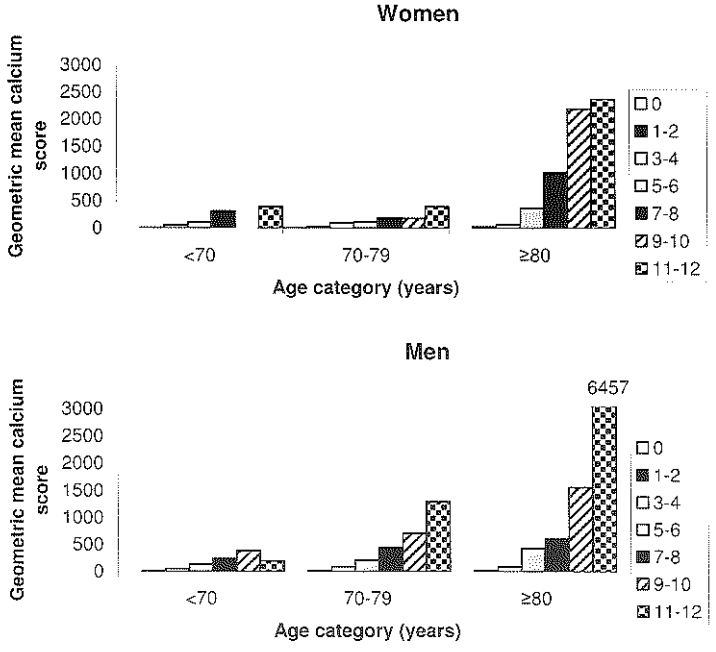


Figure 3 Geometric mean MSCT calcium score in the carotid arteries by number of calcified carotid plaques, adjusted for age, gender, MSCT type, ultrasound system, and total amount of plaques

Discussion

We found a strong association between carotid calcification assessed by MSCT and carotid plaques assessed by ultrasound. The amount of carotid plaques could be accurately estimated by the calcium score. This suggests that calcification assessed by MSCT is a good indicator of the presence of plaques in the carotid arteries. Furthermore, a strong association was present between carotid calcification assessed by MSCT and calcified plaques by ultrasound independent of the total amount of plaque, supporting the validity of the assessment of calcified plaques by ultrasound.

No previous study has examined the relation between MSCT calcium score and carotid plaques. However, the association between MSCT calcium score and carotid IMT has been examined before. Forty-five subjects underwent electron beam computed tomography (EBCT) of the neck to ascertain the extent of atherosclerotic calcification in the carotid arteries followed by B-mode carotid ultrasound for IMT. A strong correlation was found between the 2 different measures of carotid atherosclerosis [2]. In a histopathologic study, a strong correlation was found between coronary calcification and coronary plaque, indicating that calcification is a good measure of the amount of atherosclerosis in the coronary arteries [1]. Given our strong and graded associations between carotid calcification and carotid plaques, we can now pose that this is also true for the carotid artery.

Plaque echogenicity has been reported to be associated with cerebrovascular events [8]. In our study, in subjects without calcified plaques, a very low geometric mean calcium score was found. However, with increasing number of calcified plaques, the MSCT calcium score sharply increased. We found a moderately strong correlation between MSCT calcium score and carotid calcified plaques. Assuming that MSCT provides an accurate measure of calcification, these findings suggest that calcified plaques measured by ultrasound is a good indicator of presence of calcification.

There are some drawbacks of MSCT compared to ultrasound. Ultrasound of the carotid arteries is non-invasive, relatively inexpensive and frequently used. In contrast, MSCT is more expensive and ionizing radiation is used to visualize calcification. No data are yet available on the predictive value of carotid calcification measured by MSCT for the risk of cardiovascular events. Before deciding on the value of MSCT as compared to ultrasound for assessment of carotid atherosclerosis, large prospective studies should be performed to examine the predictive value of carotid calcification for cardiovascular events.

The advantage of our study is that it is large and population-based. Since both MSCT carotid calcification and carotid plaques were measured without knowledge of the other variable,

information bias is not likely to have influenced our results. However, some limitations of our study need to be discussed. A major limitation of our study is that we used carotid plaques assessed by ultrasound as the golden standard for our first research question. The measurement of carotid plaques was only semiquantative using the number of plaques, determined by eyeballing, as a measure of plaque burden. Probably, the assessment would have been stronger, if we would have used a more accurate assessment of carotid plaques. Furthermore, our subjects were elderly (mean age 72 years) and the results might have been different in a younger population, as the composition of atherosclerotic plaques change over time. Our study may be further limited by the fact that non-participation of diseased subjects may have resulted in a relatively healthy study population, with a more restricted range of variables studied, which may lead to an underestimation of the associations. We restricted the analysis to subjects with assessment of both measures of carotid atherosclerosis. Since the overall vascular risk profile was not much different between people with and without complete data on both measures, we think that this did not affect our results. Finally, the measurement of calcification assessed by MSCT and plaques assessed by ultrasound were not performed during the same session. However, since the median time between the measurements was only 96 days, it is not likely that this affected our results.

In conclusion, we observed a strong association between carotid calcification assessed by MSCT and carotid plaques assessed by ultrasound. The amount of carotid plaque could be accurately estimated by the calcium score. This suggests that calcification assessed by MSCT is a good measure of the presence of plaques in the carotid arteries.

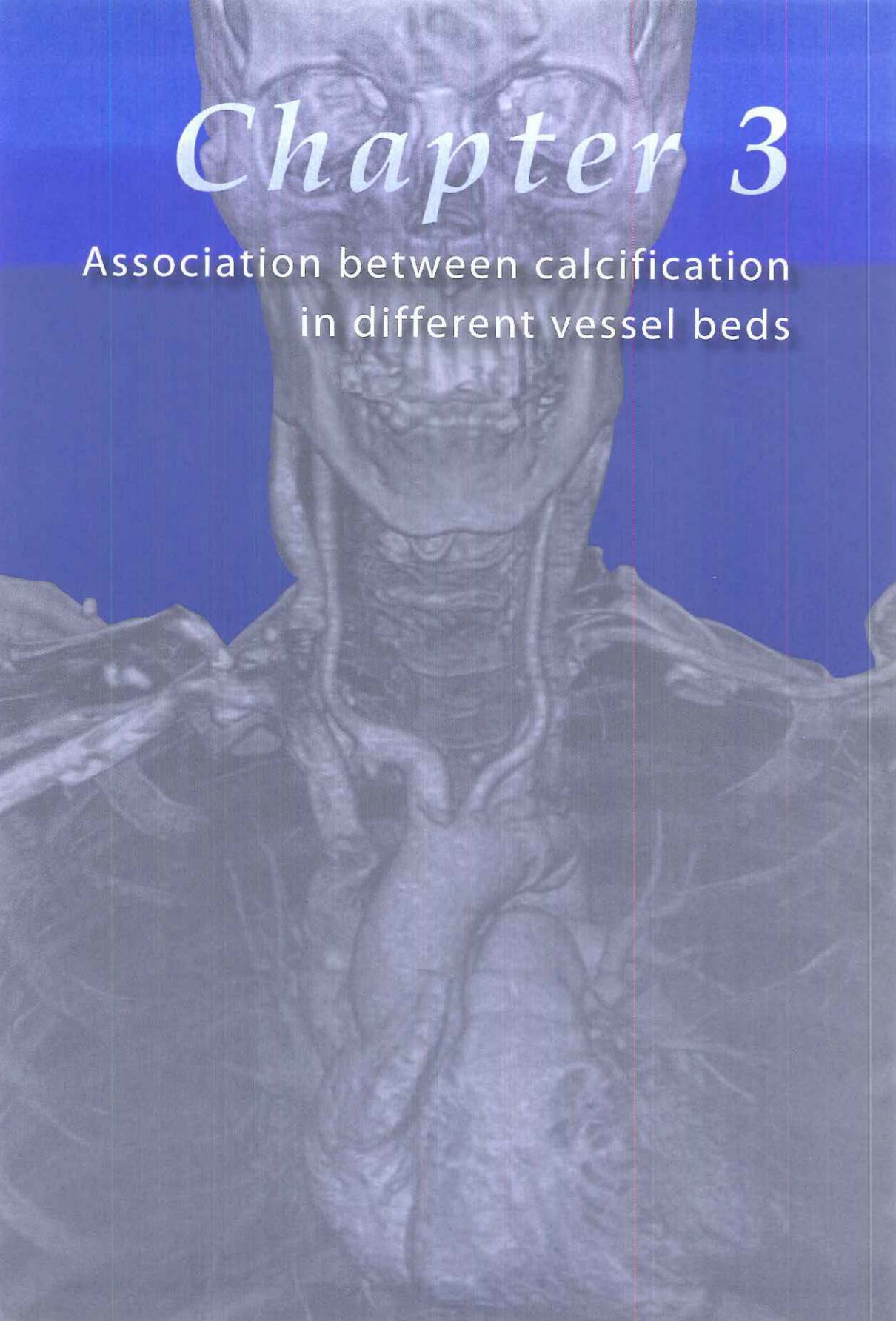
References

1. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.
2. Allison MA, Tiefenbrun J, Langer RD, Wright CM. Atherosclerotic calcification and intimal medial thickness of the carotid arteries. Int J Cardiol. 2005;103:98-104.
3. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
4. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.
5. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109:1089-1094.

6. Hollander M, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Breteler MM. Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study. J Neurol Neurosurg Psychiatry. 2003;74:317-321.

7. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993;39:561-577.

8. Mathiesen EB, Bonaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the tromso study. Circulation. 2001;103:2171-2175.



Chapter 3

Association between calcification in different vessel beds

3.1

Association between arterial calcification in different vessel beds, assessed by multislice CT

Abstract

Objective

The present study was performed to examine the prevalence of and associations between calcification in the coronary arteries, aortic arch and carotid arteries, assessed by multislice computed tomography (MSCT), in an elderly population.

Methods and Results

This study was part of the population-based Rotterdam Study. From October 2003 until July 2004, subjects underwent a 16-slice MSCT scan. Calcification was quantified by calculating the Agatston, volume, and mass score. Analyses were performed in 600 subjects (mean age 74 years). The prevalence of calcification in the coronary and carotid arteries was higher in men compared to women. However, aortic arch calcification was more prevalent among women. In men, multivariate adjusted correlation coefficients based on the Agatston score ranged from 0.40 (between coronary and aortic arch calcification) to 0.54 (between aortic arch and carotid calcification) ($p<0.001$). Multivariate adjusted correlation coefficients for women ranged from 0.30 (between coronary and aortic arch calcification) to 0.40 (between coronary and carotid calcification) ($p<0.001$).

Conclusions

While the prevalence of calcification in the coronary and the carotid arteries was higher in men compared to women, aortic arch calcification was more prevalent among women. Moderate to strong correlations between calcification in different vessel beds were found.

Introduction

Atherosclerosis is a generalized process and is the major cause of cardiovascular disease (CVD). Atherosclerotic lesions have typical histological and histochemical compositions at different stages of their natural history [1]. The more advanced lesions also contain calcium deposits. Since calcification in the coronary arteries is directly related to the severity and extent of underlying plaque burden, the amount of calcification can be used as a measure of atherosclerosis [2,3]. Most studies have focused on the presence of calcification in the coronary arteries. However, the use of multislice computed tomography (MSCT) enables non-invasive quantification of arterial calcification also in other vessels and allows examination of intervessel correlations. This may give insight in whether the assessment of calcification in one vessel bed predicts calcification in other vessels and allows examination of the concept of generalized atherosclerosis.

Data on the prevalence of calcification in different vessel beds and on correlations between calcification in these vessel beds are scarce. One study examined patterns of systemic calcified atherosclerosis [4]. With electron beam computed tomography (EBCT), this study evaluated in 650 subjects (mean age 57.6 years, 53% men) the extent of calcified atherosclerosis in the coronary arteries, the right and left carotid artery, the proximal aorta, the distal aorta and the iliac vessels. However, their study population was younger than ours and to our knowledge no similar study has been performed in an elderly population.

Therefore, in a large population-based study among older subjects, we examined the prevalence of calcification in the coronary arteries, aortic arch and the carotid arteries and the associations between calcification in these vessel beds.

Methods

Study population

The study is embedded in the Rotterdam Study, a population-based study, which started with a baseline visit in 1990-1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited and 7,983 agreed to participate. The design and rationale of the Rotterdam Study have been described elsewhere [5]. Study center visits took place approximately every three years.

From September 2003 onwards all participants who completed the fourth visit to the study center were invited to participate in the present study and to undergo a MSCT scan

of the heart, the aortic arch and the carotid arteries. Present analyses were restricted to the 600 participants who were scanned until July 2004. In 6 subjects the cardiac scan was not performed because they had a pacemaker. Additionally, 12 subjects had a history of previous stenting in the coronary arteries. Therefore, data on coronary calcification were available for 582 subjects. Due to severe artefacts in the image acquisition, the aortic arch of one subject could not be evaluated, hence data on calcification in the aortic arch were available for 599 subjects. In all subjects data on the calcification in the carotid arteries were available. The mean duration between the study center visit and the MSCT scan was 68 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

Imaging was performed with a 16-slice MSCT scanner (Somatom Sensation 16, Siemens, Forcheim, Germany). Two scans were performed: a cardiac scan and a scan which included the aortic arch and the carotid arteries. No contrast medium was used. The cardiac scan reached from the apex of the heart to the tracheal bifurcation. Before performing the cardiac scan, the participants exercised breath holding. Within a single breath hold consecutive non-overlapping 3-mm thick slices were acquired and reconstructed with 12×1.5 mm collimation, 120 kVp, effective 30 mAs, and prospective ECG triggering at 50% of the cardiac cycle. If during the exercise of the breath holding the heart rate was irregular or above 105 beats per minute, the cardiac scan was performed with 12×0.75 mm collimation, 150 effective mAs and retrospective ECG gating. For this scan, images were reconstructed at an optimal position within the cardiac cycle (with the least motion artefacts) with 3 mm effective slice width and 1.5-mm reconstruction interval. For both cardiac scans reconstructions were performed with 180 mm field-of-view (FOV) and medium sharp convolution kernel ("B35f"). The second scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Scan parameters were: 16×0.75 mm collimation, 120 kVp, 100 effective mAs, 0.5 s rotation time and normalized pitch of 1. Images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5 mm, 120 mm FOV and medium sharp convolution kernel ("B35f").

Analysis of calcification

Trained scan readers, who were blinded to the clinical data of the participants, performed the scoring of the calcifications. Three vessel beds were analyzed: the coronary arteries, the aortic arch and the carotid arteries. The examination of coronary arteries comprised the left main, left anterior descending, left circumflex and the right coronary artery. The examination of 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 examination of carotid arteries comprised both right and left carotid artery within 3 cm proximal and distal of the bifurcation.

Atherosclerotic calcifications were identified based on a threshold of 130 Hounsfield Units (HU) for all the applied scoring methods and the calcifications were scored using dedicated software (syngo Calcium Scoring, Siemens, Forcheim, Germany). Calcification was quantified by calculating the Agatston, volume, and mass scores. For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion (the number of voxels with an attenuation value ≥ 130 HU times the volume of one voxel) and a factor assigned according to the maximum attenuation value of the lesion [6]. For image data with an overlapping reconstruction increment, Agatston scores were normalized with the ratio of increment and slice width. The volume score (mm^3) for each lesion was calculated as the product of the area of a calcified lesion, the increment and an isotropic interpolation factor. The interpolation factor takes information from the adjacent slices into account and modifies the contribution of a single image voxel to the volume of an individual lesion [7]. The (calcium) mass score (mg CaHA) was calculated as the product of the area of a calcified lesion, the increment, the mean attenuation value of the lesion and a calibration factor [8].

The total score per vessel bed was calculated by adding up the scores of all lesions. This was performed separately for the 3 quantification methods.

Cardiovascular risk factors

Information on smoking, blood pressure and lipid lowering medication use was obtained during the home interview of the Rotterdam Study. Subjects were categorized as current, past and never smokers. Clinical measures were obtained during a visit at the study center. Height and weight were measured and the body mass index was calculated (weight[kg]/height [m]^2). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were obtained using an automatic enzymatic procedure (Hitachi 911, Roche CHOD PAP). Diabetes was defined as the use of antidiabetic medication or a fasting glucose level of ≥ 7 mmol/l[9]. Information on cardiovascular disease (myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass graft surgery (CABG) and stroke) was collected at baseline in 1990-1993 and during follow-up as described previously [10, 11]. The missing values of covariates were handled by single imputation using an expectation-maximization algorithm [12].

Statistical analysis

The prevalence of calcification within each age category (60-69, 70-79, ≥80) was calculated for all vessel beds. The distribution of calcium scores was examined for subjects in the age category 60 to 69 years, for men and women separately. Median calcium scores for each quantification method were computed per age category. Age-adjusted correlation coefficients were computed using Spearman's correlation coefficients. Additionally, we adjusted for cardiovascular risk factors. All analyses were performed for the three quantification methods. Data was analyzed using SPSS for Windows, release 11.0.

Results

Table 1 describes the characteristics of the 600 study participants. The mean age (± sd) of the study population was 74 years (4.8) and 52% were male.

Table 1 Characteristics of the study population

Variable	Men (n=314)	Women (n=286)
Age (years)	73.8 ± 5.3	73.9 ± 5.5
Body mass index (kg/m²)	27.0 ± 3.4	27.6 ± 4.7
Systolic blood pressure (mm Hg)	151 ± 21	154 ± 22
Diastolic blood pressure (mm Hg)	80 ± 11	79 ± 11
Total cholesterol (mmol/l)	5.3 ± 0.9	5.9 ± 0.9
HDL-cholesterol (mmol/l)	1.3 ± 0.3	1.5 ± 0.4
Serum glucose (mmol/l)	5.9 ± 1.3	5.7 ± 1.2
Diabetes mellitus (%)	14	12
Smokers (%)		
Current	11	9
Past	75	42
Never	14	49
Use of blood pressure lowering medication (%)	47	48
Use of lipid lowering medication (%)	26	24
History of myocardial infarction (%)	14	5
History of stroke (%)	5	4
Coronary Artery Bypass Graft (%)	7	1
Percutaneous Transluminal Coronary Angioplasty (%)	3	1

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

The prevalence of calcification increases with age throughout the vascular bed. In the eldest group, all men had calcifications in the coronary arteries and all women had calcifications in the aortic arch. At all ages, the prevalence of calcification in the coronary and the carotid arteries was higher in men (overall prevalence 89% and 83%, respectively) compared to women (overall prevalence 80% and 77%, respectively), although this difference decreased with age. In contrast, the prevalence of calcification in the aortic arch was

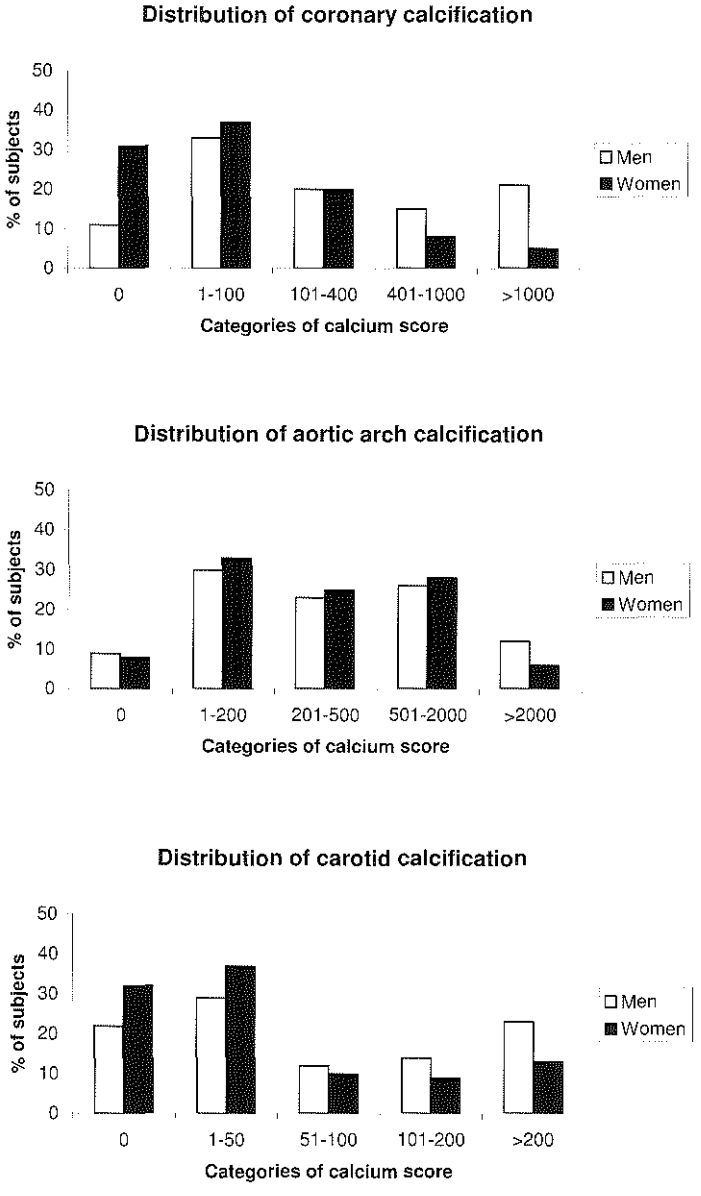


Figure 1 *The distribution of calcium scores in all vessel beds, in subjects aged 60 - 69*

somewhat higher in women (96%) compared to men (94%). Only 12 (2%) subjects were found to have no calcification in any vessel bed. Figure 1 displays the distribution of calcium scores in all vessel beds, in subjects aged 60-69. Although the prevalence of calcification in the aortic arch was higher among women, the calcium score in subjects with calcium was higher in men. For example, 12% of men but only 6% women had an aortic arch calcium score > 2000.

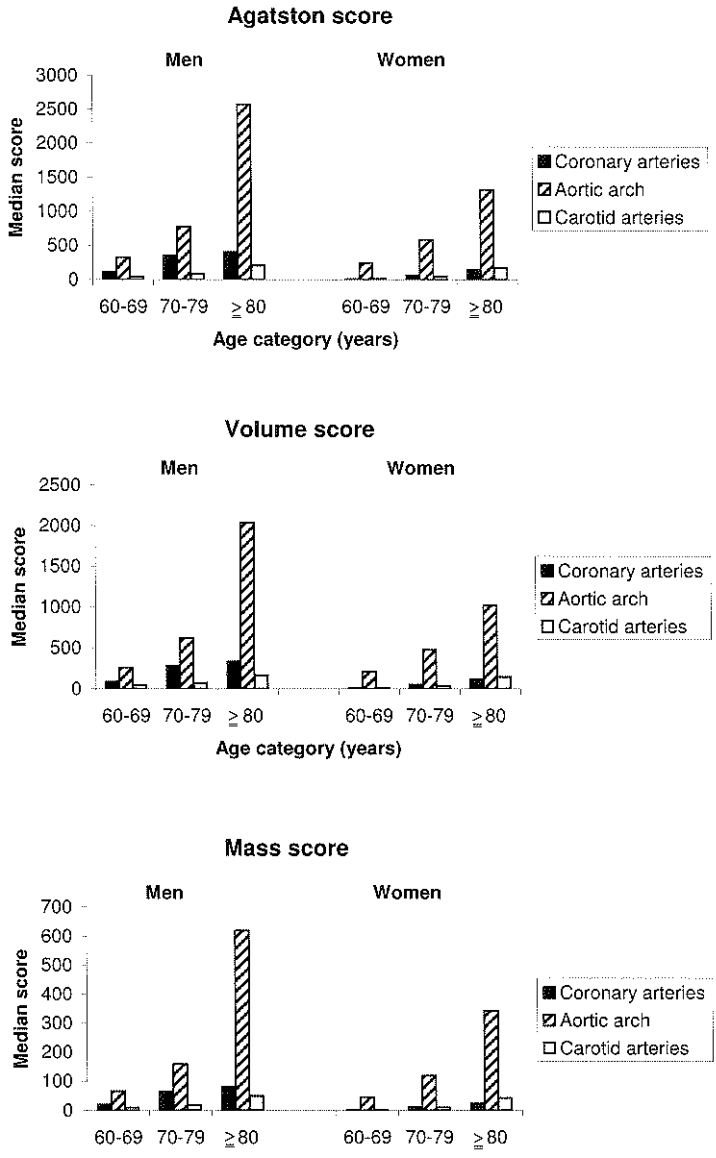


***Figure 2** Median calcium scores according to quantification method*

Figure 2 shows the median calcium scores per quantification method and vessel bed. Men had a higher median calcium score than women, regardless of age. The prevalence of calcification in the aortic arch was higher in women than in men.

Table 2 shows age-adjusted correlation coefficients for the intervessel comparisons for men and women, using the Agatston score. In men, the strongest correlation was found between calcification in the aortic arch and the carotid arteries (r=0.60). In women the relations were somewhat weaker, the strongest correlation was found between calcification in the coronary arteries and the carotid arteries (r=0.47). Additional adjustment for cardiovascular risk factors slightly attenuated the strength of the associations. We found similar trends for the volume and the mass score. By using the volume score, we found age-adjusted correlations between coronary arteries and the aortic arch, coronary arteries and carotid arteries and between aortic arch and carotid arteries in men of 0.50, 0.56 and 0.60, respectively; and in women of 0.34, 0.47 and 0.42, respectively. Corresponding correlation coefficients for the mass score were 0.50, 0.56 and 0.59 in men and 0.33, 0.47 and 0.41 in women.

Table 2 *Intervessel correlation coefficients*

	Coronary		Aortic arch		Carotid	
	Model 1*	Model 2†	Model 1*	Model 2†	Model 1*	Model 2†
Men						
Coronary	-	-	0.50	0.40	0.55	0.46
Aortic arch	0.50	0.40	-	-	0.60	0.54
Carotid	0.55	0.46	0.60	0.54	-	-
Women						
Coronary	-	-	0.34	0.30	0.47	0.40
Aortic arch	0.34	0.30	-	-	0.42	0.39
Carotid	0.47	0.40	0.42	0.39	-	-

$p < 0.001$ for all correlations.
All correlation coefficients are based on Agatston scores.
* *Adjusted for age.*
† *Additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, smoking, diabetes mellitus, total cholesterol, HDL-cholesterol, blood pressure lowering medication, lipid loweringmedication and history of cardiovascular disease.*

Discussion

The prevalence of calcification increases with age throughout the vascular bed. In the eldest group, all men had calcification in the coronary arteries and all women had calcification in the aortic arch. At all ages, the prevalence of calcification in the coronary and the carotid arteries was higher in men compared to women, although this difference decreased with age. In contrast, the prevalence of calcification in the aortic arch was higher in women than in men. Overall, moderate correlations were found in the intervessel bed comparisons, regardless of the quantification method.

One study [4] previously reported prevalences of vessel calcification among 650 subjects, who were self-referred or referred by their primary care provider. In this study, calcifications in the coronary arteries, the right and the left carotid artery, and in the proximal and the distal aorta were measured using EBCT. In the youngest age group (<50 years), calcification was most prevalent in the coronary arteries in both genders, while the lowest prevalences were found in the carotid arteries. In men aged 70 and over, the prevalence of calcification was most common in the proximal aorta and the coronary arteries (both 98%) and lowest in the carotid arteries (67-80%). In women aged 70 and over, the prevalence of calcification was highest in the proximal aorta (96%) and lowest in the carotid arteries (60-64%). The prevalence patterns in men and women are comparable to those of our study, although overall we had a higher prevalence of calcification. A study among postmenopausal women [13] showed a higher prevalence of aortic calcification compared to coronary calcification. We found equivalent prevalences in men and women for calcification in the aortic arch. However, in men the prevalence of calcification in the coronary arteries was higher compared to women. Although this pattern is confirmed by the results of Allison et al, explanations for these gender differences still need to be explored.

Several studies have examined associations between atherosclerosis in 2 vessel beds [13-20]. Two studies examined correlations in more vessel beds [4, 21]. In one study [21] based on 1,567 autopsy reports, atherosclerosis in the coronary arteries, the proximal aorta and the carotid arteries was examined. In men, the highest correlation was found between atherosclerosis in the aorta and in the carotid arteries (r=0.50) and in women the highest correlation was found between atherosclerosis in the aorta and in the coronary arteries (r=0.43). Allison et al found the highest correlations between the carotid arteries and the proximal aorta (men, r=0.38 (left carotid artery and the aorta) and r=0.42 (right carotid artery and the aorta), women, r=0.37 (right carotid artery and the aorta) and r=0.38 (left carotid artery and aorta). In men, we found stronger intervessel correlations compared to the results of the study by Allison et al, but also in our study, the strongest correlation was found between calcification in the aortic arch and the carotid arteries. Our results among

women differ from the two previous studies. However, we limited our measurement to the aortic arch, in contrast to the autopsy study by Sternby et al [21], in which the thoracic and abdominal aorta were assessed, and the study by Allison et al [4] in which calcification was scored from the aortic root until the diaphragm.

A limitation of our study is that we measured calcification, not plaque per se. Although calcification is not a direct measure of plaque, coronary calcification determined by EBCT has been correlated with the total area of coronary plaque [2]. Also, the presence of aortic calcification has been shown to indicate aortic atherosclerosis [22]. To the best of our knowledge, there is 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.

In conclusion, the prevalence of calcification was generally high and increased with increasing age. In the highest age group, all men had calcification in the coronary arteries and all women had calcification in the aortic arch. We found moderate to strong correlations between calcification in different vessel beds. This implies that assessment of the amount of calcification in one vessel bed is only a proxy and not an accurate measure of calcification in other vessels. However, our findings do support the concept of generalized atherosclerosis.

References

1. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol. 1995;15:1512-1531.
2. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.
3. Rifkin RD, Parisi AF, Folland E. Coronary calcification in the diagnosis of coronary artery disease. Am J Cardiol. 1979;44:141-147.
4. Allison MA, Criqui MH, Wright CM. Patterns and risk factors for systemic calcified atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24:331-336.
5. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
6. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.

7. Callister TQ, Cooil B, Raya SP, Lippolis NJ, Russo DJ, Raggi P. Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method. Radiology. 1998;208:807-814.

8. Ohnesorge B, Flohr T, Fischbach R, Kopp AF, Knez A, Schroder S, Schopf UJ, Crispin A, Klotz E, Reiser MF, Becker CR. Reproducibility of coronary calcium quantification in repeat examinations with retrospectively ECG-gated multisection spiral CT. Eur Radiol. 2002;12:1532-1540.

9. Standards of medical care in diabetes. Diabetes Care. 2005;28 Suppl 1:S4-S36.

10. Hollander M, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Breteler MM. Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study. J Neurol Neurosurg Psychiatry. 2003;74:317-321.

11. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109:1089-1094.

12. Dempster AP, Laird, N.M. and Rubin, D.B. Maximum Likelihood From Incomplete Data via the EM Algoritm. Journal of the Royal Statistical Society. 1977;Ser.B.:1-39.

13. Kuller LH, Matthews KA, Sutton-Tyrrell K, Edmundowicz D, Bunker CH. Coronary and aortic calcification among women 8 years after menopause and their premenopausal risk factors : the healthy women study. Arterioscler Thromb Vasc Biol. 1999;19:2189-2198.

14. Megnien JL, Sene V, Jeannin S, Hernigou A, Plainfosse MC, Merli I, Atger V, Moatti N, Levenson J, Simon A. Coronary calcification and its relation to extracoronary atherosclerosis in asymptomatic hypercholesterolemic men. The PCV METRA Group. Circulation. 1992;85:1799-1807.

15. Davis PH, Dawson JD, Mahoney LT, Lauer RM. Increased carotid intimal-medial thickness and coronary calcification are related in young and middle-aged adults. The Muscatine study. Circulation. 1999;100:838-842.

16. Seese B, Brandt-Pohlmann M, Moshage W, Achenbach S, Schwarz T, Bachmann K. Evaluation of the Association Between Coronary Calcification Detected by Electron Beam Computed Tomography and Atherosclerosis of Extracranial Carotid Arteries In Vivo. International Journal of Angiology. 1998;7:301-306.

17. Arad Y, Spadaro LA, Roth M, Scordo J, Goodman K, Sherman S, Lledo A, Lerner G, Guerci AD. Correlations between vascular calcification and atherosclerosis: a comparative electron beam CT study of the coronary and carotid arteries. J Comput Assist Tomogr. 1998;22:207-211.

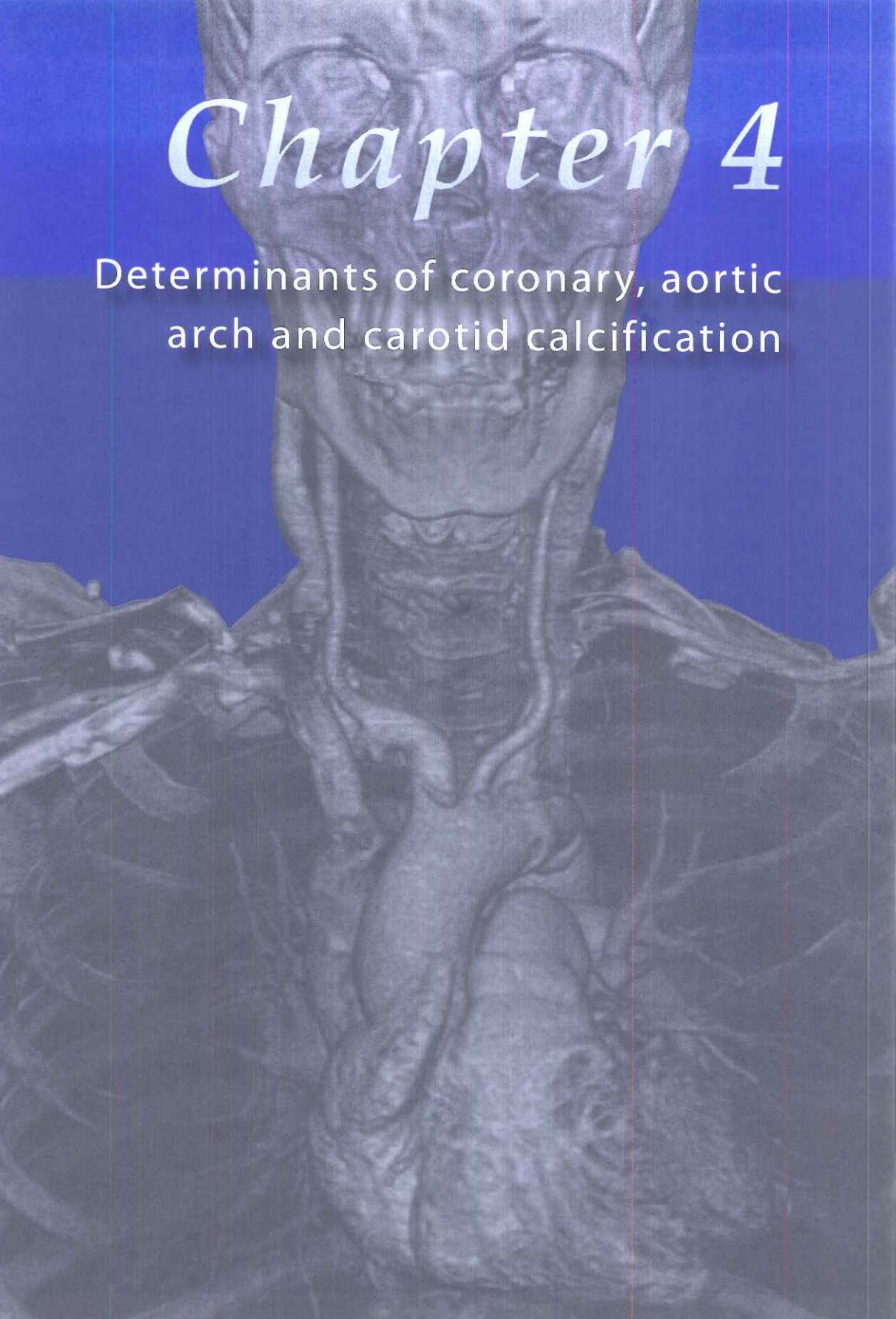
18. Newman AB, Naydeck BL, Sutton-Tyrrell K, Edmundowicz D, O'Leary D, Kronmal R, Burke GL, Kuller LH. Relationship between coronary artery calcification and other measures of subclinical cardiovascular disease in older adults. Arterioscler Thromb Vasc Biol. 2002;22:1674-1679.

19. Adler Y, Fisman EZ, Shemesh J, Schwammenthal E, Tanne D, Batavraham IR, Motro M, Tenenbaum A. Spiral computed tomography evidence of close correlation between coronary and thoracic aorta calcifications. Atherosclerosis. 2004;176:133-138.

20. Rohani M, Jogestrand T, Ekberg M, van der Linden J, Kallner G, Jussila R, Agewall S. Interrelation between the extent of atherosclerosis in the thoracic aorta, carotid intima-media thickness and the extent of coronary artery disease. Atherosclerosis. 2005;179:311-316.

21. Sternby NH. Atherosclerosis in a defined population. An autopsy survey in Malmo, Sweden. Acta Pathol Microbiol Scand. 1968;Suppl 194:195+.

22. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and post-mortem calcification of the aorta. Am Heart J. 1954;48:540-543.



Chapter 4

Determinants of coronary, aortic arch and carotid calcification

4.1

Risk factors for arterial calcification

Abstract

Background

This study was performed to examine the association of cardiovascular risk factors with calcification in the coronary arteries, aortic arch and carotid arteries, assessed by multislice computed tomography (MSCT).

Methods and results

This study was part of the Rotterdam Study, a population-based study in subjects aged 55 years and over. From October 2003 until December 2004, subjects were invited to undergo a MSCT scan. Coronary, aortic arch, and carotid calcification was quantified according to the Agatston score. Analyses were performed in the first 1003 subjects. Age and current smoking were the strongest independent risk factors for arterial calcification. Hypertension, hypercholesterolemia, and diabetes were also independently related to calcification, although not consistent across all vessel beds and for men and women. Obesity tended to be inversely related to arterial calcification in women, while low HDL-cholesterol showed no relation with arterial calcification.

Conclusion

Although associations were not completely consistent across the different vessel beds and for men and women, our results indicate that generally the same risk factors are present for atherosclerosis in the coronary, aortic arch, and carotid circulation.

Introduction

Atherosclerotic lesions have typical histological and histochemical compositions at different stages of their natural history [1]. More advanced atherosclerotic lesions contain calcification, although calcification may also be present in small amounts in the earlier stages of atherosclerosis [2]. Calcified lesions in the coronary arteries are correlated with the total area of coronary plaque, suggesting that calcification can be used as a measure of atherosclerosis [3].

Several studies examined the association between cardiovascular risk factors and the presence of arterial calcification [4-12]. Until now, only one study examined associations between cardiovascular risk factors and calcification in several vessel beds, including the coronary arteries, the proximal aorta, and the carotid arteries [10]. This study was performed among relatively young subjects.

We investigated the association of cardiovascular risk factors with calcification in the coronary arteries, aortic arch, and the carotid arteries in a population-based study among elderly subjects.

Methods

Study population

The study is embedded in the Rotterdam Study, a population-based study, which started in 1990-1993. All inhabitants aged 55 years and older and living in a suburb of Rotterdam were invited and 7,983 agreed to participate (78% response). In 2000-2001, the cohort was extended with 3,011 subjects (67% response) with the same inclusion criteria. The design and rationale of the Rotterdam Study have been described elsewhere [13]. Study center visits took place approximately every three years, during which cardiovascular risk factors were measured.

From September 2003 onwards, all participants who completed the center visit (the fourth for the original cohort and the second for the extended cohort) were invited to participate in the present study and to undergo a multislice computed tomography (MSCT) scan of the heart, the aortic arch and the carotid arteries. We restricted the present analyses to the first 1,003 participants who were scanned until December 2004.

This 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

In the first 708 subjects imaging was performed with a 16-slice MSCT scanner (SOMATOM Sensation 16, Siemens, Forcheim, Germany). Two scans were performed: a cardiac scan and a scan which included the aortic arch and the carotid arteries. The cardiac scan reached from the apex of the heart to the tracheal bifurcation. Before performing the cardiac scan, the participants exercised breath holding. Within a single breath hold consecutive non-overlapping 3-mm thick slices were acquired with 12 × 1.5 mm collimation, 120 kVp, effective 30 mAs, and prospective ECG triggering at 50% of the cardiac cycle. If during the exercise of the breath holding the heart rate was irregular or above 105 beats per minute, the cardiac scan was performed with 12 × 0.75 mm collimation, 150 effective mAs and retrospective ECG gating. For this scan, images were reconstructed at an optimal position within the cardiac cycle (defined at the position with the least motion artefacts) with 3 mm effective slice width and 1.5-mm reconstruction interval. For both cardiac scans reconstructions were performed with 180 mm field-of-view (FOV) and medium sharp convolution kernel ("B35f"). The second scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Scan parameters were: 16 × 0.75 mm collimation, 120kVp, 100 effective mAs, 0.5 s rotation time and normalized pitch of 1. Images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5 mm, 120 mm FOV and medium sharp convolution kernel ("B35f"). In another 295 subjects image acquisition was performed with a 64-slice MSCT scanner (SOMATOM Sensation 64, Siemens, Forcheim, Germany). Scan parameters were similar for both MSCT scanners, except for collimation and effective mAs. For the 64-slice MSCT scanner the collimation was 32 × 0.6 mm and the mAs value was real time adapted to body weight (CARE DOSE, Siemens, Forcheim, Germany) with a reference value of 50, 190, and 100 mAs for the prospectively triggered cardiac scan, retrospectively gated cardiac scan, and the aorta-carotid scan, respectively.

Analysis of calcification

Two reviewers, with a medical background, were trained by an experienced radiologist and scored arterial calcification using a standardized protocol. They were blinded to the clinical data of the participants. Three vessel beds were analyzed: the coronary arteries, the aortic arch and the carotid arteries. The examination of coronary arteries comprised the left main, left anterior descending, left circumflex, and the right coronary artery. The examination of 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 examination of 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. For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion (the number of voxels with an attenuation value ≥ 130 HU times the volume of one voxel) and a factor assigned according to the maximum attenuation value of the lesion [14]. For image data with an overlapping reconstruction increment, Agatston scores were normalized with the ratio of increment and slice width. The total score per vessel bed was calculated by adding up the scores of all lesions.

Cardiovascular risk factors

Information on smoking, blood pressure and lipid lowering medication use was obtained during a home interview of the Rotterdam Study. Subjects were categorized as current, past and never smokers. Clinical measurements were conducted during a visit at the study center. Height and weight were measured and the body mass index (BMI) was calculated (weight[kg]/height [m]^2). We defined obesity as a BMI ≥ 30 kg/m^2. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. We defined hypertension as a systolic blood pressure ≥ 160 mmHg and/or a diastolic blood pressure ≥ 100 mmHg [15] and/or the use of blood pressure lowering medication. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using an automatic enzymatic procedure (Hitachi 911, Roche CHOD PAP). We defined hypercholesterolemia as a serum total cholesterol ≥ 6.2 mmol/l [16] and/or the use of lipid reducing medication, low HDL-cholesterol was defined as a HDL-cholesterol < 1.0 mmol/l [16]. Diabetes was defined as the use of anti-diabetic medication and/or a fasting glucose level ≥ 7 mmol/l [17]. Information on cardiovascular disease (myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass graft surgery (CABG) and stroke was collected at baseline in 1990-1993 (original cohort) or at baseline in 2000-2001 (extended cohort) and during follow-up as described previously [18, 19]. The missing values of covariates were handled by single imputation using an expectation-maximization algorithm [20].

Population for analysis

In 10 subjects the cardiac scan was not performed because of the presence of a pacemaker. Additionally, 21 subjects had a history of previous stenting in the coronary arteries. Therefore, data on coronary calcification were available for 972 subjects. Due to severe artefacts in the image acquisition, the aortic arch of one subject could not be evaluated, hence data on calcification in the aortic arch were available for 1002 subjects. Data on calcification in the carotid arteries was available for all subjects. The median duration between the study center visit and the MSCT scan was 75 days.

Statistical analysis

We used logistic regression to determine the association between cardiovascular risk factors and arterial calcification. The highest quartile of calcification was compared to the lower 3 quartiles, for each vessel bed separately. Since calcium scores were substantially higher in men than in women, all analyses were stratified by gender. First, the regression analysis was only adjusted for age and scanner type (model 1). In model 2, additional adjustments were made for smoking status (never, current, past), obesity, hypertension, hypercholesterolemia, low HDL-cholesterol, diabetes mellitus, history of myocardial infarction, stroke, CABG and PTCA.

After exclusion of subjects with a history of cardiovascular disease, analysis of covariance was used to compute age-adjusted geometric mean calcium scores for the number of risk factors present (0, 1, 2, > 2). The risk factors included (1) obesity, (2) hypertension, (3) hypercholesterolemia, (4) low HDL-cholesterol, (5) current smoking and (6) diabetes. All analyses were adjusted for the type of MSCT scanner (16 versus 64). SPSS 11.0 for Windows (SPSS, Inc, Chicago, Illinois) was used for data analysis.

Results

Table 1 shows the characteristics of the study population. MSCT scans were obtained in 1003 subjects. The study population consisted for 48% of men. The mean age (± sd) of the study participants was 72 years (± 6.4).

Tables 2, 3 and 4 show the age-adjusted and the multivariate adjusted odds ratios for associations between cardiovascular risk factors and calcium scores (upper quartile versus lower 3 quartiles) in the different vessel beds.

Age and current smoking were the strongest independent risk factors. Both variables were independently associated with calcification in all vessel beds except for the coronary

Table 1 Characteristics of the study population

Variable	Men (n=485)	Women (n=518)
Age (years)	72.0 ± 6.2	71.2 ± 6.6
Body mass index (kg/m²)	27.2 ± 3.4	27.8 ± 4.5
Systolic blood pressure (mm Hg)	148.8 ± 20.4	150.4 ± 21.5
Diastolic blood pressure (mm Hg)	81.3 ± 11.1	79.7 ± 11.4
Total cholesterol (mmol/l)	5.3 ± 0.9	5.9 ± 0.9
HDL-cholesterol (mmol/l)	1.3 ± 0.3	1.5 ± 0.4
Fasting glucose (mmol/l)	5.8 ± 1.3	5.7 ± 1.3
Diabetes mellitus (%)	12	10
Smokers (%)		
Current	13	15
Past	73	43
Never	14	42
Use of blood pressure lowering medication (%)	45	48
Use of lipid lowering medication (%)	26	23
History of myocardial infarction (%)	12	5
History of stroke (%)	5	4
Coronary Artery Bypass Graft (%)	6	1
Percutaneous Transluminal Coronary Angioplasty (%)	3	1
Coronary artery Agatston score[1]	207.3 (32.6-728.8)	33.7 (0.2-189.2)
Aortic arch Agatston score[1]	513.6 (83.4-1607.7)	370.9 (78.7-1245.4)
Carotid artery Agatston score[1]	64.1 (2-234.8)	25.7 (0-128.6)

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation. [1] Value is expressed as median (interquartile range) because of its skewed distribution.

arteries in men. Past smoking was only associated with arterial calcification in women in the aortic arch, and in men in the carotid arteries. Hypertension, hypercholesterolemia, diabetes were significant or near significant risk factors for arterial calcification except for hypertension in women in the aortic arch and men in the carotid arteries, hypercholesterolemia for women in the aortic arch, and diabetes for men in the aortic arch and carotid arteries.

Table 2 Relation between putative risk factors and presence of severe calcification in the coronary arteries (upper quartiles versus lower three)

		(Exposed (%))	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Men				
	Variable			
	Age (year)		1.1 (1.0-1.1)[b]	1.0 (1.0-1.1)
	BMI≥ 30 kg/m²	92 (20)	1.4 (0.8-2.3)	1.0 (0.5-1.8)
	Hypertension	263 (57)	2.1 (1.3-3.4)[b]	1.6 (1.0-2.7)
	Hypercholesterolemia	191 (41)	2.6 (1.7-4.1)[c]	1.9 (1.2-3.1)[b]
	HDL<1.0 mmol/l	67 (14)	0.8 (0.4-1.5)	0.7 (0.4-1.4)
	Current smoking	59 (13)	1.1 (0.4-2.5)	1.2 (0.5-3.1)
	Past smoking	337 (72)	1.3 (0.7-2.5)	1.1 (0.6-2.3)
	Diabetes	57 (12)	2.5 (1.4-4.4)[b]	1.9 (1.0-3.6)[a]
Women				
	Variable			
	Age (year)		1.1 (1.1-1.1)[c]	1.1 (1.0-1.1)[c]
	BMI≥ 30 kg/m²	131 (26)	1.1 (0.7-1.7)	0.8 (0.4-1.3)
	Hypertension	268 (53)	2.6 (1.6-4.1)[c]	2.3 (1.4-3.7)[c]
	Hypercholesterolemia	276 (54)	1.7 (1.1-2.6)[a]	1.5 (1.0-2.3)
	HDL<1.0 mmol/l	34 (7)	1.3 (0.6-2.8)	0.8 (0.3-2.0)
	Current smoking	76 (15)	2.0 (1.1-3.6)[a]	1.8 (1.0-3.6)
	Past smoking	212 (42)	1.2 (0.8-1.9)	1.3 (0.8-2.0)
	Diabetes	52 (10)	2.0 (1.1-3.7)[b]	2.0 (1.0-4.0)[a]

Model 1: adjusted for age and scanner type.
Model 2: additionally adjusted for smoking status (never, current, past), obesity, hypertension, hypercholesterolemia, low HDL-cholesterol, diabetes mellitus, history of myocardial infarction, stroke, CABG and PTCA.
OR= odds ratio, CI =confidence interval, BMI= body mass index

In men, obesity was not related to calcification in the coronary arteries and carotid arteries, but a significant independent association was found with aortic arch calcification. In women, obesity tended to be inversely related to arterial calcification, which was significant for carotid calcification. Low HDL-cholesterol was not associated with arterial calcification in any of the vessel beds.

Table 3 Relation between putative risk factors and presence of severe calcification in the aortic arch (upper quartiles versus lower three)

			Model 1	Model 2
		(Exposed (%))	OR (95% CI)	OR (95% CI)
Men				
	Variable			
	Age		1.1 (1.1-1.2)[c]	1.2 (1.1-1.2)[c]
	BMI≥ 30 kg/m²	97 (20)	2.6 (1.5-4.5)[c]	2.5 (1.4-4.6)[a]
	Hypertension	273 (56)	2.5 (1.5-4.1)[c]	2.1 (1.2-3.5)[b]
	Hypercholesterolemia	208 (43)	2.4 (1.5-3.8)[c]	1.6 (1.0-2.7)
	HDL<1.0 (mmol/l)	70 (14)	0.8 (0.4-1.5)	0.6 (0.3-1.1)
	Current smoking	59 (12)	3.2 (1.3-7.9)[a]	4.7 (1.8-12.6)[c]
	Past smoking	354 (73)	1.6 (0.8-3.3)	1.4 (0.6-3.1)
	Diabetes	58 (12)	1.2 (0.6-2.3)	0.8 (0.4-1.6)
Women				
	Variable			
	Age		1.1 (1.1-1.2)[c]	1.1 (1.1-1.2)[c]
	BMI≥ 30 kg/m²	134 (26)	0.8 (0.5-1.2)	0.6 (0.4-1.1)
	Hypertension	277 (53)	1.4 (0.9-2.3)	1.4 (0.8-2.2)
	Hypercholesterolemia	286 (55)	1.2 (0.8-1.8)	1.0 (0.6-1.5)
	HDL<1.0 (mmol/l)	34 (7)	1.3 (0.6-2.9)	0.9 (0.4-2.4)
	Current smoking	81 (16)	3.8 (2.0-7.1)[c]	3.5 (1.8-6.8)[c]
	Past smoking	224 (43)	1.6 (1.0-2.7)	1.7 (1.0-2.8)[a]
	Diabetes	53 (10)	1.8 (1.0-3.4)	2.2 (1.3-4.9)[a]

Model 1: adjusted for age and scanner type.
Model 2: additionally adjusted for smoking status (never, current, past), obesity, hypertension, hypercholesterolemia, low HDL-cholesterol, diabetes mellitus, history of myocardial infarction, stroke, CABG and PTCA.
OR= odds ratio, CI =confidence interval, BMI= body mass index

Figure 1 shows the geometric mean calcium score according to the number of risk factors in asymptomatic subjects. In both men and women, the number of risk factors was strongly associated with the calcium score for all vessel beds. Compared to subjects without risk factors, calcium scores in all three vessel beds were significantly elevated in subjects with 2 or more than 2 risk factors, except for calcium scores in the aortic arch in

Table 4 Relation between putative risk factors and presence of severe calcification in the carotid arteries (upper quartiles versus lower three)

		(Exposed (%))	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Men				
	Variable			
	Age		1.1 (1.1-1.1)[c]	1.1 (1.1-1.2)[c]
	BMI≥ 30 kg/m^2	97 (20)	1.5 (0.9-2.6)	1.3 (0.8-2.4)
	Hypertension	274 (56)	1.3 (0.8-2.0)	1.0 (0.6-1.7)
	Hypercholesterolemia	209 (43)	1.9 (1.2-3.0)[b]	1.4 (0.9-2.3)
	HDL<1.0 (mmol/l)	70 (14)	0.9 (0.5-1.6)	0.8 (0.4-1.5)
	Current smoking	59 (12)	3.6 (1.3-8.9)[b]	4.1 (1.5-10.8)[b]
	Past smoking	355 (73)	2.6 (1.2-5.8)[a]	2.4 (1.1-5.4)[a]
	Diabetes	58 (12)	1.5 (0.8-2.8)	1.2 (0.6-2.4)
Women				
	Variable			
	Age		1.1 (1.1-1.1)[c]	1.1 (1.1-1.1)[c]
	BMI≥ 30 kg/m^2	134 (26)	0.7 (0.4-1.1)	0.5 (0.3-0.9)[b]
	Hypertension	277 (53)	2.2 (1.4-3.5)[c]	2.0 (1.2-3.3)[a]
	Hypercholesterolemia	286 (55)	2.8 (1.8-4.4)[c]	2.5 (1.6-4.1)[c]
	HDL<1.0 (mmol/l)	34 (7)	1.8 (0.8-3.8)	1.4 (0.6-3.4)
	Current smoking	81 (16)	2.6 (1.4-4.7)[c]	2.1 (1.1-4.1)[a]
	Past smoking	224 (43)	1.1 (0.7-1.8)	1.1 (0.7-1.9)
	Diabetes	53 (10)	1.8 (1.0-3.4)	2.1 (1.0-4.1)[a]

Model 1: adjusted for age and scanner type.
Model 2: additionally adjusted for smoking status (never, current, past), obesity, hypertension, hypercholesterolemia, low HDL-cholesterol, diabetes mellitus, history of myocardial infarction, stroke, CABG and PTCA.
OR= odds ratio, CI =confidence interval, BMI= body mass index

men with more than 2 risk factors. In women with 1 risk factor, calcium scores in the aortic arch and in the carotid arteries were significantly elevated compared to women without risk factors.

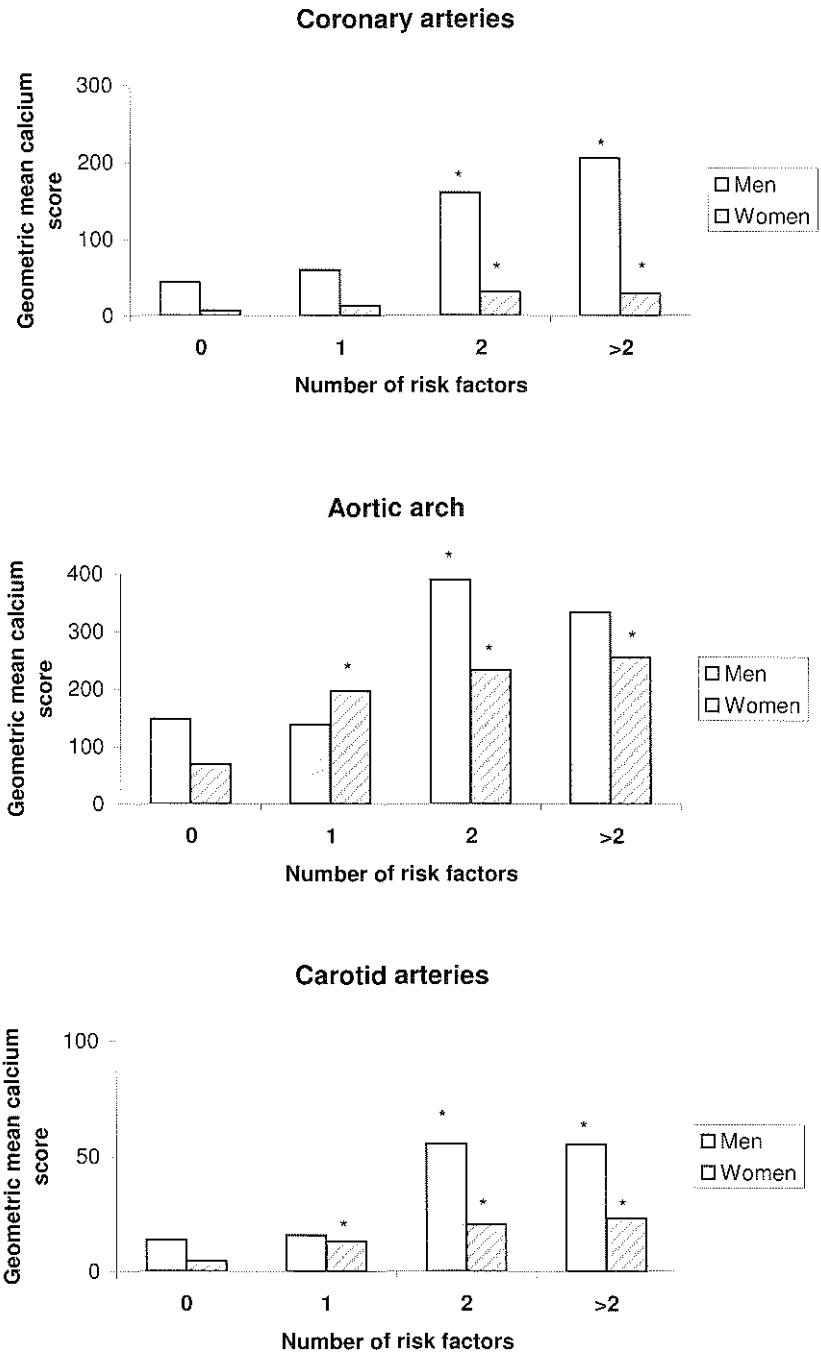


*Figure 1 Geometric mean calcium score according to the number of risk factors. * $p < 0.05$ with no risk factors as the reference category.*

Discussion

In this study, we examined the relation between cardiovascular risk factors and coronary, aortic arch and carotid calcification. Our results showed that current smoking, hypertension, hypercholesterolemia, and diabetes were independently related to arterial calcification, although associations were not consistent across all vessel beds and for men and women. A tendency was present for an inverse association with obesity in women, while no association with low HDL-cholesterol and arterial calcification was present.

Associations between cardiovascular risk factors and arterial calcification have been studied before [4-12]. The study population was relatively young in most studies, except for 2 of the studies [5, 7]. Most of these studies investigated the association between cardiovascular risk factors and the presence of calcification in one vessel bed only [4,5,7-9,11]. In postmenopausal women [12] and in asymptomatic patients who were self or physician-referred [6], the association of cardiovascular risk factors with coronary and aortic calcification was examined. In one study [10] among subjects who were self or physician-referred, cardiovascular risk factors were related to coronary, aortic, carotid and iliac calcification. The study population of this study consisted of 650 subjects with an average age of 57.6 years. To our knowledge, our study is the first that examined the association of cardiovascular risk factors with coronary, aortic arch and carotid calcification in a non-selected population of elderly subjects.

In the present study, we found that age and current smoking were the strongest risk factors for arterial calcification in all vessel beds, except for coronary calcification in men. Possibly men who smoke are prone to die from coronary heart disease at an earlier age due to selective mortality. Other studies show independent associations of smoking with coronary, aortic arch and carotid calcification in generally younger populations [4,5,7-9,11].

Hypertension, hypercholesterolemia and diabetes were independent risk factors, although associations were not consistent across all vessel beds and for men and women. Other studies found independent associations between blood pressure variables and coronary calcification [4, 8, 10, 11], aortic arch calcification [11] and carotid calcification [10]. A study by Hoff et al [4] among 30,908 asymptomatic individuals showed an independent association of hypercholesterolemia with coronary calcification in both men and women. A study among family members of cardiac patients [9] found an independent association between statin use and coronary calcification in both genders and an independent association between total cholesterol and coronary calcification in women. Iribarren et al [11] found an independent association between serum cholesterol above 6.6 mmol/l and aortic arch calcification only in women. A study by Allison et al [10] examining risk factors

for coronary, proximal aorta and carotid calcification, found age-adjusted associations in both genders between hypercholesterolemia and arterial calcification in all vessels, except for coronary calcification in men, but all the associations lost significance after multivariate adjustment. In summary, relations between cholesterol and calcification in studies were not consistent across vessel beds and across men and women, but generally the association was present.

Data on diabetes and its relation to arterial calcification are inconsistent. A study by Hoff et al [4] showed an association of diabetes with coronary calcification in both men and women, Reilly et al [9] found an association between fasting glucose and coronary calcification in women only, while no association of fasting glucose with both coronary and aortic calcification was found in a study among postmenopausal women [12]. Iribarren et al did not find an association between diabetes and aortic arch calcification in both men and women [11]. Allison et al [10] examined the association between diabetes and calcification in several arteries, and only found a univariate association with calcification in the proximal aorta in men.

Obesity in men was not related to arterial calcification, except for aortic arch calcification. In women, a tendency was found for an inverse association, however, this was only significant for carotid calcification. An inverse association in women, but not in men may be related to estrogen production in fat mass in women after menopause [21]. Results from the literature regarding the association between obesity and calcification are inconsistent. Irrespective of gender, some studies found an association of body mass index with coronary calcification [9, 10] and one study found a high BMI to be inversely associated with aortic calcification [11]. However, other studies found no association with coronary calcification [8], aortic calcification [10] and carotid calcification [10] in both genders.

We found no clear association of low-HDL with calcification for all vessel beds. It is possible that this is due to small numbers because only a small percentage of our subjects had a low HDL-cholesterol. Data on the relation between HDL-cholesterol and arterial calcification are not only scarce but also contradictory. Reilly et al, in a study among family members of cardiac patients, found an age-adjusted association of low HDL with coronary calcification in both men and women [9], Oei et al [5] found an independent association with coronary calcification only in women, while Allison et al [8] found an independent relation between HDL-cholesterol and coronary calcification in men and women. In a study of Kuller et al [12], an independent association of HDL-cholesterol with coronary calcification but not with aortic calcification was observed.

The advantages of our study are its large population and the inclusion of three vessel beds measured by the same diagnostic tool. Since calcification was measured without knowledge of risk factor status, information bias is not likely to have influenced our results. However, some limitations of our study need to be discussed. In our study, we measured calcification, not plaque per se. Although calcification is not a direct measure of plaque, coronary calcification determined by electron beam computed tomography (EBCT) has been correlated with the total area of coronary plaque [3]. Also, the presence of aortic calcification on plain radiographs has been shown to indicate aortic atherosclerosis [22]. To the best of our knowledge, there is 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. Our study may be further limited by the fact that non-participation of diseased subjects may have resulted in a relatively healthy study population, with a more restricted range of variables studied, which may lead to an underestimation of the associations. Finally, our cut-off points for high calcification depend on the distribution of calcification per vessel bed and gender and may be different in other populations.

In conclusion, our results showed that current smoking, hypertension, hypercholesterolemia, and diabetes were independently related to arterial calcification, although associations were not consistent across all vessel beds and for men and women. A tendency was present for an inverse association with obesity in women, while no association between low HDL-cholesterol and arterial calcification was present.

References

1. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol. 1995;15:1512-1531.
2. Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J. 1990;11 Suppl E:3-19.
3. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.
4. Hoff JA, Daviglus ML, Chomka EV, Krainik AJ, Sevrukov A, Kondos GT. Conventional coronary artery disease risk factors and coronary artery calcium detected by electron beam tomography in 30,908 healthy individuals. Ann Epidemiol. 2003;13:163-169.

5. Oei HH, Vliegenthart R, Hofman A, Oudkerk M, Witteman JC. Risk factors for coronary calcification in older subjects. The Rotterdam Coronary Calcification Study. Eur Heart J. 2004;25:48-55.

6. Wong ND, Sciammarella M, Arad Y, Miranda-Peats R, Polk D, Hachamovich R, Friedman J, Hayes S, Daniell A, Berman DS. Relation of thoracic aortic and aortic valve calcium to coronary artery calcium and risk assessment. Am J Cardiol. 2003;92:951-955.

7. Newman AB, Naydeck BL, Sutton-Tyrrell K, Feldman A, Edmundowicz D, Kuller LH. Coronary artery calcification in older adults to age 99: prevalence and risk factors. Circulation. 2001;104:2679-2684.

8. Allison MA, Wright CM. Age and gender are the strongest clinical correlates of prevalent coronary calcification (R1). Int J Cardiol. 2005;98:325-330.

9. Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular risk factors: impact of the analytic approach. Atherosclerosis. 2004;173:69-78.

10. Allison MA, Criqui MH, Wright CM. Patterns and risk factors for systemic calcified atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24:331-336.

11. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama. 2000;283:2810-2815.

12. Kuller LH, Matthews KA, Sutton-Tyrrell K, Edmundowicz D, Bunker CH. Coronary and aortic calcification among women 8 years after menopause and their premenopausal risk factors : the healthy women study. Arterioscler Thromb Vasc Biol. 1999;19:2189-2198.

13. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.

14. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.

15. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens. 2003;21:1983-1992.

16. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama. 2001;285:2486-2497.

17. Standards of medical care in diabetes. Diabetes Care. 2005;28 Suppl 1:S4-S36.

18. Hollander M, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Breteler MM. Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study. J Neurol Neurosurg Psychiatry. 2003;74:317-321.

19. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109:1089-1094.

20. Dempster AP, Laird, N.M. and Rubin, D.B. Maximum Likelihood From Incomplete Data via the EM Algoritm. Journal of the Royal Statistical Society. 1977;Ser.B.:1-39.

21. Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson HE, Jr., Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB,

Miller SR. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst. 2003;95:1218-1226.

22. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and post-mortem calcification of the aorta. Am Heart J. 1954;48:540-543.

4.2

Alcohol consumption and carotid calcification

Abstract

Background

Some studies have shown a J-shaped relation between alcohol consumption and atherosclerosis. However, few data are present for the association between alcohol consumption and carotid atherosclerosis. With the use of multislice computed tomography (MSCT) carotid calcification can be measured non-invasively and used as a measure of carotid atherosclerosis.

Objective

This study was performed to examine the association between alcohol consumption and carotid calcification assessed by MSCT in an elderly population.

Methods

This study was part of the Rotterdam Study, a population-based study in subjects aged 55 years and over. From October 2003 until December 2004 subjects were invited to undergo an MSCT scan. Carotid calcification was quantified according to the Agatston score. Analyses were performed among subjects without a history of cardiovascular disease (mean age 71 years, standard deviation 6.4, 45% men). Alcohol consumption was categorized in 3 types of alcoholic beverages (beer, wine, and liquor) and expressed in units per day. Age-adjusted and multivariate adjusted logistic regression was performed to determine whether alcohol consumption was associated with carotid calcification.

Results

For wine consumption, a trend towards a decreasing risk of carotid calcification with increasing consumption was found, but risk estimates lacked statistical significance. Beer and liquor consumption of more than 2 drinks a day were associated with increased, but non-significant, risks of carotid calcification.

Conclusion

In this study on risk of carotid calcification, opposite trends were found for wine drinking (inverse) and beer and liquor drinking (positive).

Introduction

Several studies have examined the association between alcohol consumption and atherosclerosis. Some studies have shown that light-to-moderate alcohol consumption is inversely associated with atherosclerosis [1-3]. Protection of the development of atherosclerosis through alcohol consumption is partly due to increasing high density lipoprotein cholesterol (HDL-cholesterol) and decreasing low density lipoprotein cholesterol (LDL cholesterol) [4,5].

Atherosclerotic lesions have typical histological and histochemical compositions at different stages of their natural history [6]. More advanced atherosclerotic lesions contain calcification, although calcification may also be present in small amounts in the earlier stages of atherosclerosis [7]. Since calcification in the coronary arteries is directly related to the severity and extent of underlying plaque burden, the amount of calcification can be used as a measure of atherosclerosis [8]. The development of electron beam computed tomography (EBCT) and multislice computed tomography (MSCT) scan has enabled the possibility to visualize and quantify the amount of arterial calcification. Data on the association of alcohol consumption with arterial calcification [9-11] are scarce and until now there are no data on the relation between alcohol consumption and carotid calcification.

We undertook this study to investigate the relation between alcohol consumption and carotid artery calcification detected by MSCT in an elderly population.

Methods

Study population

This study is embedded in the Rotterdam Study, a population-based study, which started with a baseline visit in 1990-1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited and 7,983 agreed to participate (78% response). In 2000-2001, the cohort was extended with 3,011 subjects (67% response), also aged 55 years and over. The design and rationale of the Rotterdam Study have been described elsewhere [12]. Study center visits took place approximately every three years.

From September 2003 onwards, all participants who completed the center visit (the fourth for the original cohort and the second center visit of the extended cohort) were invited to participate in the present study and to undergo a MSCT scan of the carotid arteries. Until December 2004, we scanned 815 participants without cardiovascular disease and complete data on alcohol consumption. In all subjects data on calcification

in the carotid arteries was available. The mean duration between the study center visit and the MSCT scan was 88 days. Subjects in whom cardiovascular disease was present at the time of scanning, were excluded from the analysis. This 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

In the first 563 subjects imaging was performed with a 16-slice MSCT scanner (SOMATOM Sensation 16, Siemens, Forcheim, Germany). The scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Scan parameters were: 16 × 0.75 mm collimation, 120 kVp, 100 effective mAs, 0.5 s rotation time and normalized pitch of 1. Images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5 mm, 120 mm FOV and medium sharp convolution kernel ("B35f"). In the other 252 subjects image acquisition was performed with a 64-slice MSCT scanner (SOMATOM Sensation 64, Siemens, Forcheim, Germany). Scan parameters were similar for both MSCT scanners, except for collimation and effective mAs. For the use of the 64-slice MSCT scanner the collimation was 32 × 0.6 mm and the mAs value was real time adapted to body weight (CARE DOSE, Siemens, Forcheim, Germany) with a reference value of 100 mAs.

Analysis of calcification

Two reviewers, with a medical background, were trained by an experienced radiologist and scored arterial calcification using a standardized protocol. They were blinded to the clinical data of the participants. The examination of carotid arteries comprised both right and left carotid artery within 3 cm proximal and distal of the bifurcation.

Atherosclerotic calcifications were identified based on threshold of 130 Hounsfield Units (HU) for all the applied scoring methods and the calcifications were scored using dedicated software (syngo Calcium Scoring, Siemens, Forcheim, Germany). Calcification was quantified by calculating the Agatston score. For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion (the number of voxels with an attenuation value ≥ 130 HU times the volume of one voxel) and a factor assigned according to the maximum attenuation value of the lesion [13]. Due to an overlapping reconstruction increment, Agatston scores were normalized with the ratio of increment and slice width. The total score per vessel bed was calculated by adding up the scores of all lesions.

Risk factors and medical history

Information on smoking, blood pressure, and lipid lowering medication use was obtained during a home interview of the Rotterdam Study. Subjects were categorized as current, past, and never smokers. Clinical measures were obtained during a visit at the study center. Height and weight were measured and body mass index was calculated (weight[kg]/ height [m]^2). We defined overweight as BMI $\geq$ 30 kg/m^2. Diabetes mellitus was defined as the use of anti-diabetic medication and/or a fasting glucose level of $\geq$ 7 mmol/l [14]. We used information about the highest attained level of education as a proxy of social economic status. Education was categorized into low (primary education), intermediate (secondary general and vocational education), and higher (higher vocational education or university).

Information on cardiovascular disease (myocardial infarction (MI), percutaneous transluminal coronary angiography (PTCA), coronary artery bypass graft surgery (CABG)), and stroke was collected at baseline in 1990-1993 (original cohort) or at baseline in 2000-2001 (extended cohort) and during follow-up as described previously [15,16]. Subjects in whom cardiovascular disease was present at the time of scanning, were excluded from the analysis. The missing values of covariates were handled by single imputation using an expectation-maximization algorithm[17].

Alcohol consumption

Alcohol consumption was assessed as part of the home interview prior to the study center visit. Participants were asked for their weekly alcohol drinking habits. Alcohol consumption was reported in 4 categories: beer, wine, moderately strong alcoholic beverages (like port and sherry), and liquor. The moderately strong alcoholic beverages contain predominantly fortified wines and were grouped together with the wine group. Non-drinkers were asked whether they had been alcohol consumers in the past. By adding the number of drinks of specific alcoholic beverages consumed per week and dividing by 7, the total daily consumption of alcoholic beverages was computed. Both the total alcohol consumption and the consumption of specific alcoholic beverages were divided into 4 categories of daily alcohol consumption: 0 drinks, up to 1 drink, 1 to 2 drinks and more than 2 drinks per day.

Statistical analysis

Logistic regression analysis was used to determine whether daily consumption of alcohol was associated with the highest quartile of carotid calcium. Since calcium scores were much higher in men than in women, gender specific quartiles were used. Initially, the regression analysis was adjusted for age, gender and scanner type (model 1). In model 2, additional adjustments were made for smoking (never, current, former), overweight, diabetes mellitus and educational level (low, intermediate, higher). Furthermore, the two

logistic regression models were performed for the different alcoholic beverages separately, with additional adjustment for total alcohol consumption. Both regression models were also performed after exclusion of former drinkers. Analysis of covariance was used to compute age-adjusted geometric mean calcium scores for categories of total alcohol consumption. The distribution of daily alcohol consumption was calculated for categories of total alcohol consumption and for each alcoholic type of beverage separately in men and in women. All analyses were adjusted for the type of MSCT scanner (16 versus 64). SPSS 11.0 for Windows (SPSS, Inc, Chicago, Illinois) was used for data analysis.

Results

Table 1 shows the characteristics of the study population. The study population consisted for 48% of men. The mean age (± sd) of the study participants was 71 years (± 6.4). Drinkers comprised 89 % of the population. The percentage of beer, wine and liquor drinkers of the total population were respectively 42, 84, and 38. Overall, the calcium scores were higher in men than in women. The median calcium score (interquartile range) in the carotid arteries was 49.8 (0-188.9) in men and 20.8 (0-107.6) in women. Among the men and women who did not drink alcohol at the time of the home interview, 64% (n=14) and 36% (n=25), respectively, had consumed alcohol in the past.

Figure 1 shows the distribution of total alcohol consumption and the consumption of specific beverages.

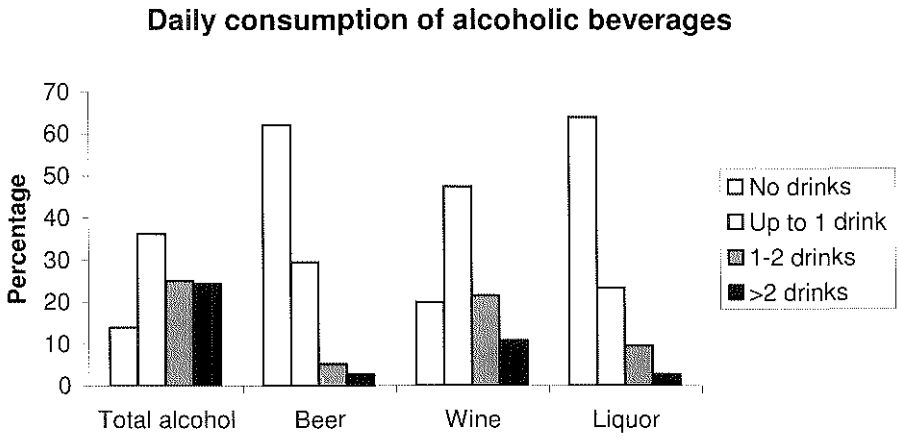


Figure 1 *Distribution of consumption of alcoholic beverages*

Table 1 Characteristics of the study population

Variable	n=815
Age (years)	71.2 ± 6.4
Men (%)	45
Body mass index (kg/m²)	27.5 ± 4.0
Systolic blood pressure (mm Hg)	149.7 ± 20.7
Diastolic blood pressure (mm Hg)	81.1 ± 11.0
Total cholesterol (mmol/l)	5.8 ± 0.9
HDL-cholesterol (mmol/l)	1.4 ± 0.4
Serum glucose (mmol/l)	5.7 ± 1.2
DM (%)	10
Smokers (%)	
Current	12
Past	56
Never	32
Use of blood pressure lowering medication (%)	39
Use of lipid lowering medication (%)	18
Education level (%)	
Low	20
Intermediate	60
Higher	20
Alcohol drinkers (%)	89
Daily intake of total alcohol in drinkers (glasses per day)[1]	1.07 (0.29-2.14)
Beer drinkers (%)	42
Daily intake of beer in drinkers (glasses per day)[1]	0.14 (0.03-0.57)
Wine drinkers (%)	84
Daily intake of wine in drinkers (glasses per day)[1]	0.57 (0.14-1.29)
Liquor drinkers (%)	38
Daily intake of liquor in drinkers (glasses per day)[1]	0.43 (0.07-1.00)

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation. [1] Value is expressed as median (interquartile range) because of its skewed distribution.

Table 2 presents the age-adjusted and the multivariate-adjusted odds ratios (OR) for risk of carotid calcification, associated with daily consumption of alcohol. A tendency for a J-shaped association was seen, but estimates lacked statistical significance.

Figure 2 shows the age and gender-adjusted geometric mean calcium score in categories of total alcohol consumption. We found the highest geometric mean calcium score in subjects in the highest alcohol consumption category but differences were not significant.

Table 2 *Risk of carotid calcification according to daily total alcohol consumption*

	n	OR[1] (95% CI)	OR[2] (95% CI)
Total alcohol consumption			
No drinks	114	1.0 (reference)	1.0 (reference)
Up to 1 drink	295	0.8 (0.5-1.4)	0.8 (0.5-1.4)
1-2 drinks	206	1.0 (0.6-1.7)	1.0 (0.6-1.8)
>2 drinks	200	1.5 (0.9-2.6)	1.5 (0.8-2.6)

[1] adjusted for age, gender and scanner type, [2] additionally adjusted for overweight, diabetes mellitus, smoking status and educational level.

OR= odds ratio, CI =confidence interval

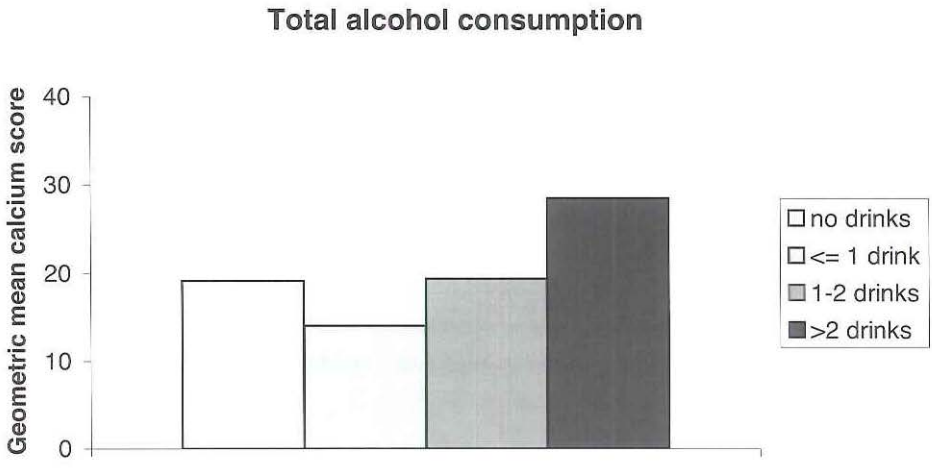


Figure 2 *Geometric mean calcium score by daily consumption of total alcohol.*

Table 3 presents the age-adjusted and multivariate adjusted ORs for the risk of carotid calcification, associated with the use of specific alcoholic beverages. For wine consumption, a trend towards a decreasing risk with increasing consumption was found, but risk estimates lacked statistical significance. Beer and liquor consumption were associated with increased, but non-significant, risks for the highest category of alcohol consumption. After exclusion of former drinkers, results were similar (data not shown).

Table 3 *Risk of carotid calcification according to level of daily alcoholic consumption for different alcoholic beverages*

	n	OR[1] (95% CI)	OR[2] (95% CI)
Beer			
No drinks	507	1.0 (reference)	1.0 (reference)
Up to 1 drink	241	0.8 (0.5-1.2)	0.8 (0.5-1.2)
1-2 drinks	43	1.3 (0.6-2.9)	1.5 (0.6-3.0)
>2 drinks	24	2.1 (0.8-5.6)	2.3 (0.8-6.2)
Wine			
No drinks	163	1.0 (reference)	1.0 (reference)
Up to 1 drink	387	0.8 (0.4-1.7)	0.8 (0.4-1.7)
1-2 drinks	176	0.6 (0.3-1.4)	0.6 (0.3-1.3)
>2 drinks	89	0.7 (0.3-1.7)	0.6 (0.3-1.6)
Liquor			
No drinks	522	1.0 (reference)	1.0 (reference)
Up to 1 drink	191	0.6 (0.4-1.0)	0.6 (0.4-1.0)
1-2 drinks	79	1.1 (0.6-2.1)	1.2 (0.6-2.2)
>2 drinks	23	2.6 (1.0-6.8)	2.4 (0.9-6.5)

[1] adjusted for age, gender, scanner type and total alcohol intake, [2]additionally adjusted for overweight, diabetes mellitus, smoking status and educational level.

OR= odds ratio, CI =confidence interval

Discussion

In this study on risk of carotid calcification, opposite trends were found for wine drinking (inverse) and beer and liquor drinking (positive). Association, however, lacked statistical significance.

Several studies examined the association between alcohol consumption and carotid atherosclerosis, two studies reported positive associations in men [18, 19] and one study found a positive association in women [20]. Some studies reported a J-shaped association [2, 3]. Kiechl et al examined the association of alcohol intake with 5-year progression of carotid atherosclerosis, defined as the occurrence of new plaques and/or the development on vessel stenosis, and found a J-shaped relation in both men and women [2]. In the Brunek study, a J-shaped relation was found between alcohol consumption and carotid intima-media thickness (IMT) [3]. In the Cardiovascular Health Study [1], the association of weekly alcohol consumption with carotid IMT was examined. Drinking 1-6 units per week, was inversely associated with IMT and drinking more than 14 units per week was positively associated with IMT, indicating a J-shaped association.

Until now, no study examined the association of alcohol consumption with carotid calcification. Three studies [9-11] examined the association of alcohol intake with coronary calcification and one study [21] with aortic arch calcification. A study by Vliegenthart et al found an inverse association between daily alcohol consumption up to 2 units and coronary calcification [10]. The CARDIA study reported a positive association between coronary calcification and alcohol consumption [9]. However, a study among active-duty US army personnel found no association of alcohol consumption with coronary calcification [11]. Finally, a study among self-referred subjects for calcium assessment [21] found no associations between alcohol consumption and aortic calcification. It is possible that the discrepancy between studies is due to different drinking patterns, lifestyle or socio-economic aspects correlated with choice of drinking behavior in certain populations. Only one study [18] investigated the association of specific alcoholic (beer and spirit consumption) types with carotid atherosclerosis. This study among men reported a positive association of spirits with progression of carotid IMT and with increase in plaque height. In this study, also subjects with a high beer consumption had a higher risk of progression of IMT, compared to the lowest drinking category. We found a similar tendency for beer and liquor consumption.

Wine consumption showed an inverse association with risk of carotid calcification, although estimates lacked significance. Many studies examined the relation between alcohol intake and manifest cardiovascular disease and found moderate alcohol

consumption to be associated with a reduction in the risk of myocardial infarction [22], coronary heart disease [23] and total and ischemic stroke [24]. In a large population based cohort study, there was a significant decrease in all-cause mortality in wine drinkers as compared with non-wine drinkers [25]. However, wine consumption has been associated with a healthy diet and associations therefore can be confounded by a healthy lifestyle [26, 27]. Because we did not take healthy lifestyle factors like diet and physical activity into account, we cannot exclude that our results on wine consumption are biased by these factors.

The advantages of our study include a large population in a population-based setting and the inclusion of arterial calcification in different vessel beds. However, some limitations of our study need to be discussed. We measured calcification, not plaque per se. Although calcification is not a direct measure of plaque, coronary calcification determined by EBCT has been correlated with the total area of coronary plaque [8]. Also, the presence of aortic calcification on plain radiographs has been shown to indicate aortic atherosclerosis[28]. To the best of our knowledge, there is 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. Our study may be further limited by the fact that non-participation of diseased subjects may have resulted in a relatively healthy study population, with a more restricted range of variables studied, which may lead to an underestimation of the associations. Also, it is impossible to correctly assess the absolute levels of alcohol consumption, partly because subjects are susceptible to giving socially desirable answers. This misclassification may have led to underestimation of the effect estimates. Finally, our definition of calcification depends on the distribution of calcification per gender and may therefore be different in other populations.

In this study on risk of carotid calcification, opposite trends were found for wine drinking (inverse) and beer and liquor drinking (positive). The association, however, lacked statistical significance and larger numbers are needed to confirm the results.

References

1. Mukamal KJ, Kronmal RA, Mittleman MA, O'Leary DH, Polak JF, Cushman M, Siscovick DS. Alcohol consumption and carotid atherosclerosis in older adults: the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 2003;23:2252-2259.

2. Kiechl S, Willeit J, Rungger G, Egger G, Oberhollenzer F, Bonora E. Alcohol consumption and atherosclerosis: what is the relation? Prospective results from the Bruneck Study. Stroke. 1998;29:900-907.

3. Schminke U, Luedemann J, Berger K, Alte D, Mitusch R, Wood WG, Jaschinski A, Barnow S, John U, Kessler C. Association between alcohol consumption and subclinical carotid atherosclerosis: the Study of Health in Pomerania. Stroke. 2005;36:1746-1752.

4. Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, Cohn R. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. Am J Epidemiol. 1987;126:629-637.

5. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation. 1992;85:910-915.

6. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol. 1995;15:1512-1531.

7. Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J. 1990;11 Suppl E:3-19.

8. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.

9. Pletcher MJ, Varosy P, Kiefe CI, Lewis CE, Sidney S, Hulley SB. Alcohol consumption, binge drinking, and early coronary calcification: findings from the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Epidemiol. 2005;161:423-433.

10. Vliegenthart R, Oei HH, van den Elzen AP, van Rooij FJ, Hofman A, Oudkerk M, Witteman JC. Alcohol consumption and coronary calcification in a general population. Arch Intern Med. 2004;164:2355-2360.

11. Tofferi JK, Taylor AJ, Feuerstein IM, O'Malley PG. Alcohol intake is not associated with subclinical coronary atherosclerosis. Am Heart J. 2004;148:803-809.

12. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.

13. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.

14. Standards of medical care in diabetes. Diabetes Care. 2005;28 Suppl 1:S4-S36.

15. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109:1089-1094.

16. Hollander M, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Breteler MM. Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study. J Neurol Neurosurg Psychiatry. 2003;74:317-321.

17. Dempster AP, Laird, N.M. and Rubin, D.B. Maximum Likelihood From Incomplete Data via the EM Algoritm. Journal of the Royal Statistical Society. 1977;Ser.B.:1-39.

18. Kauhanen J, Kaplan GA, Goldberg DE, Salonen R, Salonen JT. Pattern of alcohol drinking and progression of atherosclerosis. Arterioscler Thromb Vasc Biol. 1999;19:3001-3006.

19. Zureik M, Gariepy J, Courbon D, Dartigues JF, Ritchie K, Tzourio C, Alperovitch A, Simon A, Ducimetiere P. Alcohol consumption and carotid artery structure in older French adults: the Three-City Study. Stroke. 2004;35:2770-2775.

20. Demirovic J, Nabulsi A, Folsom AR, Carpenter MA, Szklo M, Sorlie PD, Barnes RW. Alcohol consumption and ultrasonographically assessed carotid artery wall thickness and distensibility. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1993;88:2787-2793.

21. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama. 2000;283:2810-2815.

22. Cleophas TJ. Wine, beer and spirits and the risk of myocardial infarction: a systematic review. Biomed Pharmacother. 1999;53:417-423.

23. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. Bmj. 1996;312:731-736.

24. Reynolds K, Lewis B, Nolen JD, Kinney GL, Sathya B, He J. Alcohol consumption and risk of stroke: a meta-analysis. Jama. 2003;289:579-588.

25. Gronbaek M, Becker U, Johansen D, Gottschau A, Schnohr P, Hein HO, Jensen G, Sorensen TI. Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. Ann Intern Med. 2000;133:411-419.

26. Barefoot JC, Gronbaek M, Feaganes JR, McPherson RS, Williams RB, Siegler IC. Alcoholic beverage preference, diet, and health habits in the UNC Alumni Heart Study. Am J Clin Nutr. 2002;76:466-472.

27. Tjonneland A, Gronbaek M, Stripp C, Overvad K. Wine intake and diet in a random sample of 48763 Danish men and women. Am J Clin Nutr. 1999;69:49-54.

28. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and post-mortem calcification of the aorta. Am Heart J. 1954;48:540-543.

4.3

The association of arterial stiffness and arterial calcification

Abstract

Background

The purpose of this study was to examine the association between arterial stiffness and arterial calcification.

Methods

This study was part of the Rotterdam Study, a population-based study in subjects aged 55 years and over. Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity (PWV) and carotid stiffness by assessment of common carotid distensibility using ultrasound. Aortic arch and carotid calcification was assessed by multislice computed tomography (MSCT) and quantified according to the Agatston score. Measurements on arterial stiffness and arterial calcification were conducted during the third (1997-1999) and fourth round (2002-2004) of the Rotterdam Study, respectively. Associations were analyzed using logistic regression, adjusted for age, gender, type of MSCT, mean arterial pressure, heart rate, body mass index, serum total cholesterol, HDL-cholesterol, smoking, diabetes mellitus, and lipid and blood pressure lowering medication. Adjusted geometric mean calcium scores were computed for quartiles of aortic stiffness and carotid stiffness.

Results

We found an independent association of aortic stiffness with carotid calcification with odds ratios (OR) (95% CI) of 2.1 (1.1-3.9) and 2.7 (1.3-5.3) for subjects in the third and fourth quartile, respectively, compared to subjects in the first quartile. The association of aortic stiffness with aortic arch calcification was also positive but lacked significance. Carotid stiffness was independently associated with both aortic arch and carotid calcification (aortic arch calcification 2.5 (1.2-5.4), fourth versus first quartile; carotid calcification, 2.3 (1.0-4.0), 3.2 (1.4-6.0), third and fourth versus first quartile).

Conclusion

This population-based study shows that arterial stiffness is strongly associated with arterial calcification.

Introduction

Age-related changes in the vascular elasticity of the vessel wall lead to arterial stiffness. The extent of arterial stiffness may depend on cardiovascular risk factors. Different measures of arterial stiffness have been shown to predict cardiovascular events [1]. Some studies found associations of arterial stiffness with carotid intima-media thickness (IMT) [2] and carotid plaques [2,3], however, data on the association between arterial stiffness and calcified atherosclerosis are limited [2,4,5].

Atherosclerotic lesions have different manifestations. More advanced atherosclerotic lesions contain calcification, although calcification may also be present in small amounts in the earlier stages of atherosclerosis [6]. With the use of multislice computer tomography (MSCT) it is possible to quantify the amount of arterial calcification. Recently, a study by Kullo et al examined the association between arterial stiffness and coronary calcification among 401 healthy participants. They found an independent association of arterial stiffness with coronary calcification. However, until now no studies have investigated the association between arterial stiffness and aortic arch and carotid calcification.

The aim of this study was to investigate the associations of arterial stiffness, expressed as carotid-femoral pulse wave velocity (PWV) and common carotid distensibility, with aortic arch and carotid calcification assessed by MSCT.

Methods

Study population

This study is embedded in the Rotterdam Study, a population-based study, which started with a baseline visit in 1990-1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited and 7,983 agreed to participate (78% response). The design and rationale of the Rotterdam Study have been described elsewhere [7].

The third examination phase took place from 1997 until 1999. During this phase, measurements of cardiovascular risk factors, atherosclerosis, and arterial stiffness were conducted. The fourth examination took place from 2002 to 2004. From September 2003 onwards, all participants who completed the fourth examination phase were invited to undergo a MSCT scan of the carotid arteries. We restricted the present analyses to the participants who were scanned until December 2004. In 698 subjects, data of at least one measurement of arterial stiffness was available. Due to severe artefacts in the image acquisition, the aortic arch of one subject could not be evaluated, hence data on

calcification in the aortic arch were available for 697 subjects. In all subjects data on the calcification in the carotid arteries were available.The median duration between the third examination phase and the MSCT scan was 4.7 years.

This 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.

Measures of arterial stiffness

Pulse wave velocity

Aortic stiffness was assessed by measuring carotid-femoral pulse wave velocity (PWV). PWV was measured with the subjects in supine position. PWV was assessed with an automatic device (Complior ® Artech Medicla, France) [8] that measures the time delay between the feet of simultaneously recorded pulse waves . The distance traveled by the pulse wave between the carotid artery and the femoral artery was measured over the surface of the body using a tape measure. PWV was calculated as the ratio of the distance traveled by the pulse wave and the foot-to-foot time delay and was expressed in meters per second. Data on aortic stiffness were available for 667 subjects (96%).

Carotid distensibility

Carotid stiffness was assessed by measuring common carotid distensibility and expressed as the distensibility coefficient. A lower distensibility coefficient indicates increased arterial stiffness. Common carotid distensibility was assessed with the subjects in supine position, the head tilted slightly to the contralateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere [9]. After five minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasound. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = 2ΔD/(D* pulse pressure) (1/MPa) [10]. Data on carotid stiffness were available for 607 subjects (87%).

Scan Protocol

In the first 583 subjects imaging was performed with a 16-slice MSCT scanner (SOMATOM Sensation 16, Siemens, Forcheim, Germany). The scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Scan parameters were: 16 × 0.75 mm collimation, 120 kVp, 100 effective mAs, 0.5 s rotation time and normalized pitch of 1. Images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5 mm, 120 mm FOV and medium sharp convolution kernel ("B35f"). In the other 115 subjects image acquisition was performed with a 64-slice MSCT scanner (SOMATOM Sensation 64, Siemens, Forcheim, Germany). Scan parameters were similar for both MSCT scanners, except for collimation and effective mAs. With the use of the 64-slice MSCT scanner the collimation was 32 × 0.6 mm and the mAs value was real time adapted to body weight (CARE DOSE, Siemens, Forcheim, Germany) with a reference value of 100 mAs.

Analysis of calcification

Two reviewers, with a medical background, were trained by an experienced radiologist and scored arterial calcification using a standardized protocol. They were blinded to the clinical data of the participants. Two vessel beds were analyzed: the aortic arch and the carotid arteries. The examination of 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 examination of carotid arteries comprised both right and the left carotid artery within 3 cm proximal and distal of the bifurcation.

Calcified lesions were identified based on a threshold of 130 Hounsfield Units (HU) for all applied scoring methods and the amount of calcification was scored using dedicated software (syngo Calcium Scoring, Siemens, Forcheim, Germany). For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion (the number of voxels with an attenuation value ≥ 130 HU times the volume of one voxel) and a factor assigned according to the maximum attenuation value of the lesion [11]. For image data with an overlapping reconstruction increment, Agatston scores were normalized with the ratio of increment and slice width. The total score per vessel bed was calculated by adding up the scores of all lesions.

Cardiovascular risk factors

Information on cardiovascular risk factors was collected during the third follow up examination. Data on drug use and smoking habits were obtained during the home interview. Smoking was classified as former or current smoking. Systolic (first Korotkoff phase) and diastolic (fifth Korotkoff phase) blood pressure was measured twice on the

right arm using a random-zero sphygmomanometer, after the participant had been seated for at least five minutes. The mean of the two blood pressure values was used in the analyses. Pulse pressure was defined as the difference between systolic and diastolic blood pressure. Mean arterial pressure was calculated as diastolic blood pressure + 1/3 pulse pressure. Body mass index [weight (kg)/height $(m)^2$] was calculated. Serum total cholesterol and high-density lipoproteins (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Diabetes mellitus was defined as the use of blood glucose lowering medication and/or a fasting serum glucose level equal to or greater than 7.0 mmol/l [12]. History of myocardial infarction and stroke was defined as described previously [13,14]. The missing values of covariates were handled by single imputation using an expectation-maximization algorithm [15].

Statistical analysis

The associations were examined using logistic regression with aortic arch or carotid calcifications as dependent variables and aortic stiffness and carotid stiffness as the independent variables. Since calcium scores were much higher in men than in women, gender specific quartiles were used. The highest quartile of calcification was compared to the lower 3 quartiles, for both genders together and for each vessel bed separately. Both aortic stiffness and carotid stiffness were categorized in quartiles, irrespective of gender. The cut-off values for aortic stiffness were 10.7, 12.2, 13.9, 25.2; and for carotid stiffness were 31.1, 15.2, 11.6, 8.7. The first quartile, indicating the lowest stiffness, was used as the reference category. Initially, the regression analysis was adjusted for age, gender, mean arterial pressure, heart rate and type of MSCT (model 1). In model 2, additional adjustments were made for body mass index, serum total cholesterol, HDL-cholesterol, smoking, diabetes mellitus, and lipid and blood pressure lowering medication.

Analysis of covariance was used to test for trend and was used to compute geometric mean calcium scores for quartiles of aortic stiffness and carotid stiffness, for both vessel beds. SPSS 11.0 for Windows (SPSS, Inc, Chicago, Illinois) was used for data analysis.

Results

Table 1 shows the characteristics of the study population. Fifty percent of the study population was men. The mean age (± sd) of the study participants during the arterial stiffness measurement was 68.7 years (± 5.4). Overall, calcium scores were higher in men than in women. The median calcium score (interquartile range) for the aortic arch was 670 (149-1848) for men and 469 (128-1386) for women. Corresponding scores for the carotid arteries were 83 (9-297) and 36 (0-167).

Table 1 Characteristics of the study population

Variable	n = 698
Age (years)	68.7 ± 5.4
Men (%)	50.4
Systolic blood pressure (mmHg)	14.3 ± 21.3
Diastolic blood pressure (mmHg)	81.0 ± 10.7
Mean arterial pressure (mmHg)	99 ± 13
Pulse pressure (mmHg)	66 ± 16
Heart rate (bpm)	70 ± 11
Body mass index (kg/m^2)	26.7 ± 3.9
Total cholesterol (mmol/l)	5.8 ± 1.0
High-density lipoprotein cholesterol (mmol/l)	1.4 ± 0.4
Serum glucose (mmol/l)	5.8 ± 1.1
Current smokers (%)	13.8
Diabetes mellitus (%)	9.6
Use of blood pressure lowering medication (%)	34.9
Use of lipid lowering medication (%)	16.6
History of myocardial infarction (%)	10
History of stroke (%)	5
Aortic arch calcification according to Agatston[1]	547 (134-1612)
Carotid artery calcification according to Agatston[1]	58 (2-222)
Pulse wave velocity index (m/s)	12.5 ± 2.5
Distensibility coefficient (10^{-3}/kPa)	12.2 ± 4.7

[1] *Value is expressed as median (interquartile range) because of its skewed distribution.*

Table 2 Relation between aortic stiffness and calcification in the aortic arch and the carotid arteries.*

	Aortic arch calcification (n=666)	
	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Aortic stiffness		
1st quartile	1.0 (reference)	1.0 (reference)
2nd quartile	1.2 (0.6-2.2)	1.2 (0.6-2.2)
3rd quartile	1.5 (0.8-2.7)	1.4 (0.7-2.7)
4th quartile	1.8 (0.9-3.3)	1.8 (0.9-3.5)

	Carotid artery calcification (n=667)	
	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Aortic stiffness		
1st quartile	1.0 (reference)	1.0 (reference)
2nd quartile	1.4 (0.7-2.5)	1.4 (0.7-2.8)
3rd quartile	2.2 (1.2-4.0)[a]	2.1 (1.1-3.9)[a]
4th quartile	2.7 (1.4-5.0)[b]	2.7 (1.3-5.3)[b]

Model 1 adjusted for age, gender, type of MSCT, mean arterial pressure and heart rate. Model 2 additionally adjusted for body mass index, serum total cholesterol, HDL-cholesterol, smoking, diabetes mellitus, and lipid and blood pressure lowering medication.

[a] *$0.01 < p < 0.05$*

[b] *$0.001 < p < 0.01$*

[c] *$p < 0.001$*

** highest quartile of calcification versus the lower three quartiles*

Table 2 shows the adjusted ORs (odds ratio) for risk of arterial calcification associated with ortic stiffness. We found an independent association of aortic stiffness with carotid calcification with odds ratios (OR (95% CI) of 2.1 (1.1-3.9) and 2.7 (1.3 5.3) for subjects in the third and fourth quartile, respectively, compared to subjects in the first quartile. The association of aortic stiffness with aortic arch calcification was also positive but lacked significance.

Table 3 Relation between carotid stiffness and calcification in the aortic arch and the carotid arteries.*

	Aortic arch calcification (n=606)	
	Model 1 OR (95% CI)	Model 1 OR (95% CI)
Carotid stiffness		
1st quartile	1.0 (reference)	1.0 (reference)
2nd quartile	1.3 (0.6-2.5)	1.2 (0.6-2.4)
3rd quartile	2.2 (1.1-4.2)[a]	1.9 (1.0-4.0)
4th quartile	3.0 (1.5-6.2)[b]	2.5 (1.2-5.4)[a]

	Carotid artery calcification (n=607)	
	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Carotid stiffness		
1st quartile	1.0 (reference)	1.0 (reference)
2nd quartile	1.9 (1.0-3.5)	1.9 (0.9-3.4)
3rd quartile	2.3 (1.2-4.3)[a]	2.3 (1.0-4.0)[a]
4th quartile	3.6 (1.7-7.1)[c]	3.2 (1.4-6.0)[b]

Model 1 adjusted for age, gender, type of MSCT, mean arterial pressure and heart rate. Model 2 additionally adjusted for body mass index, serum total cholesterol, HDL-cholesterol, smoking, diabetes mellitus, and lipid and blood pressure lowering medication.

[a] *$0.01 < p < 0.05$*

[b] *$0.001 < p < 0.01$*

[c] *$p < 0.001$*

** highest quartile of calcification versus the lower three quartiles*

Table 3 shows the adjusted ORs for quartiles of carotid stiffness and the risk for arterial calcification, for each vessel separately. Carotid stiffness was independently associated with both aortic arch and carotid calcification (aortic arch calcification 2.6 (1.2-5.4), fourth versus first quartile; carotid calcification, 2.0 (1.0-4.0), 2.9 (1.4-6.0), third and fourth versus first quartile).

Figure 1 shows the geometric mean calcium score of the aortic arch and carotid arteries for quartiles of aortic stiffness. With increasing quartiles of aortic stiffness, the geometric mean calcium score increased in both vessel beds, however, only for carotid artery calcification a significant trend (p=0.001) was found.

Figure 2 shows the geometric mean calcium score of the aortic arch and carotid arteries for quartiles of carotid stiffness. With increasing quartiles of carotid stiffness, the geometric mean calcium score increased in both vessel beds, a significant trend test was found with aortic arch (p<0.0001) and with carotid calcification (p=0.002).

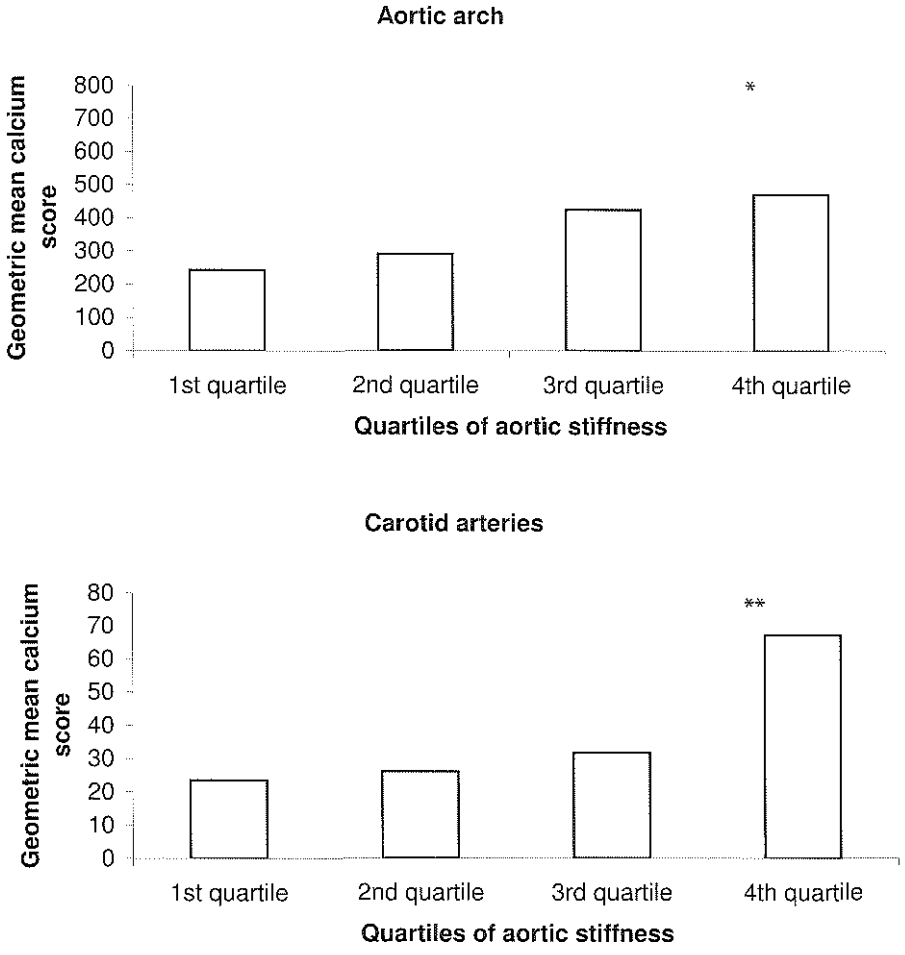


*Figure 1 Geometric mean calcium scores for quartiles of aortic stiffness, for the aortic arch and carotid arteries *p-trend (aortic arch calcification)=0.054; ** p-trend (carotid calcification)=0.001*

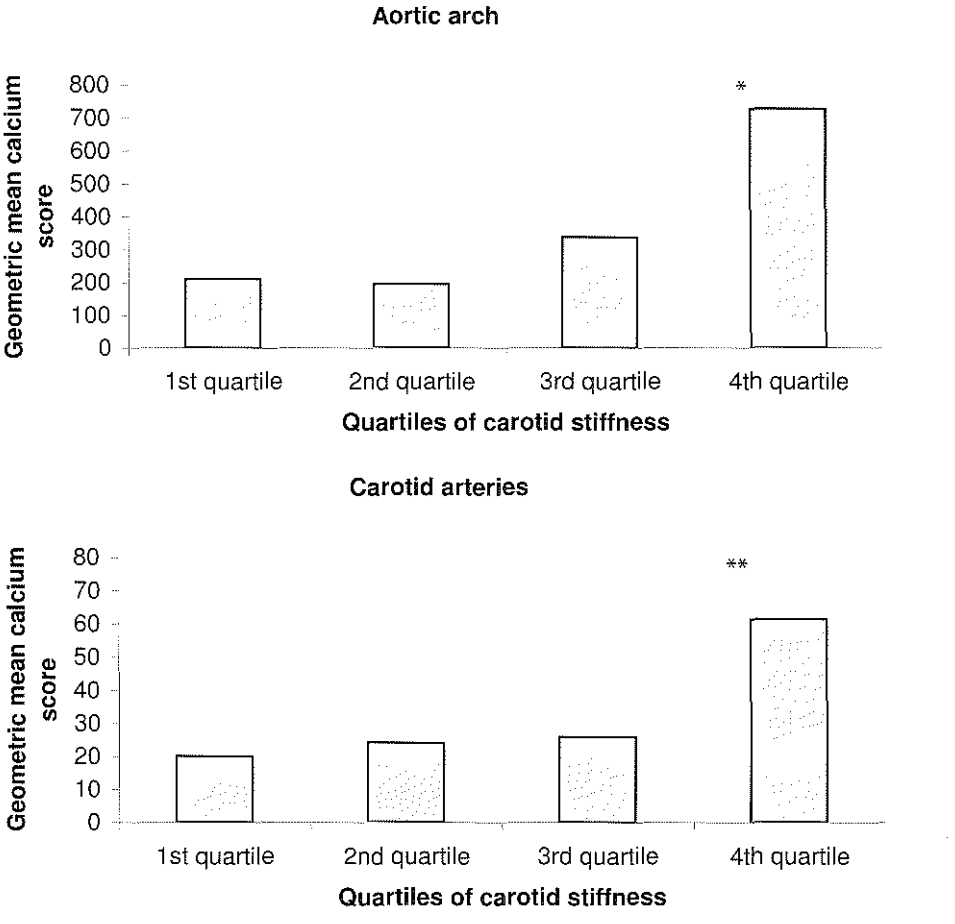


*Figure 2 Geometric mean calcium scores for quartiles of carotid stiffness, for the aortic arch and carotid arteries *p-trend (aortic arch calcification)<0.0001; ** p-trend (carotid calcification)=0.002.*

Discussion

We found aortic stiffness and carotid stiffness to be independently associated with carotid calcification and carotid stiffness to be independently associated with aortic arch calcification. The association between aortic stiffness and aortic arch calcification was in the same direction, but lacked statistical significance.

Several studies have found an association between arterial stiffness and atherosclerosis [2-5]. However, data on the association between arterial stiffness and calcified atherosclerosis are scarce [2, 4, 5]. A previous analysis performed in the Rotterdam Study showed that arterial stiffness, measured as PWV and carotid distensibility, was associated with several

measures of atherosclerosis, among which aortic calcification measured on a lateral lumbar radiograph [2]. A study by Nakamura et al found the length of abdominal aortic calcification, visualized on a lateral lumbar radiograph, to be independently related to PWV among 97 patients [4]. Until now, one study investigated the association between aortic PWV and coronary calcification assessed by electron beam computed tomography (EBCT) in a community-based population [5]. In this study, aortic PWV was independently associated with the presence and quantity of coronary calcification. We are the first to examine arterial stiffness with arterial calcification quantified by MSCT in other vessel beds.

Several explanations for our findings are possible. Firstly, both arterial stiffness and arterial calcification are processes influenced by age, so it is possible that both processes accidentally occur together in older age. However, associations remained after adjustment for age, so this does not seem a likely explanation. Secondly, the presence of atherosclerosis may lead to arterial stiffening. It is likely that calcified plaques affect stiffness more than non calcified plaques. However, this cannot be concluded from our data, since we were not able to examine the relation of stiffness with non-calcified plaques. Thirdly, stiffening of the arterial wall increases shear stress and may lead to vessel wall damage and atherosclerosis. Since our study was cross-sectional, our data provide no information on which of the two latter mechanisms are more likely.

The advantages of our study include a large population in a population-based setting and the inclusion of two measures of arterial stiffness in different vessel beds. However, some limitations of our study need to be discussed. For determining arterial stiffness we used data from the third examination phase, which was 4.7 years prior to the MSCT scan. Using data on arterial stiffness from a prior examination phase may have led to misclassification in the severity of arterial stiffness at the time of measurement of arterial calcification. However, we expect the misclassification to be non-differential with respect to arterial calcification and therefore, might have led, if anything, to an underestimation of the associations. Our study may be further limited by the fact that non participation of diseased subjects may have resulted in a relatively healthy study population, with a more restricted range of calcium scores and values of arterial stiffness, which may lead to an underestimation of the associations. Our cut-off points for high calcification and for quartiles of arterial stiffness depend on the distribution of calcification per vessel bed and gender and may be different in other populations. Finally, to the best of our knowledge there is no data on the relation between carotid artery calcification and carotid plaque burden. However, coronary and aortic calcifications have been found to be related to the presence of atherosclerosis [16] in these vessels. 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.

In conclusion, aortic stiffness and carotid stiffness were independently associated with carotid calcification and carotid stiffness was also independently associated with aortic arch calcification. The association between aortic stiffness and aortic arch calcification was in the same direction, but lacked statistical significance. These results support the view that arterial stiffness and arterial calcification are concurrent processes.

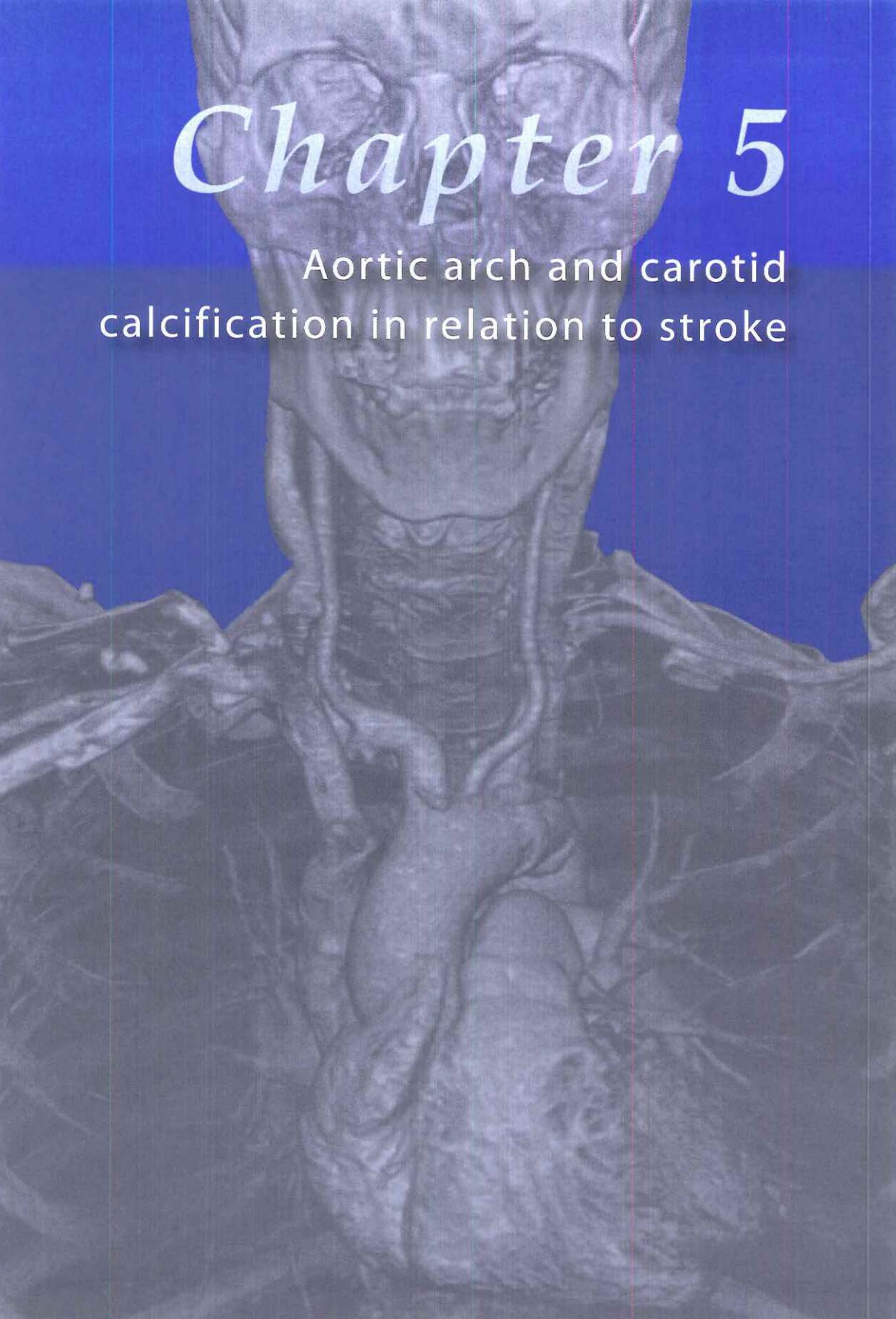
References

1. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. 2006;113:657-663.
2. van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, van der Kuip DA, Hofman A, Witteman JC. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke. 2001;32:454-460.
3. Zureik M, Temmar M, Adamopoulos C, Bureau JM, Courbon D, Thomas F, Bean K, Touboul PJ, Ducimetiere P, Benetos A. Carotid plaques, but not common carotid intima-media thickness, are independently associated with aortic stiffness. J Hypertens. 2002;20:85-93.
4. Nakamura U, Iwase M, Nohara S, Kanai H, Ichikawa K, Iida M. Usefulness of brachial-ankle pulse wave velocity measurement: correlation with abdominal aortic calcification. Hypertens Res. 2003;26:163-167.
5. Kullo IJ, Bielak LF, Turner ST, Sheedy PF, 2nd, Peyser PA. Aortic pulse wave velocity is associated with the presence and quantity of coronary artery calcium: a community-based study. Hypertension. 2006;47:174-179.
6. Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J. 1990;11 Suppl E:3-19.
7. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
8. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. 1995;26:485-490.
9. Hoeks AP, Brands PJ, Smeets FA, Reneman RS. Assessment of the distensibility of superficial arteries. Ultrasound Med Biol. 1990;16:121-128.
10. Reneman RS, van Merode T, Hick P, Muytjens AM, Hoeks AP. Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol. 1986;12:465-471.
11. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.
12. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997;20:1183-1197.
13. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109:1089-1094.

14. Hollander M, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Breteler MM. Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study. J Neurol Neurosurg Psychiatry. 2003;74:317-321.

15. Dempster AP, Laird, N.M. and Rubin, D.B. Maximum Likelihood From Incomplete Data via the EM Algoritm. Journal of the Royal Statistical Society. 1977;Ser.B.:1-39.

16. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and post-mortem calcification of the aorta. Am Heart J. 1954;48:540-543.



Chapter 5

Aortic arch and carotid calcification in relation to stroke

5.1

Aortic arch and carotid calcification and history of stroke

Abstract

Background

The purpose of this study was to investigate the association of calcification in the aortic arch and the carotid arteries with a history of stroke.

Methods

This study was part of the Rotterdam Study, a population-based study in subjects aged 55 years and over. From October 2003 until December 2004, participants were invited to undergo a multislice computed tomography (MSCT) scan. Analyses were performed in the first 1003 subjects (mean age 71.6 ± 6 years, 48% male). Calcification in the aortic arch and carotid arteries was quantified according to the Agatston score. At the time of the scan, a history of stroke was reported by 42 subjects. Analyses were performed by means of logistic regression.

Results

History of stroke was associated with a high aortic arch calcification score (upper quartile) (age- and sex adjusted OR=2.3, 95% CI: 1.2-4.6) and with a high carotid artery calcification score (2.2, 1.2-4.3). After additional adjustment for cardiovascular risk factors, risk estimates slightly decreased for both aortic arch calcification (2.3, 1.0-4.1) and carotid calcification (1.8, 0.9-3.6).

Conclusion

In this population-based study, we found that subjects with a history of stroke had an increased risk of having high calcium scores in the aortic arch and the carotid arteries compared to subjects without a history of stroke. Associations, however, were not completely independent of conventional cardiovascular risk factors.

Introduction

Atherosclerosis in the aortic arch and in the carotid arteries has been related to risk of cerebrovascular events [1-7]. Strong associations were found between atherosclerosis in the aortic arch and risk of stroke [1-3]. Most studies, however, were based on transesophageal echocardiographic (TEE) assessment of aortic arch plaque, which is an invasive method and is not suitable for population-based studies[2,3]. Several studies have found associations of carotid intima-media thickness (IMT) [4, 6, 7] and carotid plaques [5] with risk of stroke. Although ultrasound is a non-invasive measure, plaques cannot be accurately quantified, whereas elevated IMT reflects an early phase of the atherosclerotic process.

With the development of electron beam computed tomography (EBCT) and multislice computed tomography (MSCT) it is possible to quantify the amount of arterial calcification as a measure of atherosclerosis. Calcification in coronary arteries has been found to predict coronary heart disease [8]. Less is known about the predictive value of calcification in other vessels. One study [9] quantified carotid calcification by the use of MSCT. The study found calcium scores to be related to transient ischemic attack (TIA) and stroke. However, their study population was small and not population-based.

In a large population-based study we examined the association of a history of stroke with aortic arch and carotid calcification assessed by MSCT.

Methods

Study population

The study is embedded in the Rotterdam Study, a population-based study that started in 1990-1993. All inhabitants aged 55 years and older and living in a suburb of Rotterdam were invited and 7,983 agreed to participate (78% response). In 2000-2001, the cohort was extended with 3,011 subjects (67% response) with the same inclusion criteria. The design and rationale of the Rotterdam Study have been described elsewhere [10]. Study center visits took place approximately every three years.

From September 2003 onwards, 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 to undergo a MSCT scan of the aortic arch and the carotid arteries. Present analyses were restricted to the first 1,003 participants who were scanned until December 2004. Due to severe artefacts in the image acquisition, the aortic arch of one subject could not be evaluated, hence data on calcification in the aortic arch

were available for 1002 subjects. Data on the calcification in the carotid arteries were available for all subjects. The median duration between the study center visit and the MSCT scan was 75 days. This 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

In the first 708 subjects, imaging was performed with a 16-slice MSCT scanner (SOMATOM Sensation 16, Siemens, Forcheim, Germany). The scan reached from the aortic arch to the intracranial circulation (1 cm above the sella turcica). Scan parameters were: 16 × 0.75 mm collimation, 120 kVp, 100 effective mAs, 0.5 s rotation time and normalized pitch of 1. Images were reconstructed with effective slice width 1 mm, reconstruction interval 0.5 mm, 120 mm FOV and medium sharp convolution kernel ("B35f"). In another 295 subjects image acquisition was performed with a 64-slice MSCT scanner (SOMATOM Sensation 64, Siemens, Forcheim, Germany). Scan parameters were similar for both MSCT scanners, except for collimation and effective mAs. With the use of the 64-slice MSCT scanner the collimation was 32 × 0.6 mm and the mAs value was real time adapted to body weight (CARE DOSE, Siemens, Forcheim, Germany) with a reference value of 100 mAs.

Analysis of calcification

Two reviewers, with a medical background, were trained by an experienced radiologist and scored arterial calcification using a standardized protocol. They were blinded to the clinical data of the participants. The examination of 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 examination of 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. For each calcified lesion, the Agatston score was calculated as the product of the area of a calcified lesion (the number of voxels with an attenuation value ≥ 130 HU times the volume of one voxel) and a factor assigned according to the maximum attenuation value of the lesion [11]. For image data with an overlapping reconstruction increment, Agatston scores were normalized with the ratio of increment and slice width. The total score per vessel bed was calculated by adding the scores of all lesions.

Covariates

Information on smoking, blood pressure, and lipid lowering medication use was obtained during a home interview of the Rotterdam Study. Subjects were categorized as current, past and never smokers. Clinical measures were obtained during a visit at the study center. Height and weight were measured and the body mass index was calculated (weight[kg]/height [m]^2). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The mean of two consecutive measurements was used in the analyses. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were obtained using an automatic enzymatic procedure (Hitachi 911, Roche CHOD PAP). Diabetes was defined as the use of anti-diabetic medication or a fasting glucose level of ≥ 7 mmol/l. Information on cardiovascular disease (myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft surgery (CABG) was collected at baseline in 1990-1993 (original cohort) or at baseline in 2000-2001 (extended cohort) and during follow-up as described previously [12]. The missing values of covariates were handled by single imputation using an expectation-maximization algorithm[13].

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 and for the extended cohort was assessed by asking 'did you ever suffer from a stroke, diagnosed by a physician?'. Medical records of subjects who answered 'yes' were verified [14]. After entrance into the Rotterdam Study, participants are continuously monitored for major events through automated linkage of the study database with files from general practitioners and the municipality. Also nursing home physicians' files and files from general practitioners of participants who moved out of the district were scrutinized. For reported events, additional information (including brain imaging) was obtained from hospital records. Research physicians discussed information on all potential strokes and TIA with an experienced stroke neurologist (P.J.K.) to verify all diagnoses. Ischemic, hemorrhage, and unspecified strokes were included and subarachnoid hemorrhages and retinal infarcts were excluded.

Statistical analysis

Logistic regression was used to determine whether people with severe aortic arch or carotid calcification (highest quartile) were more likely to have had a stroke compared to subjects in the lowest three quartiles. Since calcium scores were much higher in men than in women, gender specific quartiles were used. Initially, the logistic regression analysis was adjusted for age, gender, and type of MSCT (model 1). In model 2, additional adjustments were made for body mass index, systolic and diastolic blood pressure, total

serum cholesterol, HDL-cholesterol, diabetes, smoking status, blood pressure lowering and lipid lowering medication, and previous cardiovascular disease.

Analysis of covariance was used to compute age, gender, and MSCT type-adjusted geometric mean calcium scores stratified by the presence of stroke. SPSS 11.0 for Windows (SPSS, Inc, Chicago, Illinois) was used for data analysis.

Results

Table 1 Characteristics of the study population

Variable	n=1003
Age (years)	71.6 ± 6.4
Men (%)	48
Body mass index (kg/m^2)	27.5 ± 4.0
Systolic blood pressure (mmHg)	149.7 ± 21.0
Diastolic blood pressure (mmHg)	80.5 ± 11.1
Total cholesterol (mmol/l)	5.6 ± 1.0
HDL-cholesterol (mmol/l)	1.4 ± 0.4
Fasting glucose (mmol/l)	5.8 ± 1.3
Diabetes mellitus (%)	11
Smokers (%)	
Current	12
Past	58
Never	30
Use of blood pressure lowering medication (%)	46
Use of lipid lowering medication (%)	25
History of myocardial infarction (%)	9
History of stroke (%)	5
Coronary artery Bypass Graft (%)	3
Percutaneous Transluminal Coronary Angioplasty (%)	2
Aortic arch Agatston score[1]	430 (80.5-1363)
Carotid artery Agatston score[1]	42 (0.1-181)

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation. [1] Value is expressed as median (interquartile range) because of its skewed distribution.

Table 1 shows characteristics of the study population. The study population consisted of 48% men. The mean age (± sd) of the study participants was 71.6 years (± 6.4). Overall, calcium scores were higher in men than in women. The median calcium score (interquartile range) in the aortic arch and the carotid arteries were 513 (83-1607), 64 (2-235), respectively for men; and 371 (79-1245), 26 (0-129) respectively for women. A history of stroke prior to CT scanning was present in 42 (4%) participants.

Table 2 shows the age and gender-adjusted and the multivariate adjusted odds ratios for associations between history of stroke and high calcium scores (upper quartile) in the aortic arch and carotid artery. The age-adjusted odds ratios for the association of history of stroke with aortic arch calcification and carotid calcification were 2.3 (95% CI, 1.2-4.6) and 2.2 (95% CI, 1.2-4.3), respectively. In multivariate analyses, risk estimates were slightly reduced but lost statistical significance.

Table 2 *Association of aortic arch and carotid calcification with a history of stroke*

	Aortic arch calcification			Carotid artery calcification		
History of stroke	n	Stroke cases	OR (95% CI)	n	Stroke cases	OR (95% CI)
Model 1	1002	41	2.3 (1.2-4.6)[a]	1003	42	2.2 (1.2-4.3)[a]
Model 2	1002	41	2.0 (1.0-4.1)	1003	42	1.8 (0.9-3.6)

Model 1: adjusted for age, gender and scanner type.

Model 2: additionally adjusted for body mass index, systolic and diastolic blood pressure, total serum cholesterol, HDL-cholesterol, diabetes, smoking status, blood pressure lowering medication and lipid lowering medication, and cardiovascular history.

[a] *$p < 0.05$*

Figure 1 shows the age and gender-adjusted geometric mean calcium scores according to the presence of a history of stroke. Subjects with a history of stroke had a higher geometric mean calcium score in both vessels than those without a history of stroke, however, differences lacked statistical significance.

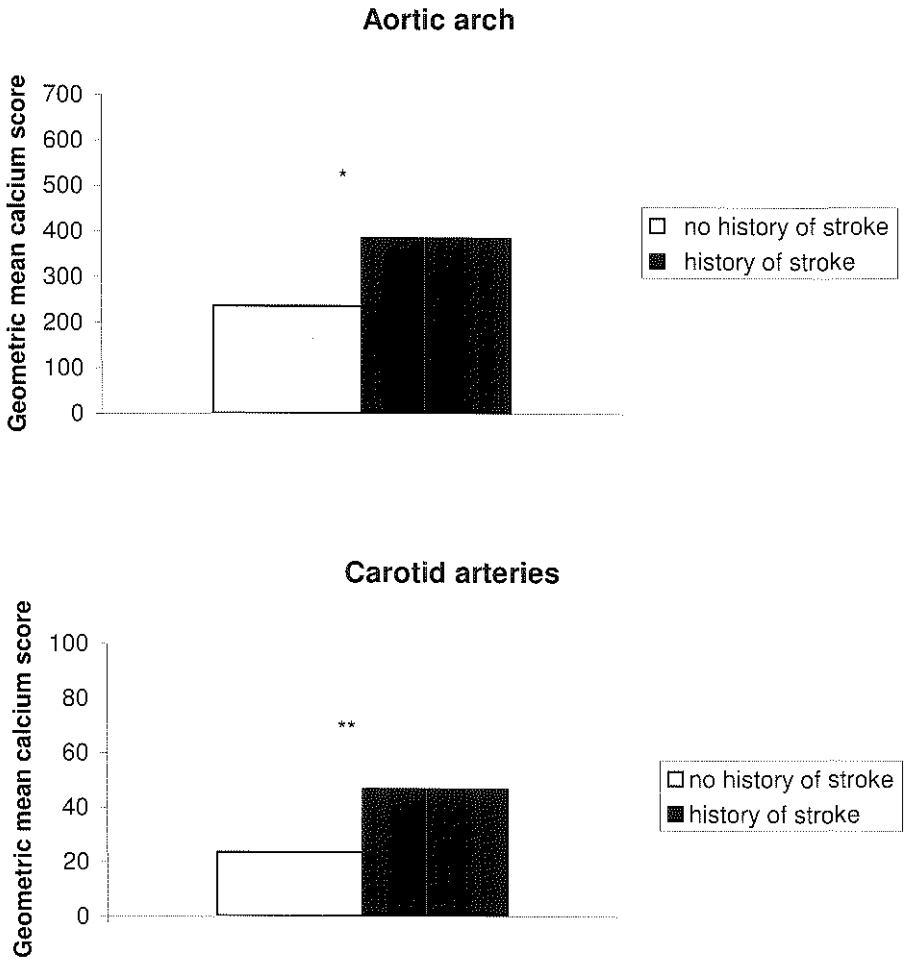


Figure 1 *Age, gender, and MSCT-adjusted geometric mean calcium score according to history of stroke.*
$^{*}p_{aortic\ arch} = 0.17$ for the difference in geometric mean calcium score in the aortic arch in subjects with a history of stroke versus no history of stroke; $^{**}p_{carotid\ arteries} = 0.06$ for the difference in geometric mean calcium score in the carotid arteries in subjects with a history of stroke versus no history of stroke.

Discussion

In this population-based study, we found that subjects with a history of stroke were more likely to have a high calcium score in the aortic arch and the carotid arteries compared with subjects without a history of stroke. After adjustment for conventional vascular risk factors, however, the associations were no longer statistically significant.

Several studies have found that atherosclerosis in the aortic arch [1-3] is associated with cerebrovascular events. Two studies [2, 3] examined the association between aortic arch plaques and a history of stroke in a case-control setting. Aortic arch plaques were visualized by means of TEE and both studies found strong relations with risk of stroke. Iribarren et al [1] examined aortic arch calcification and risk of ischemic and hemorrhagic stroke in a large prospective study among volunteers. Calcification was based on the presence or absence of calcification on plain radiographs. In women, an independent association of ischemic stroke with calcification in the aortic arch was found, but no association was present in men. No relationship was found with hemorrhagic stroke, irrespective of gender. With MSCT, calcification in the aortic arch can be measured more accurately and non-invasively. In our study, we found that history of stroke was associated with aortic arch calcification.

Also, atherosclerosis in the carotid arteries has been shown to be related to risk of cerebrovascular events [4-7]. Three prospective cohort studies found that IMT, assessed by ultrasound, was independently associated with risk of stroke [4, 6, 7]. In a prospective cohort study, carotid plaques assessed by ultrasound were dose-dependently associated with risk of stroke [5]. Until now, one study examined the association between carotid calcification with cerebrovascular events in a cross-sectional study design[9]. The study quantified carotid calcification by means of MSCT and found calcium scores to be related to TIA or stroke. However, the study population was small and not population-based. Nevertheless, our results confirm these previously published results.

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 one study [1], a plain radiograph was used to assess aortic arch calcification, but this only allows dichotomizing the presence of atherosclerosis. 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, which is not a precise quantification of the extent of plaque. Carotid IMT is a standardized and quantitative measure of atherosclerosis. However, the early stages of increased IMT appear to represent hypertrophy rather than atherosclerosis. Another limitation of ultrasound is that only a limited part of the carotid arteries can be visualized in a subgroup of subjects. The use

of MSCT 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. The use of MSCT does, however, have some drawbacks. MSCT is an expensive tool, which generates images with the use of X-rays. The radiation dose for detecting calcified atherosclerosis is relatively low, but levels accumulate when multiple vessels are assessed and assessments are repeated over time. At this point in time, it is unclear which method of measuring aortic arch or carotid atherosclerosis is best for risk stratification. Prospective data on the different measures in relation to risk of stroke are necessary for proper evaluation.

The advantages of our study are its large population and the inclusion of two 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, some limitations of our study need to be discussed. We measured calcification, not plaque per se. Although calcification is not a direct measure of plaque, coronary calcification determined by EBCT has been correlated with the total area of coronary plaque [15]. Also, the presence of aortic calcification on plain radiographs has been shown to indicate aortic atherosclerosis [16]. To the best of our knowledge, there is 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. Our study may be further limited by the fact that non-participation of diseased subjects may have resulted in a relatively healthy study population, with a more restricted range of variable studied, which may lead to an underestimation of the associations.

Also, our definition of calcification depends on the distribution of calcification per gender and may therefore be different in other populations. Finally, the occurrence of stroke may have led to changes in life-style and medication use to reduce risk of a recurrent event. The change in lifestyle and medication could have reduced the difference in arterial calcification between subjects with and without stroke, and therefore the observed risk estimates may have been underestimated.

Several mechanisms have been proposed to explain the associations of atherosclerosis in the aortic arch and carotid arteries with risk of stroke. Firstly, atherosclerotic plaques in the aortic arch [2, 17] and the carotid arteries [5] may serve as a source of cerebral emboli. One study [5], however, found carotid plaques not to be related to risk of cerebral infarction in the ipsilateral hemisphere. In our study, we did not have enough cases of stroke to examine associations with ipsilateral and contralateral stroke separately. Secondly, it is possible that atherosclerotic lesions in both the aortic arch and the carotid arteries are a

marker for generalized atherosclerosis, indicating the increased likelihood of intracranial small vessel disease.

In conclusion, we found that subjects with a history of stroke had an increased risk of having high calcium scores in the aortic arch and the carotid arteries compared with subjects without a history of stroke. Our findings need confirmation in large prospective studies.

References

1. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama. 2000;283:2810-2815.
2. Amarenco P, Cohen A, Tzourio C, Bertrand B, Hommel M, Besson G, Chauvel C, Touboul PJ, Bousser MG. Atherosclerotic disease of the aortic arch and the risk of ischemic stroke. N Engl J Med. 1994;331:1474-1479.
3. Jones EF, Kalman JM, Calafiore P, Tonkin AM, Donnan GA. Proximal aortic atheroma. An independent risk factor for cerebral ischemia. Stroke. 1995;26:218-224.
4. Hollander M, Hak AE, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Witteman JC, Breteler MM. Comparison between measures of atherosclerosis and risk of stroke: the Rotterdam Study. Stroke. 2003;34:2367-2372.
5. Hollander M, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, Hofman A, Breteler MM. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. Circulation. 2002;105:2872-2877.
6. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999;340:14-22.
7. Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad B. Incidence of stroke is related to carotid IMT even in the absence of plaque. Atherosclerosis. 2005;179:325-331.
8. Arad Y, Goodman KJ, Roth M, Newstein D, Guerci AD. Coronary calcification, coronary disease risk factors, C-reactive protein, and atherosclerotic cardiovascular disease events: the St. Francis Heart Study. J Am Coll Cardiol. 2005;46:158-165.
9. Nandalur KR, Baskurt E, Hagspiel KD, Finch M, Phillips CD, Bollampally SR, Kramer CM. Carotid artery calcification on CT may independently predict stroke risk. AJR Am J Roentgenol. 2006;186:547-552.
10. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
11. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.
12. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004;109:1089-1094.

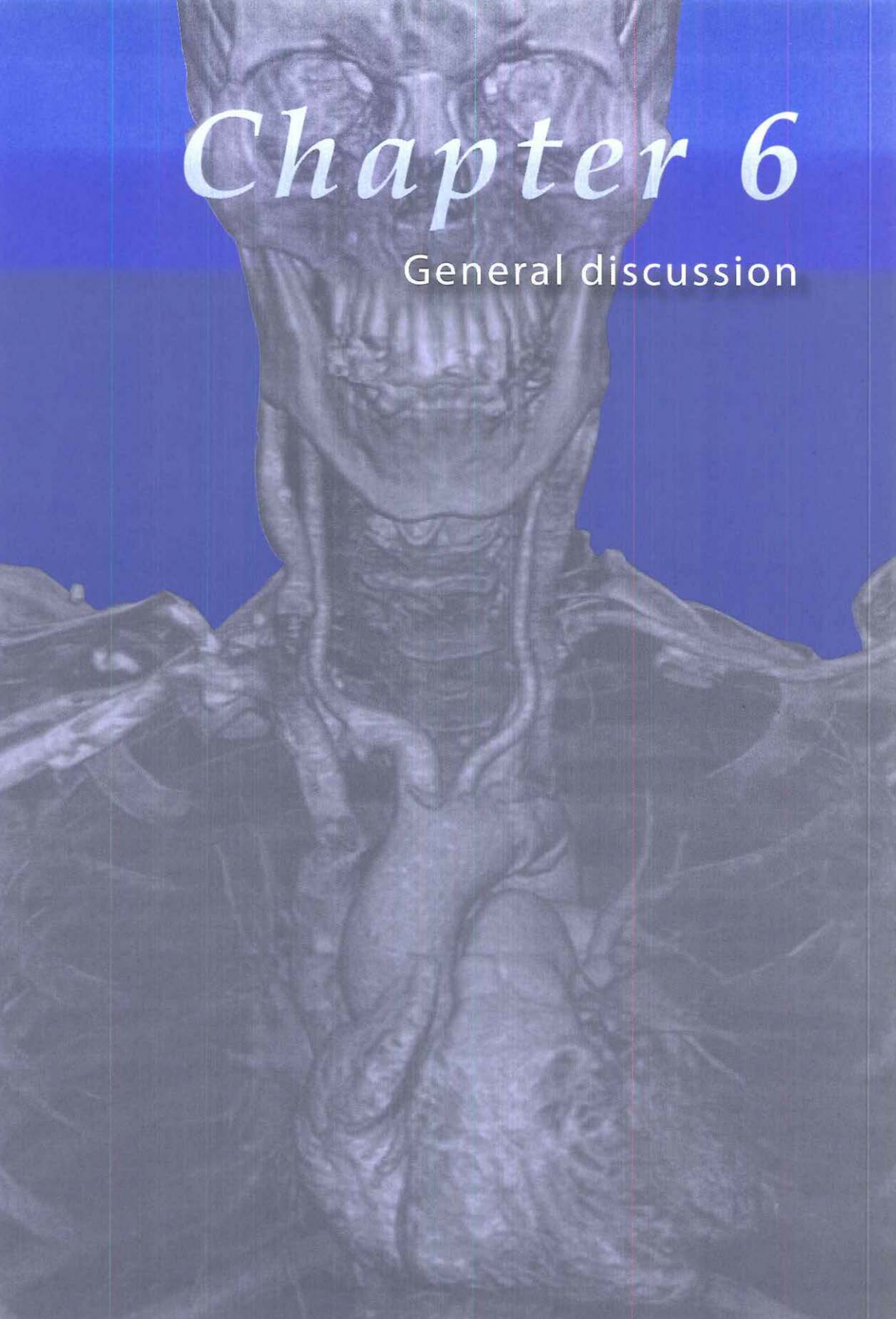
13. Dempster AP, Laird, N.M. and Rubin, D.B. Maximum Likelihood From Incomplete Data via the EM Algoritm. Journal of the Royal Statistical Society. 1977;Ser.B.:1-39.

14. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke. 1996;27:1499-1501.

15. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.

16. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and post-mortem calcification of the aorta. Am Heart J. 1954;48:540-543.

17. Amarenco P, Cohen A, Baudrimont M, Bousser MG. Transesophageal echocardiographic detection of aortic arch disease in patients with cerebral infarction. Stroke. 1992;23:1005-1009.



Chapter 6

General discussion

General discussion

This thesis was based on studies that have been performed in the Rotterdam Study, a large population-based study of elderly subjects [1]. The objectives of these studies were to examine the determinants of and the associations between coronary, aortic arch, and carotid calcification assessed with multislice computed tomography (MSCT). Also, the association of calcification in these vessels and history of stroke was examined. The merits and shortcomings of the described studies have been discussed in the previous chapters. This chapter will provide a more general discussion of the main findings, and will consider methodological issues.

Background

The presence of atherosclerosis in the coronary arteries [2-4], aorta [5-7], and the carotid arteries [8-11] is a risk factor for cardiovascular disease. Atherosclerosis is a disease of the arterial vessel bed, which can be measured in different stages during life and with several diagnostic tools. Atherosclerotic lesions have typical histological and histochemical compositions at different stages of their natural history [12]. More advanced atherosclerotic lesions contain calcification, although calcification may also be present in small amounts in the earlier stages of atherosclerosis [13].

Cardiovascular disease leads to a high morbidity and mortality and consequently puts a large burden on the health care system. Therefore, early identification of high-risk subjects is needed. Several non-invasive measures of atherosclerosis exits and may be useful in identifying subjects at high risk. Existing non-invasive measures of atherosclerosis include intima media thickness (IMT) and number of plaques of the carotid arteries by ultrasound and ankle brachial index, measuring peripheral atherosclerosis. The introduction of electron beam computed tomography (EBCT) and MSCT has enabled the non-invasive visualization and accurate quantification of atherosclerotic calcifications in arteries.

Coronary calcification assessed by EBCT has been found to be a predictor of coronary heart disease [2,3]. EBCT and MSCT also allows measurement of calcification in other vessel beds like the aorta [14] and the carotid arteries [15] .Quantification of calcification in different vessel beds might improve cardiovascular risk assessment. However, until now little data are available on the predictive value of arterial calcification in different vessel beds for the occurrence of new cardiovascular events.

and women. A tendency was present for an inverse association with obesity in women, while no association of low HDL-cholesterol with arterial calcification was present. Age and current smoking were the strongest risk factors for arterial calcification in all vessel beds, except for coronary calcification in men. Possibly men who smoke are prone to die from coronary heart disease at an earlier age due to selective mortality. The inverse association with obesity in women, but not in men, may be related to estrogen production in fat mass in women after menopause [25].

Some studies have shown that light-to-moderate alcohol consumption is inversely associated with atherosclerosis [26-28]. Data on the association of alcohol consumption with arterial calcification [29-31] are scarce and until now there are no data on the relation between alcohol consumption and carotid calcification. In chapter 4.2 we describe the association between alcohol intake and risk of carotid calcification. Several studies have examined the association between alcohol consumption and atherosclerosis [6, 26-35]. In our study, opposite trends were found for wine drinking (inverse) and beer and liquor drinking (positive). However, the associations lacked statistical significance. Although we included nearly 1,000 subjects, this study may lack power to show significant associations. Therefore, larger numbers are needed to confirm the results.

We evaluated the associations of arterial stiffness, expressed as carotid-femoral pulse wave velocity (PWV) and common carotid distensibility, with aortic arch and carotid calcification assessed by MSCT, in chapter 4.3. We found an association between aortic stiffness and carotid calcification. Furthermore, an association was found between carotid stiffness and aortic arch and carotid calcification. The results confirm the association between arterial stiffness and atherosclerosis. One possible explanation is that the presence of calcified atherosclerosis stiffens the vessel wall. However, our study was cross-sectional, and therefore, we cannot conclude of the order of events. Further studies need to be conducted to reveal the mechanism behind the association.

Aortic arch and carotid calcification and history of stroke

Aortic arch and carotid artery calcification assessed by MSCT may be useful tools to identify subjects at high risk for stroke. Atherosclerosis in the aortic arch and in the carotid arteries has been related to risk of cerebrovascular events [5-11]. However, no data are available on the (additive) predictive value of aortic arch and carotid calcification for risk prediction for stroke. In our study, we found that subjects with a history of stroke had an increased risk of having high calcium scores in the aortic arch and the carotid arteries compared with subjects without a history of stroke. After adjustment for conventional vascular risk factors, the associations slightly decreased but were no longer statistically significant. The loss of significance may be due to lack of power. Therefore, the association should be

examined in larger studies. Finally, a prospective study is needed to establish the strength of the predictive value of arterial calcification for risk of stroke.

Methodological considerations

Study design

There are three main types of non-experimental epidemiological studies, including cohort studies, case-control studies and cross-sectional studies. In a cohort study, a population is observed in time for the incidence of diseases. The study population in a case-control study is selected on the presence and absence of disease. A cross-sectional study collects all data at the same point in time and does not encounter the effect of time.

The studies described in this thesis were all cross-sectional in design. In the study on the association between carotid calcification and carotid plaques (chapter 2.1) and in the study on the association between calcification in the coronary arteries, aortic arch and carotid arteries (chapter 3.1), the cross-sectional design is most appropriate. The aim of the first study was to validate MSCT carotid calcification and the aim of the second study was to see whether calcification in one vessel bed can predict calcification in other vessel beds. For these purposes, the measures should be measured within a short time period. For the studies on determinants of coronary, aortic arch and carotid calcification (chapter 4.1, 4.2, 4.3) and the study on aortic arch and carotid calcification and history of stroke (chapter 5.1) the cross-sectional design is suitable, although not the optimal design. In the studies described in chapter 4, the research question is causal and time order is important to be able to make inferences on causality. The chapter on arterial calcification and a history of stroke provides a strong suggestion for a positive relation, which indicates that the measurement of arterial calcification may be of predictive value. Our final goal is examining the predictive value of arterial calcification for the risk of stroke, which should be examined in a prospective cohort study design. Until now little is known about associations of arterial calcification in different vessel beds with cardiovascular risk factors and cardiovascular outcomes. Therefore, our results should be seen as preliminary results waiting for confirmation from future prospective studies.

Sources of bias

Selection bias occurs when participating in a study is related to the exposure and, independent to the exposure, to the outcome. Selection bias can lead to both under- and overestimations of the effect measures. In this study, subjects who visited the research center for regular examinations of the Rotterdam Study were eligible for participation in this study. Therefore, subjects who were less healthy and unable to visit the research

center, were not included in this study, probably resulting in a healthier study population. Of 1,877 eligible subjects who visited the research center between September 2003 and December 2004, 1,003 participated. Eligible subjects that did not undergo a MSCT scan may differ from the subjects that participated concerning lifestyle, medical history, and risk factors levels. We compared characteristics of non-responders to the participants. The participants were significantly older (1.4 years older) and consisted of more men (48% versus 44%). Conventional cardiovascular risk factors did not differ between responders and non-responders.

Information bias is present when there is misclassification in obtaining information on exposure or outcome. The misclassification can be differential or non-differential. Differential misclassification is misclassification of the outcome that is related to exposure status, or vice versa, and results in a distortion (over- or underestimation) of the findings. Non-differential misclassification, which is random and independent of the other axis, leads to a dilution of the association. An example of information bias can be found in our study on alcohol drinking habits and arterial calcification as described in chapter 4.2. It is impossible to correctly assess the absolute levels of alcohol consumption because subjects are susceptible to give socially desirable answers. However, at the moment of the interview subjects were not aware of the outcome (arterial calcification) and therefore, misclassification is likely to be non-differential. This will have led to an underestimation of the reported effect estimates.

A confounding factor is a risk factor for the disease independent of the determinant under study, which is also associated with the determinant. A confounder, however, should not be an intermediate in the causal pathway between the determinant and the disease. To remove the influence of a confounding factor, the factor has to be measured and adjusted for in the statistical analyses. If the factor is not measurable, adjustment for the confounding factor is not possible and residual confounding will result. In etiologic research, we are interested in the causal relation between the determinant and an outcome, independent of confounders. For example, in the study on alcohol consumption and carotid calcification (chapter 4.2), smoking is likely to be a confounding factor. Subjects who smoke are more likely to consume alcohol and smoking is a risk factor for carotid calcification (chapter 4.1). Therefore, we adjusted for smoking in the statistical analyses. However, in the study on the validation of carotid calcification (chapter 2.1) and the study on associations between arterial calcification in different vessel beds (chapter 3.1), confounding is not an issue, because in these chapters we were not investigating causal relationships. In the study on the correlation between arterial calcification in different vessel beds, we additionally adjusted for the cardiovascular risk factors to examine which part of the associations was due to shared risk factors.

Validity of measurement

In this thesis, the first results are presented from a population-based study on prevalence, risk factors and predictive value of calcification in the coronary arteries, aortic arch and carotid arteries. Calcification in the coronary arteries assessed by EBCT has been shown to be correlated with the total area of coronary plaque [17]. Also aortic calcification as measured on a conventional radiograph has been shown to be related to aortic atherosclerosis [36]. In chapter 2.1, we investigated the association between carotid calcification assessed by MSCT and carotid plaques assessed by ultrasound, and found strong correlations between the two measurements. Therefore, carotid calcification can be used as a proxy for carotid atherosclerosis.

Clinical implications of the findings and future research

It has been shown that coronary calcification is a predictor of coronary heart disease [2, 3]. Data on the predictive value of aortic arch and carotid arteries for cardiovascular disease, however, are limited. We examined cross-sectionally the association between calcification in the aortic arch and in the carotid arteries with history of stroke. We found aortic arch and carotid calcification to be related to history of stroke, although the estimates lacked statistical significance in multivariate models. To answer the question whether calcification in the aortic arch and in the carotid arteries predicts cerebrovascular disease independent of cardiovascular risk factors, a prospective study is needed. If the predictive value of these measures can be proven, they can help to identify asymptomatic subjects at high risk of cerebrovascular disease. Whether coronary heart disease and stroke screening should be combined and which vessel bed or combination of vessel beds is most informative for determination of risk should be investigated. Also, the predictive value of arterial calcification by MSCT as compared to other non-invasive measures of atherosclerosis, such as carotid IMT by ultrasound, should be compared, and their relative advantages and disadvantages evaluated.

We presented evidence that carotid calcification is a good measure of carotid plaques. This indicates that besides coronary calcification, also carotid calcification assessed by MSCT can be used as a proxy for atherosclerosis. This supports the use of arterial calcification in studies on effects of risk factors on atherosclerosis. The finding of associations of arterial calcification with conventional cardiovascular risk factors further supports its use for these purposes. Still little is known about the reasons for differences in prevalence and in sex-dependencies of atherosclerosis in different vessel beds. The assessment of arterial calcification will especially enable the study of determinants of atherosclerosis at different sites of the arterial tree. The use of MSCT enables accurate quantification of the amount of arterial calcification as a measure of atherosclerosis. Repeating the measurement over time could provide information on progression of atherosclerosis. If this can be shown in

future studies, serial MSCT assessment of arterial calcification will be a potential tool in clinical trials to assess the effects of drug treatment.

References

1. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol. 1991;7:403-422.
2. Arad Y, Spadaro LA, Goodman K, Newstein D, Guerci AD. Prediction of coronary events with electron beam computed tomography. J Am Coll Cardiol. 2000;36:1253-1260.
3. LaMonte MJ, FitzGerald SJ, Church TS, Barlow CE, Radford NB, Levine BD, Pippin JJ, Gibbons LW, Blair SN, Nichaman MZ. Coronary artery calcium score and coronary heart disease events in a large cohort of asymptomatic men and women. Am J Epidemiol. 2005;162:421-429.
4. Kondos GT, Hoff JA, Sevrukov A, Daviglus ML, Garside DB, Devries SS, Chomka EV, Liu K. Electron-beam tomography coronary artery calcium and cardiac events: a 37-month follow-up of 5635 initially asymptomatic low- to intermediate-risk adults. Circulation. 2003;107:2571-2576.
5. Jones EF, Kalman JM, Calafiore P, Tonkin AM, Donnan GA. Proximal aortic atheroma. An independent risk factor for cerebral ischemia. Stroke. 1995;26:218-224.
6. Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. Jama. 2000;283:2810-2815.
7. Amarenco P, Cohen A, Tzourio C, Bertrand B, Hommel M, Besson G, Chauvel C, Touboul PJ, Bousser MG. Atherosclerotic disease of the aortic arch and the risk of ischemic stroke. N Engl J Med. 1994;331:1474-1479.
8. Hollander M, Bots ML, Del Sol AI, Koudstaal PJ, Witteman JC, Grobbee DE, Hofman A, Breteler MM. Carotid plaques increase the risk of stroke and subtypes of cerebral infarction in asymptomatic elderly: the Rotterdam study. Circulation. 2002;105:2872-2877.
9. Hollander M, Hak AE, Koudstaal PJ, Bots ML, Grobbee DE, Hofman A, Witteman JC, Breteler MM. Comparison between measures of atherosclerosis and risk of stroke: the Rotterdam Study. Stroke. 2003;34:2367-2372.
10. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med. 1999;340:14-22.
11. Rosvall M, Janzon L, Berglund G, Engstrom G, Hedblad B. Incidence of stroke is related to carotid IMT even in the absence of plaque. Atherosclerosis. 2005;179:325-331.
12. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Jr., Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol. 1995;15:1512-1531.
13. Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J. 1990;11 Suppl E:3-19.

14. Kuller LH, Matthews KA, Sutton-Tyrrell K, Edmundowicz D, Bunker CH. Coronary and aortic calcification among women 8 years after menopause and their premenopausal risk factors : the healthy women study. Arterioscler Thromb Vasc Biol. 1999;19:2189-2198.

15. Nandalur KR, Baskurt E, Hagspiel KD, Finch M, Phillips CD, Bollampally SR, Kramer CM. Carotid artery calcification on CT may independently predict stroke risk. AJR Am J Roentgenol. 2006;186:547-552.

16. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-832.

17. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. Circulation. 1995;92:2157-2162.

18. Allison MA, Wright CM. Age and gender are the strongest clinical correlates of prevalent coronary calcification (R1). Int J Cardiol. 2005;98:325-330.

19. Allison MA, Criqui MH, Wright CM. Patterns and risk factors for systemic calcified atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24:331-336.

20. Hoff JA, Daviglus ML, Chomka EV, Krainik AJ, Sevrukov A, Kondos GT. Conventional coronary artery disease risk factors and coronary artery calcium detected by electron beam tomography in 30,908 healthy individuals. Ann Epidemiol. 2003;13:163-169.

21. Newman AB, Naydeck BL, Sutton-Tyrrell K, Feldman A, Edmundowicz D, Kuller LH. Coronary artery calcification in older adults to age 99: prevalence and risk factors. Circulation. 2001;104:2679-2684.

22. Oei HH, Vliegenthart R, Hofman A, Oudkerk M, Witteman JC. Risk factors for coronary calcification in older subjects. The Rotterdam Coronary Calcification Study. Eur Heart J. 2004;25:48-55.

23. Wong ND, Sciammarella M, Arad Y, Miranda-Peats R, Polk D, Hachamovich R, Friedman J, Hayes S, Daniell A, Berman DS. Relation of thoracic aortic and aortic valve calcium to coronary artery calcium and risk assessment. Am J Cardiol. 2003;92:951-955.

24. Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular risk factors: impact of the analytic approach. Atherosclerosis. 2004;173:69-78.

25. Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson HE, Jr., Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB, Miller SR. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst. 2003;95:1218-1226.

26. Mukamal KJ, Kronmal RA, Mittleman MA, O'Leary DH, Polak JF, Cushman M, Siscovick DS. Alcohol consumption and carotid atherosclerosis in older adults: the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 2003;23:2252-2259.

27. Kiechl S, Willeit J, Rungger G, Egger G, Oberhollenzer F, Bonora E. Alcohol consumption and atherosclerosis: what is the relation? Prospective results from the Bruneck Study. Stroke. 1998;29:900-907.

28. Schminke U, Luedemann J, Berger K, Alte D, Mitusch R, Wood WG, Jaschinski A, Barnow S, John U, Kessler C. Association between alcohol consumption and subclinical carotid atherosclerosis: the Study of Health in Pomerania. Stroke. 2005;36:1746-1752.

Summary

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Cardiovascular disease leads to a high morbidity and mortality and consequently puts a large burden on the health care system. Therefore, early identification of high-risk subjects is needed. Several non-invasive measures of atherosclerosis exits and may be useful in identifying subjects at high risk. Existing non-invasive measures of atherosclerosis include intima-media thickness (IMT) and plaques of the carotid arteries by ultrasound and the ankle brachial index, measuring peripheral atherosclerosis.

The introduction of electron beam computed tomography (EBCT) and multislice computed tomography (MSCT) has enabled the non-invasive visualization and accurate quantification of calcification in different arteries. Quantification of calcification in different vessel beds may improve cardiovascular risk assessment. Chapter 2 describes the comparison of carotid calcification assessed by MSCT with carotid plaques measured by ultrasound. In chapter 3, the association between calcification in the different vessel beds is examined. The studies described in chapter 4 and 5 focus on determinants of arterial calcification and on its relation with history of cerebrovascular disease.

The MSCT scan study is embedded in the Rotterdam Study, a population-based study, which started with a baseline examination in 1990-1993. All inhabitants of a suburb of Rotterdam, aged 55 years and over, were invited and 7,983 agreed to participate. In 2000-2001, the cohort was extended with 3,011 subjects, also aged 55 years and over.

From September 2003 until February 2006, all participants who completed the regular examination (the fourth for the original cohort and the second center visit of the extended cohort) were invited to participate in the present study and to undergo a MSCT scan of the heart, the aortic arch and the carotid arteries. Two scans were performed: a cardiac scan and a scan which included the aortic arch and the carotid arteries.

We restricted the studies in this thesis to the first 1,003 participants who were scanned until December 2004. Data on coronary, aortic arch and carotid artery calcification were available in respectively 972, 1,002 and 1,003 subjects. Data on medical history and cardiovascular risk factors were collected during the home interview and the regular Rotterdam Study center visits, prior to the MSCT scan.

Chapter 1 is a brief introduction to the work presented in this thesis.

Chapter 2 describes the magnitude of the correlation of MSCT carotid calcification with carotid plaques assessed by ultrasound. We observed a strong association between

carotid calcification assessed by MSCT and carotid plaques assessed by ultrasound. The calcium score could accurately estimate the amount of carotid plaques. This suggests that calcification assessed by MSCT is a good measure of the presence and amount of plaques in the carotid arteries. Furthermore, a strong association was present between carotid calcification assessed by MSCT and calcified plaques by ultrasound independent of the total amount of plaque, supporting the validity of the assessment of calcified plaques by ultrasound.

Chapter 3 describes the prevalence of calcification in the coronary arteries, the aortic arch and the carotid arteries and the association between calcification in these vessel beds. The prevalence of calcification increased with age throughout the vascular bed. In the eldest group (age 80 and over), all men had calcification in the coronary arteries and all women had calcification in the aortic arch. We found moderate to strong correlations between calcification in different vessel beds. This implies that assessment of the amount of calcification in one vessel bed is only a proxy and not an accurate measure of calcification in other vessels. However, our findings support the concept of generalized atherosclerosis.

Chapter 4.1 describes the associations between cardiovascular risk factors and calcification in the coronary arteries, aortic arch and carotid arteries. We found that age, current smoking, hypertension, hypercholesterolemia, and diabetes were independently related to arterial calcification, although associations were not consistent across all vessel beds and for men and women. A tendency was present for an inverse association with obesity in women, while no association of low HDL-cholesterol with arterial calcification was present. Age and current smoking were the strongest risk factors for arterial calcification in all vessel beds, except for coronary calcification in men. Possibly, men who smoke are prone to die from coronary heart disease at an earlier age due to selective mortality. The inverse association with obesity in women, but not in men, may be related to estrogen production in fat mass in women after menopause.

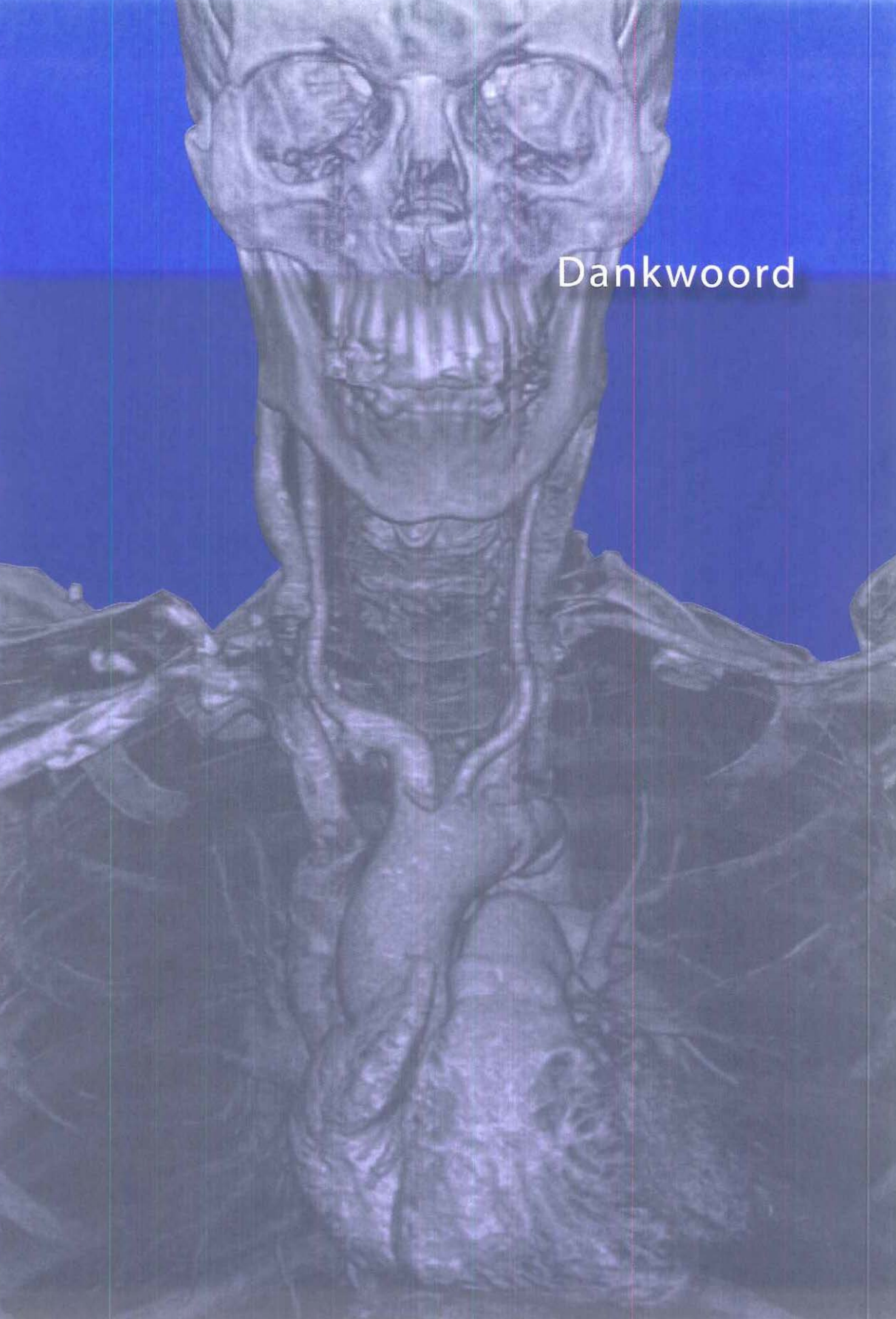
Chapter 4.2 describes the relation between alcohol consumption and carotid calcification. Opposite trends were found for wine drinking (inverse) and beer and liquor drinking (positive). However, the associations lacked statistical significance. Although we included nearly 1,000 subjects, this study may lack power to show significant associations. Therefore, larger numbers are needed to confirm the results.

Chapter 4.3 describes the association of arterial stiffness (aortic and carotid stiffness) with aortic arch and carotid calcification. We found an association between aortic stiffness and carotid calcification. Furthermore, an association was found between carotid stiffness and

aortic arch and carotid calcification. The results confirm the association between arterial stiffness and atherosclerosis.

In **chapter 5.1**, the association of aortic arch and carotid calcification with history of stroke is described. We found that subjects with a history of stroke had an increased risk of having high calcium scores in the aortic arch and the carotid arteries compared with subjects without a history of stroke. After adjustment for conventional vascular risk factors, the associations slightly decreased but were no longer statistically significant. The loss of significant associations may be due to lack of power. Therefore, our findings need confirmation in larger studies. Finally, a prospective study is needed to establish the strength of the predictive value.

In the general discussion in **chapter 6**, methodological considerations are discussed to adequately interpret the study results described in this thesis. Moreover, suggestions for future research are given.



Dankwoord

Dankwoord

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Arlette

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