Breast arterial calcifications and heart disease risk in women

Angela H.E.M. Maas

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Breast arterial calcifications and heart disease risk in women

Arteriële calcificaties in de borst en het risico op hart- en vaatziekten bij vrouwen

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Angela Helena Elisabeth Maria Maas
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"Perhaps it is better to wake up after all, even to suffer,
than to remain a dupe to illusions all one's life."

Kate Chopin (1850−1904*), The Awakening, 1899*

Voor Ernst, Arthur en Eric

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CHAPTER 1

Introduction

Atherosclerosis develops later in women compared to men. This is mainly caused by the protective role of circulating estrogens on the vascular wall during the fertile period of life.[1] Atherosclerotic plaque morphology is different and less extensively in women before menopause compared to women after menopause. In premenopausal women estrogens have a stabilizing effect on the fibrous cap of atherosclerotic plaques to prevent rupture.[2] After menopause atherosclerotic lesions develop into more complex plaques with calcifications involved.[3] Circulating estrogen levels are inversely associated with the calcium content of atherosclerotic plaques.[4]

Abdominal aortic calcifications on chest and lumbar X-rays are important markers of subclinical atherosclerosis,[5,6] showing an increase of calcium deposits in women after menopause.[7] The clinical use of conventional X-ray techniques for screening on arterial calcium has been overruled in the past decade by novel diagnosting modalities like electron beam computed tomography (EBCT) and multislice computed tomography (MSCT). The amount of calcium present in the coronary arteries has shown to be a strong marker of plaque burden and is currently used for assessment of future coronary heart disease (CHD) risk in asymptomatic patients.[8-10] In women, calcium scores are lower in all age groups compared to men and the appearance of calcium occurs later, mostly after the age of 65 years.[11]

A disadvantage of these novel screening techniques are the costs and feasibility for its widespread application. Moreover, preventive strategies in patients at risk need to start early in life, even before atherosclerotic disease is detectable, to have their maximum effect in risk reduction. Simultaneously, growing expenses in health care demand a more cost-effective use of available screening techniques.[12] In women, calcifications are often seen in the arteries on mammograms and it is not known whether mammograms may be used for screening purposes of CHD risk. As mammograms are often made in established breast cancer screening programs, its simultaneous use as a screening tool for CHD prevention could be very cost-effective. Further, breast cancer screening programs mostly start at the age of 50, when CHD risk factors in the general population of women are amply present and the age-adjusted CHD risk is still relative low.

Calcifications in breast arteries

Calcifications in the arteries on mammograms can vary from small particles in a single breast artery to thick colums of calcium along an entire artery and its side branches, looking like a railroad track. The reported prevalence of breast arterial calcifications (BAC) with conventional and evolving mammographic techniques is 3% up to 35% in women from breast cancer screening programs.[13-15] From pathological studies it is known that these calcifications are located in the media of the vessel wall, comparable with the media calcification that is often seen in the peripheral vessels of diabetics.[16,17] In some studies BAC was found to be predominantly associated with older age and diabetes mellitus, while others found the association with diabetes mellitus and various CHD risk factors to be controversial.[18-21] In a previous study in 12,239 women from a breast cancer screening program, we found that mortality in women with diabetes and BAC was 74% higher compared to diabetics without BAC.[22] In a recent study however, no association could be found between the presence of BAC and atherosclerotic lesions at coronary angiography.[23]

The clinical significance of calcium deposits in the breast arteries remains yet unclear and it has to be determined whether mammograms can be used as a screening tool for atherosclerotic disease.

Outline of this thesis

The main goal of this thesis was to investigate whether arterial calcifications on mammograms can predict cardiovascular risk in women.

In **Chapter 2** we summarize the current literature on the effects of sex hormones and hormone therapy (HT) on atherosclerotic disease in women. The discrepancies between the beneficial effects of HT in the observational studies and the randomized clinical trials are discussed.

Chapter 3 investigates the prevalence of mammographic arterial calcifications in women at high risk of coronary heart disease. For this purpose, we used data of women

participating in the Raloxifene Use for The Heart (RUTH) study. The high accumulation of cardiovascular risk factors in this cohort enables to study the association between separate CHD risk factors and the prevalence of calcifications.

In **Chapter 4** we describe the prevalence of mammographic arterial calcifications in a breast cancer screening population of women, aged 49−70, participating in the European Prospective Investigation into Cancer and Nutrition (Prospect-EPIC) study. Due to the detailed information available on reproductive factors we can investigate new risk factors for BAC, such as pregnancy and lactation.

In **Chapter 5** we investigate whether breast arterial calcifications are associated with inflammatory markers for heart disease risk, as it is now considered that atherosclerosis is an inflammatory disease.

Chapter 6 describes our study in a subset of women from the RUTH cohort to evaluate a possible relation between microalbuminuria as an established cardiovascular risk marker and calcifications in the breast arteries.

In **Chapter 7** we study nutritional vitamin K intake and breast arterial calcium in women from the Prospect-EPIC cohort as it is has been described that a low dietary vitamin K intake is associated with vascular calcification.

In **Chapter 8** we investigate whether calcifications in the breast arteries are predictive of subsequent development of calcifications in the coronary arteries, in a subset of women from the Prospect-EPIC study.

In **Chapter 9** we evaluate the follow-up mammograms from women participating in the RUTH-study with a focus on progression / regression of calcifications in the breast arteries.

Chapter 10 describes the relevance of our findings in this thesis and the aetiologic mechanisms that are involved in calcifications in the breast arteries.

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CHAPTER 2

‘Rise and fall’ of hormone therapy in postmenopausal women with cardiovascular disease

Angela H.E.M. Maas, Yvonne T. van der Schouw
Diederick E. Grobbee and Yolanda van der Graaf

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Abstract

Whereas observational data for postmenopausal women using hormone therapy (HT) have shown a protective effect against cardiovascular disease, prospective, randomized trials have demonstrated a harmful effect on the vascular system.

This study describes the effects of HT on lipids, hemostatic parameters, inflammation, and the vascular wall. Reasons for the different results of observational and experimental studies of HT are postulated. The timing of hormonal supplementation seems crucial. Used chronically, HT has no harmful effects; however, first-time use of HT after a recent cardiovascular event results in an early increase in adverse cardiovascular events. In most observational studies, women started HT for postmenopausal symptoms, whereas in experimental studies women started HT 10 to 20 years or longer after menopause.

Cumulative evidence supports the hypothesis that HT has more effect in maintaining vascular health than in alleviating endothelial dysfunction. HT has not proven beneficial in the long term in women at risk of a cardiovascular event. The interval between the menopause and the start of HT plays a crucial role in the effectiveness of HT in the vascular system.

Introduction

Cardiovascular disease develops 10 to 15 years later in women than in men and is the major cause of death in women older than 65 years of age.[1] It is assumed that exposure to endogenous estrogen has an important role in the delayed manifestation of atherosclerotic disease in women. In the perimenopausal period, estrogen levels decline to about 20% of levels in the fertile period. Data from the Framingham Heart Study and Nurses' Health Study have indicated an increased risk of cardiovascular disease in young women after bilateral oophorectomy, a risk that did not occur in women using hormone therapy (HT) after surgery.[2,3] Other observational data support the hypothesis that longer exposure to endogenous estrogens protects against cardiovascular disease (CVD)[4-6] For each year's delay in the onset of menopause, the cardiovascular mortality risk decreases by 2%. This increased risk of cardiovascular disease with menopause at a younger age is more prominent at younger biological ages and is no longer important after the age of 80.[5] In the Nurses' Health Study the adverse effect of early natural menopause on CVD risk was also found, although in this study the effect was restricted to women who smoked.[6]

The cardioprotective effects of estrogens in the fertile period support the use of HT for preventive strategies in the postmenopausal years. Thus far, however, only observational data on postmenopausal use of HT have shown a beneficial effect against the occurrence of cardiovascular disease. In contrast, prospective, randomized trials with HT have demonstrated a harmful effect on the vascular system. These conflicting results complicate the understanding of postmenopausal HT use.

Metabolic changes during menopause and metabolic effects of HT

Many physiological changes occur in the years around menopause (mean age 51 years), most importantly atherogenic metabolic changes affecting the lipid profile: total cholesterol levels rise 10%, low-density lipoprotein (LDL) cholesterol levels rise 14% and lipoprotein (a) levels increase 4 to 8%, whereas high-density lipoprotein (HDL) cholesterol

levels remain unchanged.[7] Hemostatic parameters move to a more thrombogenic state, with higher levels of fibrinogen, factor VII and plasminogen-activator inhibitor-1.[8] Homocysteine levels also increase after menopause.[9] A direct association between blood pressure, body weight, and menopause has not been demonstrated.

The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, involving 875 healthy postmenopausal women, showed that estrogen alone or in combination with a progestin caused lipoprotein levels to revert to those of the premenopausal state.[10] Depending on the type of progestin used, changes in lipoprotein levels were slightly different: medroxyprogesterone acetate (MPA) attenuated the beneficial effects of estrogen on the lipoprotein levels, whereas micronized progestin did not but had no detrimental effect. No significant changes in blood pressure, insulin levels, or fibrinogen levels were found among the different treatment groups. A possible negative effect of oral estrogens is an increase in the levels of triglycerides and very low-density lipoprotein (VLDL) cholesterol, the clinical significance of which is less clear.[11] The beneficial effects of estrogens on cholesterol metabolism are also related to the inhibition of LDL cholesterol oxidation and penetration in the vessel wall. Transdermal application of estrogens has been shown to be less beneficial to the lipid parameters than oral estrogens.[12]

Several studies have demonstrated a more than twofold rise in C-reactive protein (CRP) levels after initiation of oral HT, but the clinical significance of this finding is not yet clear.[13] As more data emerge showing that HT use by high-risk women causes an increase in cardiovascular events, it is assumed that the increase in CRP levels promotes vascular inflammation and plaque instability.[14] Transdermal HT does not increase CRP levels.

Vascular effects on HT

Vasodilatation

Alterations in serum lipids are considered to account for one third of the assumed clinical benefits of estrogen, but direct effects on the arterial wall may be at least as

important. Estrogen causes vasodilatation by a rapid (5–20 minutes) activation of nitric oxide synthesis in endothelial cells.[15] In small experimental studies, intravenous administration of estradiol caused direct vasodilatation in healthy women as well as in women with atherosclerotic disease.[16,17] Recent data from 1,636 women participating in the Cardiovascular Health Study, a longitudinal study of cardiovascular risk factors in men and women older than 65 years of age, only showed a significant association between HT and flow-mediated response in healthy postmenopausal women.[18] HT had no effect on brachial artery blood flow in older women (>80 years), in women with documented cardiovascular disease, or in women with a combination of cardiovascular risk factors. These data suggest that healthy endothelium is sensitive to estrogens whereas endothelium damaged by atherosclerotic disease is not.

Atherosclerosis

The protective effects of estrogens against atherosclerosis occur over a period of hours or days and are mediated by estrogen-α and estrogen-β receptors in the vascular wall.[15,19] In monkeys, estrogen inhibits the development of atherosclerotic plaque formation when given directly after ovariectomy.[20] When estrogens were started 2 years after ovariectomy, there was no inhibition of coronary atherosclerosis. In rats with carotid intima damage, estrogens inhibited intima hyperplasia and smooth muscle cell proliferation only when given in the early stages of vascular damage.[21] Administration of estrogens after 3 days did not have a beneficial effect on the vascular wall. In pigs, stents coated with 17β-estradiol inhibited neo-intima proliferation with a reduction in restenosis after percutaneous coronary interventions.[22] These findings of animal studies support the hypothesis that estrogen has beneficial effects, especially in the early stages of atherosclerotic disease and in the early stages of vascular wall repair.

Cardiac hypertrophy

An increasing amount of data from animal studies show protective effects of estrogen against the development of cardiac hypertrophy.[23,24] The lack of estrogen in

postmenopausal women may be responsible for the increase in ventricular hypertrophy seen in women in older age.[25]

HT and cardiovascular events

Extensive data from observational studies suggest that HT has a beneficial effect on the occurrence of cardiovascular disease in postmenopausal women, with a risk reduction of 35 to 50%.[26,27] The most pronounced risk reduction was reported for current HT users, but in past users a protective effect could still be found.[28] Most studies included healthy postmenopausal women without a history of cardiovascular disease and who had a relatively healthy lifestyle. The hormonal therapy was mostly started in the direct perimenopausal or postmenopausal period for the relief of menopausal symptoms. It is difficult to compare these studies because different types of HT preparations were used for different lengths of time.

More recent observational studies have yielded data showing that women are at increased risk of cardiovascular disease: in a retrospective subanalysis of 2,489 women from the Nurses' Health study, with a previous myocardial infarction (MI) or documented atherosclerosis, the risk reduction for a recurrent event was still 35% (95% CI, 0.45−0.95) among current HT users compared with never users, with up to 20 years of follow-up.[29] Short-term HT use (<1 year), however, was associated with a 25% increase in the rate of cardiovascular events. Observational data from the National Registry of Myocardial Infarction-3 for 114,724 women older than 55 years with confirmed MI also found a mortality risk reduction of 35% in current HT users compared with nonusers (HR 0.65, 95% CI 0.59−0.72).[30] Most of these women had started the HT in the postmenopausal period for menopausal complaints or for the prevention of osteoporosis.

The Estrogen and Prevention of Atherosclerosis Trial investigated 199 postmenopausal women older than 45 years (mean 60 years), with LDL-cholesterol levels higher than 3.37 mmol/L and no clinical evidence of coronary artery disease.[31] During 2 years of treatment, carotid intima-media thickness increased at a slower rate in women

receiving 1 mg 17ß-estradiol than in those on placebo. Although the changes in carotid intima-media thickness were reduced equally by estrogens and statins, there was no additive effect of the two treatments. This suggests that, in healthy endothelium, estrogen can inhibit atherosclerosis, and that when HT is started in the direct postmenopausal period, the vascular endothelium remains sensitive to the vasoprotective effects of estrogens.

Recently, the first data from the Women's Health Initiative (WHI) were published. This trial, started in 1993, investigates the effects of various HT regimens on the occurrence of heart disease, breast cancer, osteoporotic fractures, and colorectal cancer in healthy postmenopausal women, 50–79 years of age.[32,33] A total of 16,608 women were randomized to treatment with a combination of 0.625 mg conjugated estrogens and 2.5 mg MPA (n = 8,506) or placebo (n = 8,102). After a mean follow-up of 5.2 years, the study was stopped prematurely because overall health risks exceeded the benefits, primarily due to an increase in invasive breast cancer (26%, 166 cases), cardiovascular events (29%, 164 cases), and stroke (41%, 127 cases) in the treatment group. The excess cardiovascular events (164 in the treatment group compared with 122 on placebo) were mostly due to nonfatal MI; no significant differences in coronary heart disease (CHD) deaths or revascularization procedures were seen. As expected, a twofold (151 compared with 67) increase in the incidence of thromboembolic events was seen in women on HT. All-cause mortality was not different between both treatment arms (HR 0.98. 95% CI 0.82−1.18). Although the study was designed as a primary prevention trial, 67% of the included women were older than 60 years and had many risk factors. This confirms that early intervention with HRT is important in the progression of subclinical atherosclerosis and endothelial dysfunction.

HT in cardiovascular patients

Observational data in 337 women show that current users of HT have fewer cardiovascular events (12% versus 35%) and better survival (93% versus 75%) after elective percutaneous transluminal coronary angioplasty (PTCA) compared with

nonusers.[34] Others have confirmed the better outcome after elective PTCA and coronary stenting in current users of HT.[35,36] Among women undergoing coronary artery bypass surgery, chronic HT use was associated with a significantly improved, 5-year survival.[37] However, initiation of HT after a recent MI caused a significantly higher (44%) risk of unstable angina pectoris, death, and reinfarction compared with the risk in chronic HT users and never-users.[38] This early increase in adverse cardiovascular events is attributed to proinflammatory and thrombogenic effects of HT. In a recent randomized, placebo-controlled trial of 1,017 women, aged 50–69 years, who had experienced a first MI, unopposed 17β-estradiol was neither harmful nor beneficial in terms of frequency of reinfarction or cardiac death after 2 years of treatment.[39] Importantly, noncompliance to treatment was extremely high (>50%) after 1 year in the active treatment group, which weakened the power of this study.

Despite the benefits of HT in observational studies, the first prospective randomized trial of 2,763 postmenopausal women (mean age 67 years) with documented coronary heart disease, the Heart and Estrogen/progestin Replacement Study (HERS), revealed no benefits of a combined HT regimen with 0.625 mg conjugated equine estrogen and 2.5 mg MPA in the prevention of CHD compared with placebo after 4.1 years of treatment.[40] In the first year of treatment, however, coronary events increased by more than 50% in the treated group. This excess in events was not seen in patients who were on statin therapy.[41] It is assumed that initiation of HT destabilizes coronary plaques, whereas statins are postulated to have a stabilizing effect on atherosclerotic plaques. The combined therapy seems to attenuate the detrimental effect of HT on plaque stability. Others have confirmed that combined therapy with a statin reduces the rise in CRP levels caused by estrogen monotherapy[42] After the first year of treatment, there was a non significant trend to a reduction in cardiovascular events in treated women, but this trend never reached significance. Prolongation of the follow-up to 6.7 years (HERS II) did not show any benefit of HT on the CHD event rate.[43] An extensive post-hoc analysis of 86 subgroups in the HERS did not identify a specific subgroup in which postmenopausal HT was beneficial or harmful.[44] Genetic markers in HERS are still under investigation.

The Estrogen Replacement and Atherosclerosis (ERA) trial prospectively evaluated the angiographic progression of present coronary atherosclerosis in 309 patients, assigned to 0.625 mg conjugated estrogens alone or in combination with 2.5 mg MPA or placebo.[45] After an average follow-up of 3.2 years, no differences were found in coronary vessel diameter and clinical outcomes. As in HERS, the women included in ERA were 66 years old and had been postmenopausal on average 20 years As the aging endothelium becomes less sensitive to estrogens, one could argue that it is not appropriate to start HT in this age group.[46] Further genetic analysis revealed that HDL levels increased significantly more with HT use in women with the C/C genotype of the estrogen-α receptor than in women with other genotypes.[47] This might be the first step to a better identification of women who might benefit the most from HT. Recent data from two more randomized angiographic studies, the WAVE and WELLHART, have provided further information about the lack of beneficial effects of HT on the progression of coronary atherosclerotic lesions in older women with documented coronary artery disease.[48,49]

The Women's Estrogen for Stroke Trial (WEST) was the first randomized study to use the more natural 17β-estradiol in 664 postmenopausal women who recently had experienced a transient ischemic attack or stroke.[50] Estrogen was not found to have a beneficial effect compared with placebo on recurrent stroke or mortality after a mean follow-up of 2.8 years. In the first six months of treatment, there was a significant increase in mortality or recurrent stroke in the women treated with estrogen (HR 2.3, 95% CI 1.1−5.0), reflecting early deleterious effects in high-risk women. As in the HERS and ERA trials, patients included in WEST were relatively old (71 years).

In the Postmenopausal Hormone Replacement against Atherosclerosis trial, 321 postmenopausal women at risk for a first cardiovascular event were investigated for adverse changes in carotid intima-media thickness after 48 weeks of treatment with 17β-estradiol, a combined regimen with a progestin, or placebo. In neither active treatment group was the progressive increase in carotid intima-media thickness slowed, although both active treatments decreased LDL levels and fibrinogen levels.[51] The duration of this study was too short to draw further conclusions.

In summary, in chronic HT users hormonal therapy is not harmful during coronary interventions, whereas starting HT after a recent cardiovascular event causes an early increase in adverse cardiovascular events and has no beneficial effects in secondary prevention in the long term. The concomitant activation of the thrombotic system by HT and the vulnerability of existing coronary plaques play an important role in the negative effects of HT on the vascular system. These data support the hypothesis that the vascular endothelium is susceptible to the beneficial effects of estrogens when started early after menopause, but is no longer susceptible with later administration.

Failure of HT or failure of clinical trials?

Despite the consistent benefit of HT on cardiovascular events in past and more recent observational studies, randomized trials thus far have failed to show any benefit (Table 1). On the contrary, more reports of early negative effects of initiating HT in women at risk for cardiovascular disease are emerging.[29,52] The discrepancy between the proven beneficial effects of estrogens on the cardiovascular system and the lack of positive effects in clinical practice is as yet unsolved. Recent meta-analyses of observational HT trials have shown no benefit of HT on the cardiovascular disease event rate in postmenopausal women and emphasize the healthy user bias of most observational studies.[53,54]

One important aspect in comparing observational data and randomized studies is the age of the patients and the time since menopause. Nearly all observational data are derived from women who started HT for postmenopausal symptoms, and thus therapy was started in the early postmenopausal period. In the randomized HT trials, however, the indication for inclusion was age 50–79 years without clinical signs of atherosclerotic disease (WHI) or with clinically manifest cardiovascular disease in the secondary prevention trials. In all these studies, the women no longer suffered from perimenopausal complaints and started HT 10 to 20 years or even longer after menopause.[55-57] The time when HT is started relative to the menopause appears to be crucial in the effectiveness of HT in the vascular system.

The authors of the WHI stated that the study was done in 'apparently' healthy, postmenopausal women.[32] As endothelial function declines with aging, long before clinical symptoms of atherosclerotic disease become manifest, one can argue whether the women included in the WHI were as healthy as they were assumed to be. Two-thirds (67%) of the women in the WHI were older than 60 years, and many had risk factors for coronary heart disease. Recent data from the Cardiovascular Health Study support the hypothesis that estrogens might be more important in maintaining vascular health than in treating subclinical endothelial dysfunction.[18] Therefore the first data from the WHI, although it was intended to be a primary prevention trial, might indicate again that HT does not work in 'secondary' prevention.[58]

The proinflammatory and thrombogenic effects of HT are still under investigation and are probably more complex than initially thought. In a nested case-control study of 304 participants within the still ongoing observational study of the Women's Health Initiative (WHI-OS), it was found that HT was associated with significantly elevated levels of CRP but not with other inflammatory markers such as interleukin-6.[59] Total levels of these inflammatory biomarkers seem to be even more important in predicting coronary risk than the use or nonuse of HT per se. This suggests that more factors than HT are involved in creating a systemic inflammatory state that causes the early risk of cardiovascular events in healthy, postmenopausal women.

Many have attributed the failure of HT in secondary prevention trials to the rapidly growing use of statins. Although women were less well represented in the large statin trials, most studies showed a comparable or even higher risk reduction in coronary heart disease events in women than in men.[60,61] Statin users in HERS had a lower CHD event rate than nonusers (HR 0.79, 95% CI 0.63−0.99) and a mean 30% lower rate of mortality from all causes compared with nonusers.[41] Concomitant statin and HT use diminished the early risk of cardiovascular events seen with HT in the first year of treatment but did not have an additional benefit of HT throughout the study. An interesting finding was that the use of a statin with or without HT resulted in a much lower incidence of venous thromboembolic events (HR 0.40, 95% CI 0.18−0.91).

Table 1. Randomized controlled prevention studies with HT.

Study [Ref.]	Publ. Year	Population	Type prevention	Medication* and follow-up duration	Results n = CVD event rate
HERS Heart and Estrogen/progestin Replacement Study [40]	1998	2763 women with documented CAD >55 yrs	secondary	CEE + MPA or placebo (follow-up 4.1 yrs)	No benefit HT n = 179 HT n = 182 placebo
WEST Women's Oestrogen for Stroke Trial [50]	2001	644 women >55 yrs after stroke/TIA	secondary	E or placebo (follow-up 2.8 yrs)	No benefit HT n = 99 E n = 93 placebo
ESPRIT oEStrogen in the Prevention ReInfarction Trial [39]	2002	1017 women 50–69 yrs after first MI	secondary	E2 or placebo (follow-up 2 yrs)	No benefit HT n = 83 E2 n = 91 placebo
PHOREA Postmenopausal HormOne REplacement against Atherosclerosis [51]	2001	321 women >55 yrs with atherosclerosis (IMT-carotis)	secondary	E or E + G or placebo (follow-up 48 weeks)	No benefit HT
ERA Estrogen Replacement and Atherosclerosis [45]	2000	309 women with ≥1 stenosis coronary artery ≥30% (QCA)	secondary	CEE or CEE + MPA or placebo (follow-up 3.2 yrs)	No benefit HT

Table 1 (continued).

Study [Ref.]	Publ. year	Population	Type prevention	Medication* and follow-up duration	Results n = CVD event rate
WAVE Women's Angiographic Vitamin and Estrogen trial [48]	2002	423 women with ≥1 stenosis coronary artery 15−75% (QCA)	secondary	CEE or CEE + MPA and/or Vit. E,C or placebo (follow-up 2.8 yrs)	No benefit HT No benefit vit. C and E
WELLHART Women's Lipid Lowering Heart Atherosclerosis Trial [49]	2002	226 women with ≥1 stenosis coronary artery ≥50% (QCA)	secondary	CEE or CEE + MPA or placebo (follow-up 3 yrs)	No benefit HT
EPAT Estrogen in the Prevention of Atherosclerosis [31]	2001	199 healthy postmenopausal women >45 years with LDL-C >3.37 mmol/L (IMT-carotis)	primary	E or placebo, with statin if LDL-C >4.15 mmol/L (follow-up 2 yrs)	Benefit HT equal to statin
WHI Women's Health Initiative [32]	2002	16.608 healthy postmenopausal women 50−79 yrs	primary	CEE + MPA or placebo (follow-up 5.2 yrs)	No benefit HT n = 694 HT n = 546 placebo

*CEE= 0.625 mg conjugated estrogens; MPA= 2.5 mg medroxyprogesterone acetate.
E2 = 2 mg estradiol valerate; E = 1 mg 17β-estradiol; G = 0.025 mg gestodene.

Will alternatives to HT fulfill the promise?

The recent negative results of the WHI and secondary prevention trials indicate that postmenopausal therapy with estrogen and progestin results in increased risks of disease, does not make asymptomatic women feel better, and does not improve cognition. There seems to be no role for HT in the treatment of women without menopausal symptoms. Women with vasomotor symptoms must weigh the risks associated with treatment against the benefit of symptom relief.[62-64]

Selective Estrogen Receptor Modulators such as raloxifene are not suited for the treatment of perimenopausal symptoms but are effective in the treatment of osteoporosis and are likely to reduce the incidence of invasive breast cancer.[65] Raloxifene decreases levels of LDL-cholesterol while levels of HDL-cholesterol and triglycerides remain unchanged.[66] Other changes in biochemical markers are a reduction in lipoprotein (a), fibrinogen, and homocysteine with no change in plasma activator inhibitor-1. Thrombogenic side effects are comparable to those of HT. An important difference with HT is that raloxifene does not increase CRP levels.[67]

Although in experimental animals the cardioprotective effect of raloxifene on endothelial function is less well documented compared with that of HT, the first clinical data in women are promising. In a secondary retrospective subgroup analysis of 1,035 women with increased cardiovascular risk included in the Multiple Outcomes of Raloxifene Evaluation trial, a large osteoporosis study, no early increase in adverse cardiovascular events was noted in the first year of treatment, whereas there was a significant reduction in cardiovascular events of 40% (RR 0.60, 95% CI, 0.38−0.95) after 4 years of treatment with raloxifene.[68] The effects of raloxifene are currently being investigated in the Raloxifene Use for The Heart randomized trial involving 10,101 postmenopausal women older than 55 years at risk of a cardiovascular event. Primary end points of the study are invasive breast cancer, acute coronary syndromes, and cardiovascular mortality.[69] As in the other secondary prevention trials, the mean age of the participants in the study is relatively high at 68 years.[70]

Conclusion

Cumulative evidence supports the hypothesis that HT has more effect in maintaining vascular health than in restoring endothelial function. The initiation of HT in patients at risk of a cardiovascular event results in more cardiovascular complications and has not been proven beneficial in the long term. The time when HT is started relative to the menopause plays a crucial role in the effectiveness of the hormones in the vascular system. It is still not known whether starting HT shortly after the menopause can delay clinical signs of atherosclerotic disease.

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CHAPTER 3

Prevalence and determinants of breast arterial calcium in women at high risk of cardiovascular disease

Angela H.E.M. Maas, Yvonne T. van der Schouw

Willem P.Th.M. Mali, Yolanda van der Graaf

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Abstract

BACKGROUND: Breast arterial calcifications (BAC) are commonly seen on mammograms and its frequency increases with age, especially after menopause. Several studies based on data from breast cancer screening programs have suggested that BAC may be used as a risk marker of coronary heart disease (CHD).

METHODS: We studied the prevalence of BAC in 600 women at high risk for cardiovascular events and assessed whether classical CHD risk factors are independently related to these calcium deposits. Baseline mammograms were scored for the presence of BAC in one or both breasts by two independent observers. Logistic regression analysis was used to investigate the independent effect of CHD risk factors on the occurrence of BAC.

RESULTS: BAC was present in 138 of 600 (23%) women. The prevalence of BAC was highest (35.4%) in women older than 70 years (OR 2.9; 95% CI 1.96−4.33) and increased significantly with increasing cardiovascular risk score ($p = 0.03$). Multivariate logistic analysis showed that age (OR 1.1, 95% CI 1.07−1.15) and diabetes (OR 1.6, 95% CI 1.01−2.49) were independently associated with BAC. Parity was highly correlated with the prevalence of BAC, with a relative risk of 6.1 (95% CI 2.26−16.39) in women with 6 or more children compared with that of women with no or 1 child.

CONCLUSION: BAC is common in women at high risk of cardiovascular disease and increases with age, diabetes, parity, and the total atherosclerotic burden.

Introduction

Breast cancer screening programs may offer a new way to identify women at high risk of cardiovascular events and have the advantage that these programs are already running in many western countries. Accumulating evidence suggests that the presence of breast arterial calcium (BAC) in mammograms may serve as a marker for generalized vascular disease.[1,2] In a large cross-sectional study involving 12,239 women, 50 to 69 years of age, who participated in a breast cancer-screening program, we found a prevalence of BAC of 9% with a significantly higher prevalence of 15.4% in diabetic subjects.[2-5] At follow-up, overall mortality was significantly higher in women with BAC (OR 1.29, 95% CI 1.06−1.58) than in women without BAC, with an excess mortality in diabetic women of 74% (OR 1.74, 95% CI 1.19−2.56). However, to date it is not established to what extent the various determinants of cardiovascular risk contribute to the presence of these calcifications. We analyzed the mammograms of 600 participants included in the high-risk cohort of the Raloxifene Use for The Heart (RUTH) study.

Methods

Population

The 610 participants of the Dutch subset of patients from the RUTH study were included in this cross-sectional study. The RUTH trial was started in 1998 and is a multicenter, randomized, double-blind, placebo-controlled clinical trial involving women from 26 countries. Its aim is to evaluate treatment with 60 mg raloxifene versus placebo in 10,101 postmenopausal women at high risk for major cardiovascular events. The two separate primary endpoints are coronary events and invasive breast cancer. The trial design, methods, and participant characteristics at baseline have been described in detail elsewhere.[6,7] Briefly, inclusion criteria were age ≥55 years, ≥1 year postmenopausal with established coronary heart disease (i.e., prior myocardial infarction (MI), coronary artery

bypass grafting, percutaneous coronary intervention (PCI), or angiographic evidence of a 50% occlusion of one or more major coronary arteries) or an increased risk of a major coronary event, based on the presence of multiple cardiovascular risk factors. Among the main exclusion criteria were recent MI (<3 months), recent PCI (<6 months), New York Heart Association class III or IV heart failure, need for estrogen replacement therapy, or a history of breast cancer, endometrial carcinoma, or deep venous thrombosis or pulmonary embolism. An inclusion criterion for women without established coronary heart disease was the presence of multiple factors (minimum score 4) shown in epidemiologic studies to increase the risk of MI and coronary death, such as smoking, hypertension, hyperlipidemia, and diabetes mellitus (Table 1).

Table 1. RUTH trial inclusion criteria (at least ≥4 points for inclusion)*

Criteria	**Points**
- Myocardial infarction (MI) 3−36 months before randomization	4
- Angina pectoris with documented coronary disease on angiogram	4
- Coronary angioplasty 6−36 months before randomization	4
- Coronary bypass 3−36 months before randomization	4
- Lower extremity arterial disease, documented by symptoms, or ankle/brachial index <0.9, revascularization or nontraumatic amputation	4
- Current smoker AND hypertension AND hyperlipidemia	4
- Diabetes mellitus (fasting glucose >140 mg/dl OR on medication)	3
- MI > 36 months before randomization	2
- Coronary angioplasty >36 months before randomization	2
- Coronary bypass >36 months before randomization	2
- Age ≥70 years	2
- Age >65 years and <70 years	1
- Current smoker by self report (≥10 cigarettes for 6 months before enrollment)	1
- Systolic blood pressure >160 or diastolic blood pressure >95 mmHg OR on medication	1
- Low-density lipoprotein cholesterol >160 mg/dl or high-density lipoprotein cholesterol >45 mg/dl with triglycerides >250 mg/dl OR on medication)	1

* Adapted from: Mosca L et al. *Am J Cardiol* 2001; 88: 392-395.

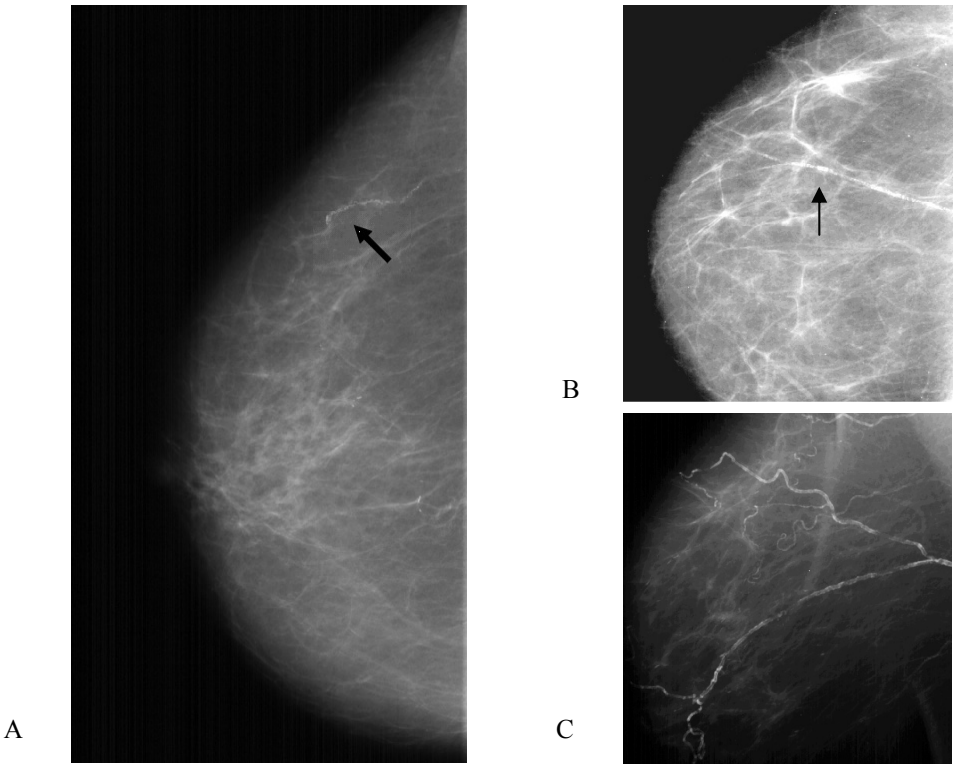

Figure 1. Photographs representing mammograms: (A) mild breast arterial calcium (BAC); (B) moderate BAC; (C) severe BAC.

Mammograms

Standard mammograms (cranio-caudal and lateral views) were made for all women included in the RUTH trial at baseline, within 1 year of randomization, before study entry. The mammograms were stored at the radiology departments of the participating hospitals. The baseline mammograms of 600 of the 610 women included in the Dutch arm of the study could be retrieved by the Department of Radiology at the University Medical Center Utrecht and BAC was scored by 2 independent observers, blinded to the clinical data of the patients, using the criteria of Kemmeren et al.[3] If there was disagreement, the 2 observers reviewed the mammograms together to reach consensus.

BAC was characterized by 2 parallel lines or rings of calcification on mammograms of the right, the left, or both breasts (Fig. 1). The intensity of BAC was graded as follows: grade 1, mild BAC (the artery(s) was faintly outlined by calcium) (Fig. 1A); grade 2, moderate BAC (the artery(s) was distinctly outlined by calcium) (Fig. 1B); and grade 3, severe BAC (the artery(s) was distinctly outlined by thick columns of calcium) (Fig. 1C).

Baseline measurements

The baseline data on cardiovascular risk factors were recorded at randomization before study entry. Body mass index was calculated as the ratio of body weight to height (in meters) squared (kg/m²). Blood pressure was measured twice with the subject in sitting position after 5 minutes of rest. Fasting venous blood samples were collected locally and analyzed centrally. Diabetes mellitus was defined as a fasting serum glucose level >7.8 mmol/l (140 mg/dl) or use of oral hypoglycemic medications or insulin. Hypertension was defined as use of antihypertensive medication or a systolic blood pressure >160 mmHg or a diastolic blood pressure >95 mmHg on at least two occasions before randomization.

Hyperlipidemia was defined as use of lipid-lowering medications or a fasting low-density lipoprotein cholesterol level >4.14 mmol/l (>160 mg/dl) or a fasting high-density lipoprotein cholesterol level <1.16 mmol/l (<45 mg/dl) with fasting triglycerides >2.82 mmol/l (>250 mg/dl). Smoking was defined as smoking an average of 10 or more cigarettes a day in the 6 months before randomization. All enrolled patients were ≥55 years and had not had menses for >1 year. Parity was defined as the total number of live and still births after pregnancy.

Data analyses

BAC was defined as the presence of calcium in either one or both breasts. The prevalence of BAC was determined and 95% confidence intervals were calculated. Classical cardiovascular risk factors (age, body mass index, blood pressure, hyperglycemia, diabetes, smoking, hyperlipidemia) and reproductive factors (parity and age at menopause) were evaluated for their independent effect on the prevalence of BAC. Univariate

differences in patient characteristics at baseline were tested by unpaired Student *t* test and chi-square analyses. Logistic regression was used to estimate the independent effect of potential determinants of the presence of BAC and expressed as odds ratios with 95% confidence intervals as an approximation of relative risk.

Table 2. Prevalence of risk factors in women with and without breast arterial calcium (BAC) (n = 600).

	BAC	
	Yes 23% (n = 138)	**No. 77% (n = 462)**
Age (years)	70.4 ± 6	66.5 ± 6
Age ≥ 70 years	35.4%	15.5%
Age at menopause (years)	47.9	48.0
Parity		
0 and 1	8 (6%)	50 (11%)
2 and 3	60 (44%)	260 (56%)
4 and 5	46 (33%)	87 (19%)
≥6	22 (16%)	21 (5%)
Body mass index (kg/m²)	28 ± 4	28 ± 4
Systolic blood pressure (mmHg)	156 ± 20	150 ± 22
Diastolic blood pressure (mmHg)	85 ± 10	85 ± 10
Total cholesterol (mmol/L) (mg/dl)	5.3 ± 1.0 (205 ± 39)	5.2 ± 1 (201 ± 39)
High-density lipoprotein cholesterol (mmol/L) (mg/dl)	1.3 ± 0.3 (50 ± 12)	1.3 ± 0.3 (50 ± 12)
Low-density lipoprotein cholesterol (mmol/L) (mg/dl)	3.0 ± 1.0 (116 ± 39)	2.9 ± 0.9 (112 ± 35)
Triglyceride (mmol/L) (mg/dl)	1.5 ± 0.9 (58 ± 35)	1.7 ± 1.0 (66 ± 39)
Lipid lowering medication	80%	82%
Current smoking	11%	22%
Hypertension medicine use	63%	63%
Diabetes mellitus	34%	26%
Healed MI	15%	20%
Coronary bypass	19%	18%
Coronary angioplasty	12%	17%
Angina pectoris	79%	73%

Results

The baseline characteristics of women with and without BAC are shown in Table 2. BAC was detected in 138 of 600 women (23%). The calcifications were graded as mild in 100 women (72.5%), moderate in 28 women (20.3%), and severe in 10 women (7.2%). The prevalence of BAC per age group is shown in Figure 2. The prevalence of BAC (35.4%; 80 of 226) was highest in women ≥70 years of age (OR 2.9, 95% CI 1.96−4.33). In 17 of 138 women (12%) BAC was seen in one breast only; in all other women the calcification was symmetrical. Previous coronary heart disease events or angina pectoris with documented CAD was not independently associated with BAC. Age at menopause was not related to the presence of BAC, whereas parity and BAC were strongly associated. Within the different parity categories (Table 2), the prevalence of BAC increased by 14% in women with no or 1 child, 19% in women with 2 and 3 children, 35% in women with 4 and 5 children, and 51% in women with 6 children or more ($p<0.001$).

The prevalence of BAC increased significantly ($p = 0.03$, continuously) with a higher cardiovascular risk score (Fig. 3), but multivariate analysis showed only age, diabetes, and parity to be separate independent predictors of BAC (Table 3). With each year of increasing age the risk of BAC increased by 11% (OR 1.1, 95% CI 1.07−1.15). The risk of BAC was 58% higher in diabetics than in non-diabetics (OR 1.6, 95% CI 1.01−2.49).

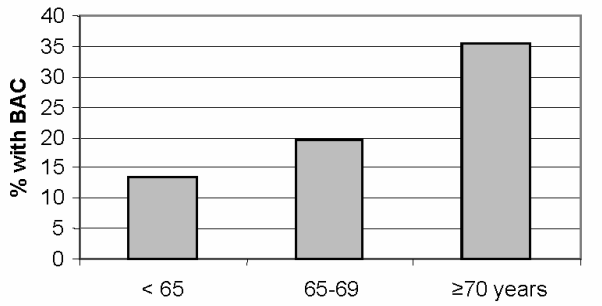

Figure 2. Percentage of women with BAC (n = 138) per age group.

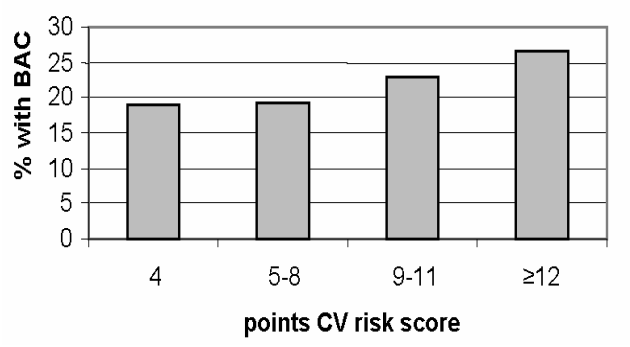

Figure 3. Prevalence (%) of BAC (n = 138) related to cardiovascular (CV) risk score (n = 600).

Multivariate analysis adjusted for age, body mass index, and diabetes did not change the magnitude of the association between the prevalence of BAC and parity. The odds ratio for BAC was 1.8 (95% CI 0.81−4.18) in women with 3-4 children, 3.8 (95% CI 1.62−8.96) in women with 4−5 children, and 6.1 (95% CI 2.26−16.39) in women with 6 or more children compared to that of women with 0 or 1 child, respectively (p value for trend 0.000).

Table 3. Multivariate relation between cardiovascular risk factors and breast arterial calcium (BAC).

Variable	Odds Ratio	95% CI
Age	1.1	1.07−1.15
Diabetes	1.6	1.01−2.49
Parity		
2−3 children	1.8	0.81−4.18
4−5 children	3.8	1.62−8.96
≥6 children	6.1	2.26−16.39

Discussion

In this cross-sectional study of postmenopausal women 'at risk' for coronary heart disease, we found a high prevalence of BAC (23%) in mammograms recorded at baseline. The prevalence of BAC was highest in women ≥70 years (35.4%). Although BAC was significantly more common among women with a higher cardiovascular risk score ($p = 0.03$) and in women with diabetes mellitus ($p = 0.01$), no specific cardiovascular risk factors besides age and diabetes could be identified to explain the calcium. The significant positive trend between the magnitude of the cardiovascular risk score and the presence or absence of BAC supports the hypothesis that cardiovascular risk factors are related to these calcium deposits and that BAC may be a marker of atherosclerotic disease. The lack of a correlation between BAC and individual traditional cardiovascular risk factors in this cohort is possibly due to the high prevalence of risk factors and the relatively old age of the patients. An interesting finding in our study was the strong and independent association between BAC and parity (Table 2), confirming the results of an earlier study.[5] This association may be due to hormonal changes in breast tissue and its vascularization during pregnancy and the regression of breast tissue thereafter. In our study population we had no information on breastfeeding.

Calcium deposits in breast arteries are located in the media of the vessel wall, rather than in the intima like calcium in the aorta and the coronary arteries.[8] It is described as an non-inflammatory age-related phenomenon and differs from atherosclerotic lesions of the intima primarily in the size and type of arteries involved.[3,8,9] In some reports it has been exclusively associated with older age and severe diabetes mellitus,[1,2] but others also found a higher prevalence of hypertension, family history and documented coronary artery disease.[10] A lower prevalence of BAC has been reported in women using hormone replacement therapy[11,12] As we found the strongest relation with parity, the biologic pathway of these calcium deposits is unclear and possibly related to gestational hormones during pregnancy.

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CHAPTER 4

Mammographic arterial calcifications: Cardiovascular risk factors, pregnancy and lactation

Angela H.E.M. Maas, Yvonne T. van der Schouw
David Beijerinck, Jan J.M. Deurenberg
Willem P.Th.M. Mali, Yolanda van der Graaf

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Abstract

PURPOSE: The purpose of our study was to retrospectively assess if mammographic calcium deposits are related to coronary heart disease (CHD) risk factors and reproductive parameters in a subset of women participating in the European Prospective Investigation into Cancer and nutrition (EPIC) study.

MATERIALS AND METHODS: The mammograms were evaluated by two radiologists for the presence of breast arterial calcifications (BAC) in PROSPECT, a breast cancer screening population of women aged 49−70 years (mean 57 years), within the European Prospective Investigation into Cancer and nutrition study. Cardiovascular risk factors and reproductive factors were examined for their independent effect on the prevalence of BAC.

RESULTS: BAC was present in 194 of 1699 women (11%) and increased with age to 20% in the highest quartile of age (mean 66 years). The OR was 4.7 in the highest versus lowest quartile of age (95% CI 2.9−7.6). After adjustment for age, no significant association was found between BAC and traditional cardiovascular risk factors. Smoking was inversely related to BAC (OR 0.6, 95% CI 0.4−0.9). BAC was prevalent in 2.5% of nulliparous women, in 9% of women with 1 or 2 children and in 17% of women with 3 or more children (OR 7.2, 95% CI, 2.9−18.0). Breastfeeding after pregnancy was significantly associated with BAC in women who ever were pregnant (OR 2.2, 95% CI 1.4−3.6).

CONCLUSION: Calcifications in the arteries on mammograms are associated with increasing age, pregnancy and lactation, but not with various cardiovascular risk factors.

Introduction

Arterial calcification is a common feature of degenerative atherosclerotic disease and with modern imaging techniques it provides insight in subclinical atherosclerosis.[1-3] With conventional radiology techniques, like mammograms, calcium deposits in the arterial wall can be seen, but the aetiology and clinical significance of these calcifications is still unclear.[4,5] Previously we found a prevalence of breast arterial calcium (BAC) of 23% in mammograms of women at high risk for a coronary heart disease (CHD) event, whereas the prevalence was estimated at 9% in a low risk population from a breast cancer screening program.[6,7] Age, presence of diabetes and the number of pregnancies were found to be independently associated with BAC, but we were unable to identify other factors that were associated with BAC.[6] The association between pregnancy and mammographic arterial calcifications has not been described before by others, and raises questions about the aetiology of BAC. As vascular calcification and pregnancy have in common that bone metabolism is actively involved, we hypothesized that hormonal changes during pregnancy are important in the aetiology of the calcifications in breast arteries.[8-10]

Thus, the purpose of our study was to retrospectively assess if mammographic calcium deposits are related to CHD risk factors and reproductive parameters in PROSPECT, a subset of women participating in the European Prospective Investigation into Cancer and nutrition (EPIC) study.

Materials and Methods

Population

The study population consists of participants of the PROSPECT cohort, which is one of the two Dutch contributions to the European Prospective Investigation into Cancer and nutrition (EPIC). The total cohort consists of 17,357 women from a population-based breast cancer screening program, enrolled between 1993−1997 in Utrecht and its surroundings. Women aged 49−70 years, scheduled for breast cancer screening, were invited to join the

PROSPECT study, along with their invitation for a routine mammography. Detailed information on the study population has been described elsewhere.[11] At baseline, women filled in a general questionnaire, a medical examination was performed, and a 30 ml non-fasting blood sample was donated by each participant which was fractionated into serum, plasma, buffy coat and erythrocyte aliquots. All women signed an informed consent prior to study inclusion with permission to use personal data and biologic material in substudies .

The study was approved by the institutional Review board of the University Medical Center Utrecht. For this study we drew a 10% random sample (n = 1736) for whom we collected the baseline mammogram and retrieved the frozen blood samples. We excluded 10 women for whom the baseline questionnaire was missing, and additionally 27 for whom a blood sample was not available. Thus, our study population consisted of 1699 women, mean age 57 years.

Mammograms

All 1699 baseline mammograms (medio-lateral-oblique and cranio-caudal views of both breasts) of participating women were obtained with a GE Senograph 600 or 800 using the Kodak Min_R film-screen combination. The mammograms were retrospectively analyzed by two radiologists working independently, each with 18 years of mammography experience, for the presence of BAC using the criteria of Kemmeren et al.[7]

Therefore, all mammograms were scored on the presence, location and severity of vascular calcifications by each reader. If there was disagreement (in 5% cases), the two observers reviewed the mammograms together to reach consensus. Breast artery calcium was characterized by calcium deposits along the contours of the breast arteries on mammograms of the right, the left, or both breasts (Fig. 1). The reviewing radiologists were blinded to the clinical data of the patients.

Cardiovascular risk factors

The general questionnaire (see above) contained questions on demographic characteristics, previous (myocardial infarction (MI), stroke), and current illnesses and risk factors for chronic diseases, such as reproductive history (number of pregnancies,

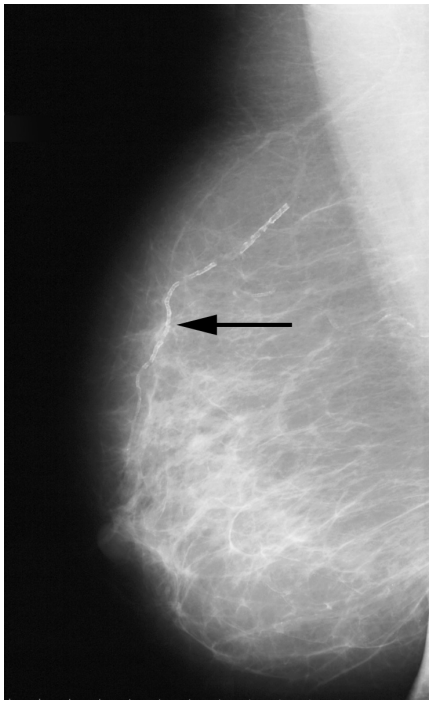

Figure 1. Medio-lateral-oblique view of the right breast with arterial calcifications.

miscarriages, breastfeeding, oral contraceptive use (OC), hormone therapy (HT), smoking habits, alcohol consumption, physical activity, family history, treatment for high blood pressure, diabetes or hypercholesterolemia. The physical examination included measurements of pulse rate, blood pressure, height, weight and waist and hip circumferences. Systolic and diastolic blood pressure were measured in duplicate, and the mean value was calculated. Furthermore, height and weight were measured without shoes in light indoor clothing to compute body mass index (BMI) defined as weight divided by height squared (kg/m²). Levels of serum total cholesterol and glucose were determined in the thawed samples using an automated enzymatic procedure on a Vitros 250 (Johnson&Johnson, Rochester, NY, USA). Hypercholesterolemia was defined as present when women had a non-fasting total cholesterol >8.0 mmol/L (309 mg/dl) and/or when women reported that a physician had diagnosed hypercholesterolemia. Diabetes mellitus was defined as present when women had a non-fasting glucose >11.1 mmol/L (202 mg/dl)

and /or when a women reported that a physician had diagnosed diabetes. Hypertension was defined as measured systolic blood pressure >160 mmHg and/or a diastolic blood pressure >95 mmHg and/or when women reported that a physician had diagnosed hypertension. Women were classified according to their smoking habits as current and past/never smokers. Previous myocardial infarctions and strokes were registered by self report. Parity was defined as all pregnancies, including (spontaneous) abortions. Breastfeeding was defined as having breastfed at least one child.

Table 1. Prevalence of risk factors in participants (n = 1699) with and without breast arterial calcium (BAC).

Risk factor	**BAC + 11% (n = 194)**	**BAC – 89% (n = 1505)**	**p-value**
Age (mean, yrs)	61	57	<0.0001
Previous MI*	3.1%	1.5%	NS
Previous stroke	1.5%	1.2%	NS
Hypertension	23%	20%	NS
Diabetes	2%	3%	NS
Current smoking	13%	24%	<0.001
Parous	97%	88%	<0.0001
Breastfeeding	88%	77%	<0.001
OC † use ever	58%	64%	NS
HT ‡ use ever	18%	26%	NS
Body mass index (mean, kg/m^2)	25.5	26.0	NS
Systolic blood pressure mmHg, mean (SD)	135 (20.0)	133 (20.4)	NS
Glucose mmol/L, (mg/dl), mean (SD)	4.5 (82) (1.3)	4.6 (84) (1.6)	NS
Total cholesterol mmol/L, (mg/dl) , mean (SD)	6.0 (232) (1.0)	5.9 (228) (1.0)	NS
LDL-chol mmol/L, (mg/dl), mean (SD)	4.08 (158) (0.9)	3.9 (151) (0.9)	NS
HDL-chol mmol/L, (mg/dl), mean (SD)	1.62 (63) (0.4)	1.57 (61) (0.4)	NS

* MI= myocardial infarction; † OC= oral contraceptives; ‡ HT= hormone therapy.

Statistical analysis

Baseline characteristics (age, previous MI, stroke, hypertension, diabetes, hyperlipidemia, smoking, reproductive history, body mass index (BMI), glucose- and lipid levels) were expressed according to the presence or absence of BAC. Means and standard deviations (SD) were computed for continuous variables, and frequency distributions for categorical variables. Risk factors for cardiovascular disease (age, BMI, blood pressure, diabetes, smoking and hyperlipidemia) and reproductive factors (parity, breastfeeding, OC- and HT use) were examined for their independent effect on the prevalence of BAC. The associations between CHD risk factors and reproductive factors with BAC were assessed by logistic regression analysis and expressed as odds ratios (OR) with 95% confidence intervals. We adjusted for age by entering age as a continuous variable in the regression model. All analyses were carried out using the SPSS 11.0 for Windows statistical package (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the study group with and without BAC (Table 1) showed BAC was present in 194 (11%) of participants who were 49−70 years of age. The prevalence of BAC increased with advancing age, from 5% in the first quartile (mean age 50), 6% in the second quartile (mean age 54), 14% in the third quartile (mean age 60) to 20% in the fourth quartile (mean age 66).

CHD events and risk factors

The number of previous CHD events (n = 29) and diabetes mellitus (n = 22) was too rare in this low risk population to estimate relationships with BAC. In current smokers BAC was significantly less prevalent compared to non-smokers (7% versus 13%, $p<0.001$). Body mass index >25 kg/m² was not associated with BAC. The prevalence of BAC was slightly higher in participants with known hypertension (13% versus 11%), but mean systolic blood pressure was not different between both groups. Mean serum glucose levels

were not different between women with and without BAC. When adjusted for age the associations with hypertension, serum glucose and lipid parameters were not significantly associated with BAC.

Table 2. Odds ratios of breast arterial calcium (BAC), adjusted for age.

Risk factors	**OR**	**95% CI**
Hypertension	0.9	0.6–1.3
Diabetes mellitus	0.5	0.2–1.4
Current smoking	0.6	0.4−0.9
Previous MI	1.5	0.6–3.8
Previous stroke	1.2	0.3–4.2
OC use ever	1.3	0.9−1.8
HT use ever	0.7	0.5−1.1
Parous, yes	5.3	2.2–13.2
1−2 children	4.8	1.9–12.0
≥3 children	7.2	2.9–18.0
Breastfeeding, yes	2.2	1.4–3.6
BMI (kg/m²)		
2nd quartile (≥20 <25)	0.9	0.4–1.7
3rd quartile (≥25 <30)	0.6	0.3–1.2
4th quartile (≥30)	0.6	0.3–1.3
Systolic blood pressure (mmHg)		
2nd quartile (119–130)	1.2	0.7–1.9
3rd quartile (130–144)	1.1	0.7–1.7
4th quartile (145–216)	1.0	0.6–1.5
Glucose (mmol/L) (mg/dl)		
2nd quartile (3.9–4.2) (71–76)	0.8	0.5–1.2
3rd quartile (4.3–4.8) (78–87)	0.7	0.5–1.1
4th quartile (4.9–23.3) (89–424)	0.8	0.6–1.3
Total cholesterol (mmol/L) (mg/dl)		
2nd quartile (5.2–5.8) (201–224)	0.9	0.6–1.4
3rd quartile (5.9–6.4) (228–248)	1.0	0.7–1.7
4th quartile (6.5–12.2) (251–472)	1.1	0.7–1.7
LDL-cholesterol (mmol/L) (mg/dl)		
2nd quartile (3.27–3.84) (127–149)	1.1	0.7–1.8
3rd quartile (3.87–4.50) (150–174)	1.1	0.7–1.7
4th quartile (4.52–7.19) (175–278)	1.5	0.9–2.3
HDL-cholesterol (mmol/L) (mg/dl)		
2nd quartile (1.32–1.50) (51–58)	1.0	0.6–1.6
3rd quartile (1.53–1.77) (59–69)	1.8	1.2–2.8
4th quartile (1.79–4.24) (69–164)	1.7	1.1–2.6

Reproductive parameters

The prevalence of BAC was significantly associated with previous pregnancies ($p< 0.0001$) and lactation ($p<0.001$). All but 5 of 194 women with BAC (97%) had been pregnant. Seventy-six of 194 women with BAC (39%) had 1 or 2 child(ren) and 112 of 194 women with BAC (58%) had 3 children or more. The prevalence of BAC increased from 2.5% in women without children, to 9% in women with 1 or 2 children (OR 4.8, 95% CI 1.9−12.0) and to 17% in women with ≥3 children (OR 7.2, 95% CI 2.9−18.0) (Table 2). The age-adjusted odds ratios for ever parous versus nulliparous women was 5.3 (95% CI 2.2−13.2). Among women who breastfed after pregnancy (78%) the prevalence of BAC was 14% compared to 7% in women who never breastfed (OR 2.2, 95% CI, 1.4−3.6). Women who ever used HT had a non-significant lower prevalence of BAC (8%), compared to never users (12%). There was no relation with previous or current OC use and BAC.

Discussion

In this cohort of women aged 49-70 years from a breast cancer screening program we found a BAC prevalence of 11%. The strongest determinants of BAC were parity (OR 5.3) and breastfeeding (OR 2.2) suggesting that the calcium deposits may be merely due to changes during pregnancy and breastfeeding than to CHD risk factors. Although there was a wide spread in the distributions of blood pressure, cholesterol and glucose levels, after adjustment for age these risk factors were not found to be related to BAC.

Thus far, mammographic arterial calcifications have been attributed to medial sclerosis of the breast arteries, comparable with the Mönckeberg's medial sclerosis which is often seen in the smaller peripheral vessels of (older) patients with diabetes mellitus.[4,12,13]

Vascular calcification is currently considered as a regulated process with similar mechanisms as in bone formation with a diversity of locations and morphology of the calcified deposits.[8,9,14,15] Bonelike tissues have been found in atherosclerotic lesions, medial sclerosis and valvular stenosis.[16-18] The appearance of calcium in the different vascular beds is 10−15 years later in women compared to men.[19] In pathologic studies, medial

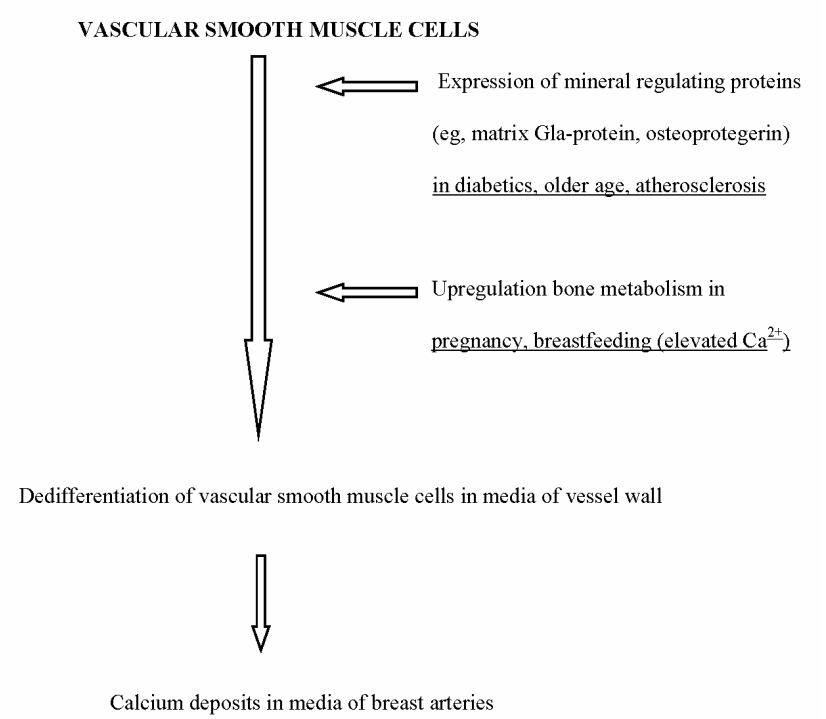

Figure 2. Mechanism of calcification in breast arteries.

calcification is different from intimal calcification by the absence of signs of inflammation and lipid deposits.[20,21] With conventional x-ray techniques it is not possible to differentiate with certainty between intimal and medial calcifications, although the appearance of medial calcifications is more fine and diffuse in smaller vessels compared to the large and discontinuous calcifications of the intima in large and medium-sized arteries.[13] In the peripheral vessels of diabetics, medial sclerosis and calcifications are an independent predictor of CHD events and severe peripheral artery occlusive disease.[22] In a previous breast cancer screening cohort of 12,239 women we found a higher prevalence of BAC in diabetics (15.4%) compared to 9% in non-diabetics and mortality in diabetics with BAC was 74% higher compared to diabetics without BAC. In another study in 600 high-risk women, we found a 58% greater risk of BAC in diabetics compared to non-diabetics (HR

1.58).[6] Hyperglycaemia may be related to medial calcium deposits by regulating the production of osteogenic proteins like osteoprotegerin (OPG), matrix Gla–protein (MGP), osteocalcin and others.[14,17,21-23] Variations in gene expression of these proteins can change vascular smooth muscle cells into a more osteogenic phenotype (Fig. 2). In women with diabetes and subsequent cardiovascular complications serum OPG-levels were found to be higher compared to controls.[23] We assume that in our present study in relative healthy women from a breast cancer screening program, the prevalence of diabetes mellitus and mean serum glucose levels were too low to enhance medial vascular calcification. The reason for the lower prevalence of mammographic calcium we found in current smokers is unclear.

Microcalcifications at mammography have been reported in a recent lactating woman, but to date, to the best of our knowledge, our study is the first to describe an association between calcifications in the arteries in mammograms and reproductive factors.[24] Given the strong associations we found with pregnancy and breastfeeding we assume that the calcium deposits in the breast arteries may be caused by the transient hypercalcaemia, which is induced by pregnancy and breastfeeding. Pregnancy is associated with major changes in the calcium metabolism to meet the high requirements for foetal growth and for breast-milk production.[10] Some biochemical proteins of bone resorption and formation (eg,osteocalcin, bone morphogenic protein) are elevated in the first months of lactation and are also found in calcified vascular tissues[25,26] Vascular smooth muscle cells of the arterial wall can migrate and dedifferentiate in response to calcifying and atherosclerotic stimuli into cells with osteocytic and chondrogenic changes in gene expression.[25,27] We hypothesize that when calcium (hydroxyapatite) is more available in the (breast) circulation, like during pregnancy and lactation, vascular smooth muscle cells can become activated and promote mineralization (Fig. 2). A comparable linkage of vascular and cardiac valve calcification and osteoclastic resorption in the skeleton has been found in women after menopause.[28,29]

These findings are in line with the observations that premenopausal women and women on HT have lower content of calcium in the coronary arteries as well as in the breast arteries suggesting that lack of estrogens are important in the calcification of the arterial wall.[19, 30-32]

Limitations of our fairly small study of relatively young women from a breast cancer screening program are the low prevalence of diabetes and previous CHD events and a lower extent of calcium deposits that is inherent to younger age, however we were able to provide detailed information on the reproductive history of the participants as well as on CHD risk factors, which we could assess due to the combination of self report and measured values.

In conclusion, we found that mammographic arterial calcifications are associated with increasing age, pregnancy and lactation, but not with traditional cardiovascular risk factors. Thus far, it is not known whether pregnancy and lactation may have a role in calcification of other vascular beds than the breast arteries.

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CHAPTER 5

Arterial calcium on mammograms is not associated with inflammatory markers for heart disease risk

Angela H.E.M. Maas, Yvonne T. van der Schouw

David Beijerinck, Jan J.M. Deurenberg

Willem P.Th.M. Mali, Yolanda van der Graaf

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Introduction

Calcifications along the wall of the breast arteries are commonly seen in mammograms, but it is still unclear whether they can be used as a tool to identify women at risk for coronary heart disease (CHD). In breast cancer screening populations the prevalence of breast arterial calcium (BAC) is 9%, while it is present in 23% women at elevated risk for CHD.[1] Although several studies have shown that classical cardiovascular risk factors for atherosclerosis are associated with mammographic arterial calcifications, thus far the correlation with clinical coronary artery disease is controversial.[2,3]. As other factors like the total number of pregnancies are important in the prevalence of BAC, its multicausative aetiology remains poorly understood.[1]

Chronic low-grade inflammation is an important aspect of atherosclerosis. Data on inflammatory markers like fibrinogen and high-sensitivity C-reactive protein (hs-CRP) in calcification of atherosclerotic arteries are conflicting and not studied in arterial calcifications on mammograms.[4] To further explore the pathophysiologic process of BAC we investigated mammograms and inflammatory markers in women from a breast cancer screening program.

Methods

We analyzed data from 1736 women, aged 49−70 years, scheduled for breast cancer screening as participants in the Europe Prospective Investigation into Cancer and Nutrition (Prospect-EPIC) study and assessed whether mammographic arterial calcifications were related to serum levels of fibrinogen and hs-CRP. Baseline information on cardiovascular risk factors was collected on the basis of a self-administered questionnaire, a medical examination was performed and a 30 ml non-fasting blood sample was donated. All women signed an informed consent and the study was approved by the institutional Review board of the University Medical Center Utrecht.

High-sensitivity C-reactive protein (CRP) was measured in citrated plasma using the Behring BNII nephelometric method (Dade Behring, Deerfield, IL, USA). CRP values below the detection limit of 0.2 mg/l (n = 50) were set to 0.1 mg/l. Total fibrinogen concentrations were determined in citrated plasma according to Clauss using the Sta-R automatic coagulation analyzer with STA Fibrinogen reagent (Diagnostica Stago, Taverny, France).

Baseline mammograms (medio-lateral-oblique and cranio-caudal views of both breasts) of 1699 participating women were available for analysis and were scored by two independent radiologists. Breast artery calcium was characterized by calcium deposits along the wall of the breast artery(-ies) on mammograms of the right, the left, or both breasts.

Table 1. Relation of risk markers with breast arterial calcium (BAC).

Variable		**+BAC (%)**	**OR***	**95% CI***
Hs-CRP (mg/l), quartiles				
I	0.40	12.4	1.0	
II	0.91	10.6	0.7	0.5−1.1
III	1.86	9.6	0.6	0.4−0.9
IV	6.80	11.4	0.8	0.5−1.2
Fibrinogen (g/l),quartiles				
I	1.88	10.3	1.0	
II	2.39	9.9	0.9	0.5−1.4
III	2.79	11.9	1.1	0.7−1.6
IV	3.61	12.0	0.9	0.5−1.4

*Adjusted for age and number of pregnancies.

Table 2. Age-adjusted multivariate relation between cardiovascular risk factors* and BAC.

Variable present	**OR**	**95% CI**
Hypertension	0.9	0.6−1.3
Hypercholesterolemia	0.9	0.5−1.7
Smoking	0.6	0.4−0.9
Pregnancy	5.3	2.2−13.2
Lactation	2.2	1.4−3.6

* Prevalence of diabetes too low (2.8%) to estimate relationships with BAC.

Results

BAC was present in 194 (11%) of 1699 of participants. Hs-CRP measurements were available in 95% of participants and fibrinogen in 93%. Mean hs-CRP concentrations were not significantly lower (2.37 mg/l) in women with BAC than in women without BAC (mean 2.52 mg/l) and no difference was seen in mean fibrinogen levels (2.67 g/l). When divided into quartiles, there was also no association between hs-CRP and fibrinogen and the prevalence of BAC (Table 1). Factors that were significantly associated with BAC were age (odds ratio [OR] 1.1 per year, 95% confidence interval [CI] 1.09−1.14), pregnancy ever (OR 5.3, 95% CI 2.2−13.2) and lactation after pregnancy (OR 2.2, 95% CI 1.4−3.6) (Table 2). Smoking was inversely related to BAC (OR 0.6, 95% CI 0.4−0.9). The prevalence of diabetes mellitus was too low in this population (2.8%) to estimate relationships with BAC. Hypertension was present in 20% of participants but after multivariate analysis it was not significantly associated with BAC.

Discussion

Our results do not support an association of CHD risk markers hs-CRP and fibrinogen with mammographic arterial calcifications. We found no significant association with CHD risk factors in this average risk population, although we did find an association of BAC with the total number of CHD risk factors in a previous study in women at high risk.[1] Only aging, a history of pregnancy and lactation after pregnancy were independently associated with BAC.

Whether hs-CRP and fibrinogen levels correlate with the extent of atherosclerosis as measured by coronary artery calcification is controversial, although both markers are clearly related to increased risk of clinical cardiovascular events.[4] Measurements of calcium deposits and inflammatory markers may reflect different pathofysiologic processes in atherogenesis.

From pathology and biochemistry studies it is known that the calcium deposits in breast arteries are located in the media of the vessel wall.[5] Medial calcification is mostly known from calcifications in the peripheral vessels in diabetics. A common pathway in vascular calcification is the activation of vascular smooth muscle cells, present in the media of all arteries, with different embryonic origins in parts of the arterial system. A variety of stimuli, such as inflammatory cytokines, lipids, glucotoxicity and hypercalcaemia, are described to induce calcification in vascular smooth muscle cells.[5] Various causative factors are likely to be involved in the calcification processes in different locations and types of the vascular tree. As pregnancies and breastfeeding are strongly associated with mammographic arterial calcifications, hypercalcaemia during pregnancy and lactation combined with the increased blood flow in the breast arteries may be a trigger for vascular smooth muscle cells to enhance calcification.

We have no explanation, however, for why the prevalence of BAC is higher in women at increased cardiovascular risk than in healthy middle-aged women.

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CHAPTER 6

Are calcifications in breast arteries related to microalbuminuria?

Angela H.E.M. Maas, Yvonne T. van der Schouw
Hans L. Hillege, Wiek H. van Gilst, Willem P.Th.M. Mali
Yolanda van der Graaf

Submitted

Abstract

OBJECTIVE: Microalbuminuria and vascular calcification are both markers of atherosclerotic disease. Recent studies indicate that urinary albumin excretion and coronary calcifications may be related, especially in patients with diabetes and hypertension. Calcifications in the breast arteries have a different etiology than coronary calcifications. It is not known whether micoalbuminuria is also associated with calcifications in the breast arteries in women.

METHODS: We performed a cross-sectional study in 509 women, participating in the Dutch contribution of the Raloxifene Use for The Heart study (RUTH). A sample of overnight morning urine was sent to a central laboratory to determine urinary albumin concentration (UAC). Mammograms at baseline were investigated on the presence of calcifications in the breast arteries (BAC) by two independent observers. The role of coronary heart disease (CHD) risk factors as possible intermediates of BAC and UAC was examined on their independent effect on UAC and BAC. Logistic regression analysis was performed to investigate the association between UAC and BAC.

RESULTS: Microalbuminuria (20−200 mg/L) was present in 49 of 509 (10%) participants. It was significantly more prevalent in women with diabetes (15%) than in women without diabetes (8%) (OR 2.0, 95% CI 1.14−3.80). BAC was present in 116 of 509 (23%) participants and increased with advancing age (OR 1.1, 95% CI 1.08−1.16). Microalbuminuria was not associated with BAC (age-adjusted OR 1.1, 95% CI 0.52–2.29). Taking into account the presence of diabetes mellitus did not change this result. The age-adjusted OR for BAC was 0.7 (95% CI 0.43−1.26) in the highest versus the lowest tertile of UAC. Multivariate logistic regression with adjustment for age, hypertension, diabetes and smoking showed no association between BAC and UAC.

CONCLUSION: We found no association between microalbuminuria and arterial calcifications in breast arteries.

Introduction

Microalbuminuria is an early sign of endothelial dysfunction and renal microvascular disease and it is considered to be a marker of atherosclerosis.[1] It clusters with other coronary heart disease (CHD) risk factors and it is associated with an increased mortality risk in diabetic and non-diabetic populations.[2-4] As microalbuminuria is related to atherosclerosis it may be associated with vascular calcification. Calcifications are more prevalent in both the media and intima layer of the vascular wall in diabetics compared to non-diabetics.[5-7] Medial calcification is often seen in the peripheral vessels of diabetics and it is strongly associated with microalbuminuria and it is a powerful predictor of CHD mortality.[6,8] Several studies have found a higher prevalence of calcifications in the breast arteries on mammograms in women with diabetes compared to non-diabetics, with a higher CHD mortality when present.[9-14] Classic cardiovascular risk factors are involved in vascular calcification and microalbuminuria, but whether there is a link between the two markers of cardiovascular risk is uncertain.[6]

We set out to investigate whether microalbuminuria is associated with breast arterial calcifications (BAC) in a subset of women, participating in the Raloxifene Use for the Heart (RUTH) study.

Methods

Population

The 610 Dutch participants of the RUTH study were included in this cross-sectional study. The RUTH trial was started in 1998 and is a multicenter, randomized, double-blind, placebo-controlled clinical trial involving women from 26 countries. Its aim is to evaluate treatment with 60 mg raloxifene versus placebo in 10,101 postmenopausal women at high risk for major cardiovascular events. The two separate primary endpoints are coronary events (coronary death, nonfatal myocardial infarction (MI) or hospitalization for acute coronary syndromes other than MI) and invasive breast cancer. The trial design, methods,

and participant characteristics at baseline have been described in detail elsewhere.[15,16]

Participants were classified according to a points risk score related to the presence of risk factors and previous CHD events. A minimum of 4 points was required for inclusion in RUTH. Briefly, inclusion criteria were age ≥55 years, ≥1 year postmenopausal with established coronary heart disease (i.e., prior myocardial infarction (MI), coronary artery bypass graft surgery (CABG), percutaneous coronary interventions (PCI), or angiographic evidence of a 50% occlusion of one or more major coronary arteries) or an increased risk of a major coronary event, based on the presence of multiple cardiovascular risk factors. An inclusion criterion for women without established CHD was the presence of multiple factors (minimum score 4) that increase the risk of MI and coronary death, such as smoking, hypertension, hyperlipidemia, and diabetes. The baseline data on cardiovascular risk factors were recorded at randomization before study entry. All participants gave written informed consent prior to inclusion and the study was approved by the local medical ethics committees of the participating hospitals. The Dutch part of the RUTH consists of 610 women and from 509 participants both the baseline mammograms and urine samples were available.

Urinary samples

A sample of overnight morning urine was available for analysis in 509 participants within 2 years of study entry and sent by mail to the central laboratory at the University Medical Center in Groningen. Urinary albumin concentration (UAC) was determined by a commercial immunoturbidimetry assay with sensitivity of 2.3 mg/L and inter- and intra-assay coefficients of variation of 4.4% and 4.3%, respectively (Dade Behring Diagnostica, Marburg, Germany). Microalbuminuria was defined as a UAC between 20 and 200 mg/L in a morning urine sample.

Risk factors

Body mass index was calculated as the ratio of body weight (in kg) to height (in meters) squared (kg/m²). Current smoking was defined as smoking an average of 10 or more cigarettes a day in the 6 months before randomization. Hypertension was present in

patients taking antihypertensive medications or in patients with a systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg on at least two measurements prior to randomization. Hyperlipidemia was present in patients with lipid-lowering medications, or in patients with a fasting LDL-cholesterol >160 mg/dL (4.14 mmol/L) or in patients with fasting HDL-cholesterol <45 mg/dL (1.16 mmol/L) with fasting triglycerides >250 mg/dL (2.82 mmol/L). Diabetes mellitus was defined as taking oral antidiabetic medication or insulin or as having fasting serum glucose >140 mg/dL (7.8 mmol/L) within 3 months prior to randomization.

Mammograms

Standard mammograms at baseline (cranio-caudal and lateral views) were made for all women before entry in the RUTH trial. Mammograms of 600 participants in the Dutch contribution of the RUTH study were read by two independent observers, blinded to the clinical data of the patients. All mammograms were scored on the presence of vascular calcifications by each reader separately. If there was disagreement, the two observers reviewed the mammograms together to reach consensus. BAC was characterized by the presence of calcium deposits along the wall of one or more arteries in the right, the left, or both breasts.

Statistical analysis

We used data on 509 participants of whom both a mammogram and an urine sample were available. Distributions of classic cardiovascular risk factors were expressed according to the tertile distribution of urinary albumin excretion.

The relationship between microalbuminuria and BAC was investigated by considering microalbuminuria as a dichotomous variable (between 20 and 200 mg/L and <20 mg/L) and categorized in tertiles. Subjects with microalbuminuria values >200 mg/L (n = 5) were excluded. Logistic regression analysis was performed to determine the association of microalbuminuria and BAC, using a model adjusted for age and a model containing age, diabetes, hypertension and smoking. Odds ratios (ORs) and corresponding 95% confidence

intervals were calculated as an approximation of relative risk. All calculations were performed with SPSS version 12.0 software (SPSS, Chicago, IL, USA).

Table 1. Cardiovascular risk factors by tertiles of albumin excretion in overnight urine samples (n = 509).

	I **(n = 169)** (mean 2.4 mg/L)	**II** **(n = 169)** (mean 5.0 mg/L)	**III** **(n = 171)** (mean 55 mg/L)
Age, yrs	67 ± 6	68 ± 7	67 ± 7
Body mass index, kg/m²	28 ± 4	28 ± 4	29 ± 4
Diabetes, %	23	27	33
Hypertension, %	60	64	70
Systolic blood pressure, mmHg	150 ± 21	153 ± 23	154±22
Diastolic blood pressure, mmHg	85 ± 9	85 ± 10	86±10
Hyperlipidemia, %	81	83	82
Total cholesterol, mmol/L (mg/dl)	5.1 ± 1 (197±39)	5.2 ± 1 (202±38)	5.2 ± 0.9 (199±36)
LDL-C, mmol/L (mg/dl)	2.9 ± 0.9 (111± 35)	3.0 ± 0.9 (114±34)	2.9 ± 0.8 (113±32)
HDL-C, mmol/L (mg/dl)	4 ± 0.3 (52±11)	1.3 ± 0.3 (51±13)	1.3 ± 0.3 (49±13)
Smoking, %	15	16	22
CV risk score, %			
≥4	8	3	5
5–8	26	30	25
9–11	34	30	32
≥12	32	38	39
Previous myocardial infaction, %	18	23	16
Coronary bypass, %	20	17	18
Coronary angioplasty, %	17	18	16
Angina pectoris, %	75	76	75
Breast arterial calcifications, %	24	20	24

Table 2. Odds Ratios for the risk of BAC by tertiles and 200 mg/L threshold of urinary albumin excretion.

Albumin	Cases (N)	Unadjusted OR (95% CI)	Age adjusted OR (95% CI)	Multivariate adjusted† OR (95% CI)
Tertiles				
1 (lowest)‡	41	1.0	1.0	1.0
2 (middle)	34	1.0 (0.62−1.67)	1.0 (0.60−1.69)	1.0 (0.60−1.72)
3 (highest)	41	0.8 (0.48−1.34)	0.7 (0.43−1.26)	0.7 (0.41−1.22)
Microalbumiuria				
No <20 mg/L	104	1.0	1.0	1.0
Yes 20−200 mg/L*	11	1.0 (0.48−1.98)	1.1 (0.52−2.29)	1.1 (0.51−2.26)

* Macroalbuminuria (>200 mg/L) excluded.
† Adjusted for age, smoking, (current/no), hypertension (yes/no), diabetes (yes/no).
‡ Ranges: 1st tertile: 1.83−3.18 mg/L; 2nd tertile 3.19−7.64 mg/L; 3rd tertile 7.65−1907.95 mg/L.

Results

Microalbuminuria (20 to 200 mg/L) was present in 49 (10%) urine samples of 509 participating women. Clinical characteristics of the participants are provided in Table 1. Women in the highest tertile of urinary albumin excretion (mean 55 mg/L) had more often diabetes (33% versus 23%), hypertension (70% versus 60%), and smoked more often (22% versus 15%), compared to those in the lowest tertile. Lipid levels were not associated with microalbuminuria. A higher cardiovascular risk points score (≥12) was also associated with higher microalbuminuria levels, but this was not significant. We found no association between microalbuminuria and the number of previous CHD events.

BAC was present in 23% of participants (116 of 509) and increased with advancing age (OR 1.11 per year of age, 95% CI 1.08−1.16). The prevalence of BAC was not different within the tertiles of UAC (24%, 20% and 24%, respectively). Microalbuminuria was not associated with BAC (age-adjusted OR 1.1, 95% CI 0.52−2.29). In diabetics, the age-adjusted OR for BAC was 1.2 (95% CI 0.49−2.88) when the highest tertile was

compared with the lowest tertile of UAC and in non-diabetics the OR was 0.6 (95% CI 0.28−1.11). Multivariate logistic regression with adjustment for age, hypertension, diabetes and smoking showed no association between the UAC and BAC (Table 2).

Discussion

In the present study we found no association between microalbuminuria (20-200 mg/L) and the prevalence of breast arterial calcifications in elderly postmenopausal women at risk for a CHD event.

To appreciate our findings, some additional remarks have to be made. First, urine sample measurements were taken in a time frame of 2 years after enrollment in the RUTH study and we cannot therefore exclude an underestimation of the urinary albumin excretion in women that were randomized to raloxifene.[17] Whether raloxifene may have changed the relation with BAC is unknown.[18] Second, the threshold definitions used for the diagnosis of diabetes, dyslipidemia and hypertension for entry in RUTH are currently considered not strictly enough. This may have caused an underestimate the relationship of albuminuria and BAC with CHD risk factors, but it is unlikely that this may have affected the relation of microalbuminuria with BAC.

The association between urinary albumen excretion and calcium in the coronary arteries (CAC) has been studied in the population-based multi-ethnic study of atherosclerosis (MESA). Participants with higher urinary albumin excretion had higher CAC scores, especially in patients with diabetes and hypertension.[19] In another study it was shown that any degree of coronary calcium in patients with diabetes mellitus implicates a higher mortality compared to non-diabetics.[20] In a recent study among patients with type 2 diabetes and a preserved renal function, a strong association was found between albuminuria and calcified plaques in both the coronary and carotid arteries.[21] These findings confirm that both microalbuminuria and coronary calcium are risk markers of atherosclerotic disease. It may be different however for calcifications in the arteries of the breasts in women. Breast arterial calcifications (BAC) are located in the media of the vessel

wall comparable with the medial calcifications in the peripheral arteries in diabetics, also called Mönckebergs atherosclerosis.[6,9-14] Both types of medial calcification are associated with diabetes and an elevated cardiovascular risk. The incidence of medial artery calcification is higher in diabetics with microalbuminuria than in those without.[22]

Prolonged exposure to hyperglycemia is now recognized a major factor in the pathogenesis of endothelial dysfunction and medial and intimal sclerosis in diabetes. Glycoxidation of the extracellular matrix of the vascular wall may enhance medial calcification.[23-25] Glucotoxicity also has an important role in transforming vascular smooth muscle cells (VSCM) into osteoblast-like cells through the regulation of osteogenic proteins like osteoprotegerin (OPG) and osteopontin (OPN).[26] Serum OPG is expressed in VSCM and may act as a vascular protective factor by inhibiting vascular calcification. However, elevated OPG levels are higher in diabetics and reflect endothelial dysfunction especially in patients with microalbuminuria and vascular complications.[27,28] It is assumed that this apparent paradox may indicate an insufficient counterregulatory mechanism in the protective role of OPG in the vascular system[29]

In our present exploratory study we were not able to find a link between microalbuminuria and mammographic arterial calcifications. An explanation may be that our study was underpowered, but it is also possible that there is just no link between the two entities. An interesting finding is that we found that smoking was strongly negatively associated with BAC while it was positively associated with microalbuminuria. Smoking has a variety of negative effects on vascular function. One major effect of nicotine is the stimulation of VSCM proliferation by enhancing platelet-derived growth factor (PDGF) release.[30] Smooth muscle cells in the breasts have apparently a different embryonic origin than elsewhere in the vascular tree that may be an explanation for a different response to certain stimuli.[31,32]

Although we found no association between microalbuminuria and mammographic arterial calcifications, both risk markers were significantly more prevalent in diabetics compared to non-diabetics suggesting overlapping aetiologic mechanisms.

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CHAPTER 7

Vitamin K intake and calcifications in breast arteries

Angela H.E.M. Maas, Yvonne T. van der Schouw
David Beijerinck, Jan J.M. Deurenberg, Willem P.Th.M. Mali
Diederick E. Grobbee, Yolanda van der Graaf

Submitted

Abstract

OBJECTIVES: Vitamin K is an important co-factor in the production of proteins that inhibit vascular calcification. A low dietary vitamin K intake has been associated with aortic and coronary calcifications and an elevated cardiovascular risk. The aetiology of arterial calcifications in the breast may be different and it is not known whether a low vitamin K intake is also associated with these calcium deposits.

METHODS: We conducted a cross-sectional study among 1689 women, aged 49−70 years. Dietary vitamin K1 and K2 intake was calculated from a validated food frequency questionnaire. Breast arterial calcifications (BAC) were assessed on standard screening mammograms by two independent radiologists. With a general linear model mean vitamin K1,K2 and vitamin K2 subtypes were calculated for women with BAC and without, adjusted for age, smoking, diabetes, intake of saturated fat, mono-unsaturated fat, poly-unsaturated fat and protein- and calcium intake.

RESULTS: BAC was less common in the highest (9 %) quartile of vitamin K2 intake, compared to the lowest (13 %) (OR 0.7, 95% CI 0.5−1.1) and not different across quartiles of vitamin K1 intake. Mean vitamin K2 levels and mean levels of vitamin K2 subtypes MK 5 through MK 10 were lower in the participants with BAC ($p = 0.01$) compared to participants without BAC. However, after adjustment for aging, smoking, diabetes and dietary factors the association of mean vitamin K2 intake with BAC was no longer significant. Only mean values of subtype MK5 of vitamin K2 remained significantly ($p = 0.03$) lower in women with BAC (0.30 µg/day) compared to women without BAC (0.33 µg/day).

CONCLUSION: Calcifications in breast arteries are not associated with a lower dietary intake of vitamin K.

Introduction

Vascular calcification is a multifactorial regulated process with several pathways and proteins involved. Vitamin K is necessary for haemostasis through the activation (carboxylation) of blood coagulation and anticoagulation factors in the liver. However, vitamin K dependent proteins are also present in extrahepatic tissues such as bone (osteocalcin) and vascular tissue (matrix-Gla-protein (MGP)).[1-3] MGP was identified in human atherosclerotic plaques where it may prevent calcium precipitation similarly as it does in cartilage.[4] Serum levels of MGP are inversely related to the severity of coronary artery calcification as visualised with electron-beam computed tomography.[5]

The main dietary sources of vitamin K1 (phylloquinone) are green vegetables and of vitamin K2 (menaquinone) meat and fermented foods.[1,6] Higher vitamin K intakes are related to a healthy dietary pattern[7] Postmenopausal women with calcified lesions in the aorta have a lower intake of vitamin K.[8] In population based studies coronary heart disease (CHD) risk has been associated with lower intakes of vitamin K1 and K2.[9,10]

It is unknown whether vitamin K intake is also related to arterial calcifications in the female breast. Breast arterial calcifications (BAC) are reported in 3% up to 29% of women undergoing mammography and they are associated with aging, diabetes, pregnancy and lactation and inversely with smoking.[11-13] As the aetiology of these calcifications is complex and not yet fully understood we sought to investigate whether vitamin K intake is associated with BAC. Therefore, we studied vitamin K intake and the prevalence of BAC in a subset of women participating in the European Prospective Investigation into Cancer and nutrition (EPIC) study.

Methods

Population

PROSPECT is one of the two Dutch contributions to the European Prospective Investigation into Cancer and nutrition (EPIC) study. The total study group consists of

17,357 women from a population-based breast cancer screening program, enrolled between 1993−1997 in Utrecht and its surroundings. Women aged 49−70 years, scheduled for breast cancer screening, were invited to join the PROSPECT study, along with their invitation for a routine mammography. Detailed information on the study population has been described before.[14] At baseline, women filled in a general questionnaire and a medical examination was performed. All women signed an informed consent prior to study inclusion with permission to use personal data and biologic material in substudies. The study was approved by the Institutional Review board of the University Medical Center Utrecht. For this study we took a 10% random sample (n = 1736) for whom we collected the baseline mammogram and retrieved the frozen blood samples. We excluded 18 women who reported use of vitamin K antagonists and additionally 29 for whom a blood sample or questionnaire was not present. A total of 1689 women were available for analysis.

Baseline measurements

At baseline a general questionnaire containing questions on demographic characteristics, presence of chronic diseases and coronary heart disease (CHD) risk factors such as family history, smoking habits, alcohol use, physical activity, blood pressure, hyperlipidemia and diabetes mellitus. Hypertension, hypercholesterolemia and diabetes mellitus were defined present when women reported that these disorders had been diagnosed by a physician. The physical examination included measurements of pulse rate, blood pressure, height, weight and waist and hip circumferences. Height and weight were measured without shoes in light indoor clothing to compute body mass index (BMI) defined as weight divided by height squared (kg/m²). Systolic and diastolic blood pressure were measured in duplicate, and the mean value was calculated. For assessment of physical activity, the Voorrips score was calculated by means of a validated questionnaire.[15]

Data on vitamin K intake

Energy and nutrient intake were estimated from a food frequency questionnaire (FFQ) that has been validated in pilot studies prior to the start of the study.[16,17] The questionnaire allows estimation of the average daily consumption of 178 foods, including sub-items on

fruit and vegetables. The concentrations of vitamin K1 and vitamin K2 (MK 4-10) in various foods were assessed at the Biochemistry Laboratory at the Maastricht University.[6] For some foods published data by others were used to update the dietary database for vitamin K.[18-21]

Mammograms

Mammograms of 1689 women, medio-lateral-oblique and cranio-caudal views of both breasts, were read by 2 independent radiologists who were blinded to the clinical data of the participants. Breast artery calcium was characterized by calcium deposits along the contours of the breast arteries in one or both breasts. All mammograms were scored on the presence of vascular calcifications by each reader separately and if there was disagreement (in 5%) the observers reviewed the mammograms together to reach consensus.

Data analysis

Vitamin K1 and K2 intake are categorized in quartiles. For each quartile we calculated mean daily dietary intake (with standard deviations (SD)). Mean vitamin K1, K2 and vitamin subtypes were calculated for women with BAC and without. Adjustment for age, smoking, diabetes, intake of saturated fat, mono-unsaturated fat, poly-unsaturated fat, protein- and calcium intake was done with univariate analysis of variance (general linear model). An alpha 0.05 level was considered to be significant.

Results

Baseline characteristics of the study population for quartiles of energy adjusted vitamin K1 and vitamin K2 intake are presented in Table I-A and I-B. The prevalence of BAC was similar (11%) across quartiles of vitamin K1 intake, while BAC was less common in the highest quartile of vitamin K2 intake (9%), compared to the lowest (13%) (OR 0.7, 95% CI 0.5−1.1). Hypercholesterolemia was more common within the lowest levels of vitamin K2 intake (7.6%), compared to the highest (2.9%), while it was less

present with low levels of vitamin K1 intake (3.8%) compared to the highest intake (6%). The presence of other risk factors for CHD was comparable within groups of vitamin K1 and vitamin K2 intake.

Table I-A. Baseline characteristics according to quartiles of energy adjusted vitamin K1 (μg/d) intake among 1689 women.

	<138 (n = 422)	**138−192 (n = 422)**	**192−261 (n = 423)**	**>261 (n = 422)**
Age, yrs	56.5 ± 6	56.9 ± 6	57.2 ± 6	58.2 ± 6
BMI, kg/m²	25.9 ± 4.1	25.7 ± 3.9	25.6 ± 3.9	26.4 ± 4.1
Systolic BP, mmHg	133.2 ± 20.3	132.9 ± 19.8	133.4 ± 20.8	132.1 ± 19.8
Diastolic BP, mmHg	79.2 ± 11.1	78.9 ± 10.2	79.7 ± 11.0	78.6 ± 10.0
Voorrips score	6.4 ± 4.6	7.2 ± 4.9	7.2 ± 5.2	6.7 ±5.1
	%	**%**	**%**	**%**
Hypertension	20.4	18.3	21.8	20.0
Diabetes	3.6	2.1	1.9	3.6
Hypercholesterolemia	3.8	3.8	5.5	6
Smoking,				
Current	23.8	22.6	19.4	26
Past	35.4	38.2	32.2	32.6
Never	40.9	39.2	48.3	40.5
Pregnancy ever	89	90	91	90
HRT use ever	25	28	22	24
OAC use ever	63	65	66	59
BAC, yes	11	12	11	10
Daily dietary intake	**mean ± SD**	**mean ± SD**	**mean ± SD**	**mean ± SD**
Energy, kcal/d	1833 ± 475	1806 ± 403	1794 ± 404	1759 ± 434
Protein, g/d	68.5 ±11.4	69.6 ± 9.6	71.2 ± 9.7	72.8 ± 9.8
Carbohydrates, g/d	196.8 ± 30.6	195.4 ± 27.4	196.1 ± 27.1	193.1 ± 27.2
Total fat, g/day	68.7 ± 11.5	68.7 ± 9.6	68.4 ± 9.7	68.6 ± 10.8
Mono-unsat.fat, g/d	25.5 ± 5.0	25.5 ± 4.4	25.3 ± 4.4	25.2 ± 4.7
Poly-unsat. fat, g/d	12.5 ± 3.4	12.9 ± 3.3	13.0 ± 3.4	13.3 ± 3.7
Saturated fat, g/d	29.7 ± 6.2	29.4 ± 5.1	29.1 ± 5.0	29.2 ± 5.3
Cholesterol, mg/d	205.4 ± 81.4	205.5 ± 64.5	205.2 ± 70.6	207.1 ±75.0
Vitamin K1, μg/d	104.2 ± 26.6	165.6 ± 15.7	223.5 ± 20.5	352.0 ± 101.6
Vitamin K2, μg/d	29.4 ± 14.2	29.0 ± 12.0	29.1 ± 12.9	28.9 ± 12.9
Alcohol, g/d	9.1 ± 13.4	9.4 ± 12.7	8.4 ± 11.1	9.5 ± 14.1
Dietary fiber, g/d	20.1 ± 4.0	21.5 ± 3.9	23.1 ± 4.1	24.5 ± 4.1
Calcium, mg/d	1056.3 ± 353.9	1071.5 ± 295.5	1090.1 ±304.2	1143.9 ± 319.1

Mean vitamin K2 levels were significantly lower in the participants with BAC ($p = 0.01$) compared to participants without BAC. The means of vitamin K2 subtypes MK 5 through MK 10 were also significantly associated with the prevalence of BAC (Table 2).

Table 1-B. Baseline characteristics according to quartiles of energy adjusted Vitamin K2 (µg/d) intake among 1689 women.

	<20 (n = 422)	**20–27 (n = 422)**	**27–36 (n = 423)**	**>36 (n = 422)**
Age, yrs	58 ± 6	57 ± 6	57 ± 6	57 ± 6
BMI, kg/m²	25.7 ± 4.0	26.2 ± 4.2	25.9 ± 3.9	25.8 ± 4.0
Systolic BP, mmHg	135.2 ± 20.8	134.4 ± 20.5	131.0 ± 19.2	130.0 ± 19.9
Diastolic BP, mmHg	80.0 ± 11.0	79.7 ± 10.5	78.5 ± 10.4	78.2 ± 10.5
Voorrips score	6.2 ± 4.6	7.1 ± 5.0	7.0 ± 5.0	7.3 ± 5.2
	%	**%**	**%**	**%**
Hypertension	21	21	17	21
Diabetes	3	1	3.6	3.6
Hypercholesterolemia	7.6	5.5	3.3	2.9
Smoking				
Current	27	21	24	21
Previous	31	34	37	37
Never	41	45	40	42
Pregnancy ever	90	92	88	89
HRT use ever	21	26	26	25
OAC use ever	62	63	64	64
BAC, yes	13	13	10	9
Daily dietary intake	**mean ± SD**	**mean ± SD**	**mean ± SD**	**mean ± SD**
Energy, kcal/d	1781 ± 413	1834 ± 415	1829 ± 462	1755 ± 423
Protein, g/d	65.1 ± 10.3	68.9 ± 8.6	72.2 ± 9.0	75.5 ± 10.2
Carbohydrates, g/d	205.1 ± 31.3	197.4 ± 25.7	194.0 ± 25.1	184.4 ± 26.3
Total fat, g/d	66.4 ± 11.7	68.7 ± 10.1	68.5 ± 9.2	70.9 ± 10.1
Mono-unsat. fat, g/d	24.8 ± 5.3	25.6 ± 4.6	25.3 ± 4.2	25.9 ± 4.4
Poly-unsat. fat, g/d	13.7 ± 3.7	13.1 ± 3.5	12.8 ± 3.3	12.2 ± 3.2
Saturated fat, g/d	27.0 ± 5.6	29.1 ± 5.1	29.5 ± 4.5	31.9 ± 5.4
Cholesterol, mg/d	185.9 ± 63.8	206.2 ± 68.5	216.8 ± 75.9	214.7 ± 78.4
Vitamin K1, µg/d	213.3 ± 115.7	207.8 ± 106.5	211.4 ± 97.3	210.2 ± 105.0
Vitamin K2, µg/d	15.0 ± 3.7	23.7 ± 2.2	31.2 ± 2.3	46.5 ± 10.8
Alcohol, g/d	9.6 ± 14.8	8.4 ± 11.6	8.7 ± 11.5	9.9 ± 13.5
Dietary fiber, g/d	22.6 ± 4.9	22.2 ± 4.3	22.4 ± 4.0	21.9 ± 4.2
Calcium, mg/d	873.8 ± 282.0	1035.0 ±256.9	1119.5± 239.5	1326.0 ± 317.8

Table 2. Crude and adjusted* mean energy adjusted intake of vitamin K1, K2 and vitamin K subtypes in 1689 women with and without breast arterial calcifications (BAC).

Vitamin K	BAC +		BAC -	
(mean µg/d)	Unadjusted	Adjusted (95% CI)	Unadjusted	Adjusted (95% CI)
Vitamin K1	216.6	209.2 (193.8-224.6)	210.7	210.9 (205.5-216.2)
Vitamin K2	26.9	28.6 (27.2-30.0)	29.4	29.3 (28.8-29.8)
MK-4	7.0	7.1 (6.9-7.4)	7.2	7.2 (7.1-7.2)
MK-5	0.30	0.31 (0.29-0.32)	0.33	0.33 (0.32-0.33)
MK-6	0.31	0.33 (0.31-0.35)	0.34	0.34 (0.33-0.35)
MK-7	0.40	0.44 (0.41-0.47)	0.46	0.45 (0.44-0.46)
MK-8	5.5	6.0 (5.6-6.4)	6.2	6.2 (6.0-6.3)
MK-9	13.4	14.5 (13.5-15.4)	15.0	15.0 (14.6-15.3)
MK-10	0.09	0.09 (0.08-0.10)	0.09	0.09 (0.09-0.09)

*Adjusted for age, smoking, diabetes, energy adjusted saturated fat, energy adjusted mono unsaturated fat, energy adjusted poly unsaturated fat, energy adjusted protein intake, and calcium intake.

After adjustment for aging, smoking, diabetes and dietary factors (energy intake, fat intake, calcium intake), the association of mean vitamin K2 intake with BAC was no longer significant. Only mean values of subtype MK5 of vitamin K2 remained significantly ($p = 0.03$) lower in women with BAC (0.30 µg/day) compared to women without BAC (0.33 µg/day).

Discussion

We found that a mean lower intake of vitamin K is not associated with the presence of mammographic arterial calcifications. After adjustment for confounders and dietary variables, only the lower intake of MK5 subtype of vitamin K2 remained significantly associated with the presence of BAC.

To better appreciate our findings some issues need to be discussed. We used an FFQ that was not specifically developed to estimate vitamin K intake. However, the FFQ included all major food sources of vitamin K and covered a one-year period of food intake to eliminate seasonal variation in intake. The intake estimates of food groups containing vitamin K2 (meat, dairy products) were shown to be adequate previously.[16] Although for foods that are rich in vitamin K1 (e.g. broccoli, cabbage, spinach), separate questions were asked and photographs presented, the validity was not optimal. However, the mean intakes of vitamin K1 and K2 were similar to that reported from the Nurses' Health Study and the Rotterdam study.[9,10] Finally, even a short vitamin K checklist provided essentially identical values of dietary phylloquinone intake as did 4-day dietary records in 36 healthy volunteers.[22] We have no explanation why we found the strongest association between BAC and lower levels of MK5 subtype of vitamin K2, whereas the relative high consumption of hard cheeses in the Netherlands is merely related to MK7 and MK 9 subtypes.[6] MK5 is present in very low concentrations in animal products and has a minor role amongst the other menaquinones.

Vascular calcification is currently considered as an active process with similar mechanisms as in bone formation. Vitamin K is important for the carboxylation of proteins, eg Matrix-Gla-protein (MGP), protein S and osteocalcin, that are present in bone and vascular tissue.[1] MGP is secreted by vascular smooth muscle cells (VSMC) and macrophages in the arteries and in chondrocytes in bone tissue. MGP acts as an inhibitor of the calcium uptake in the vascular wall and in bone tissue.[2-4] In animal experiments with MGP knock-out mice severe vascular calcification of the large arteries has been found.[23] MGP is expressed in intimal and medial calcification and both types of vascular calcification have been associated with a low vitamin K status.[1-4,24-26] It is not known whether vitamin K1 and vitamin K2 may have different effects on vascular calcification in different layers of the vascular wall.

Mammographic arterial calcifications are known to be located in the media and have thus far been associated with aging, diabetes, and endocrine factors involved with pregnancy and lactation.[11-13,27-29] Although women with BAC have a higher total burden of CHD risk factors, only diabetes is independently associated.[27] Our study is the first to

investigate mammographic arterial calcifications in relation to vitamin K intake. The absence of a clear relation between vitamin K intake and BAC may be explained by the relative limited relation of BAC with cardiovascular risk. In a recent study however, no association was found between coronary artery calcification (CAC) and vitamin K1 intake[30], although others have previously shown that the severity of CAC is correlated with low serum MGP levels and the use of oral anticoagulants.[5,31]

A number of studies have shown parallels between arterial calcifications and bone metabolism. Vitamin K metabolism is a common factor in both processes.[32] Artery calcification and osteoporosis both increase in women after menopause. The presence of aortic calcifications is associated with a lower bone mass and a marginal vitamin K status as measured by nutritional vitamin K intake and serum osteocalcin levels.[33] In the Nurses' Health Study cohort it was found that a higher intake of vitamin K was associated with a lower risk of hip fractures in women.[34] In the Framingham Offspring study a low vitamin K intake was associated with a low bone mineral density in women, but not in men.[5] No significant associations were found between vitamin K and BMD in premenopausal women and in postmenopausal women using estrogens.[36] The role of estrogens on vitamin K status is undetermined and the protective effects of estrogens on bone metabolism may overrule any effect of vitamin K intake. Mammographic arterial calcifications have recently been associated with a low bone mineral density (BMD) in postmenopausal women.[37] It needs to be further investigated whether these calcium deposits may be closer related to osteoporosis than to atherosclerosis.

In conclusion, our findings in a large group of older women do not support the view that calcifications in breast arteries are associated with a lower intake of vitamin K.

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CHAPTER 8

Breast arterial calcifications are correlated with subsequent development of coronary artery calcifications, but their aetiology is predominantly different

Angela H.E.M. Maas, Yvonne T. van der Schouw

Femke Atsma, David Beijerinck, Jan J.M. Deurenberg

Willem P.Th.M. Mali, Yolanda van der Graaf

Submitted

Abstract

OBJECTIVE: Vascular calcification is a marker of cardiovascular (CV) risk and imaging of calcium is increasingly used for CV screening. It is unknown whether calcifications in breast arteries are related to coronary artery calcifications.

METHODS: We studied 499 women, aged 49−70 years, participating in a breast cancer screening program and investigated whether arterial calcifications in the breast (BAC) are associated with coronary arterial calcifications (CAC) after 9 years follow-up. Mammograms were reviewed for the presence of BAC. CAC was assessed by multi slice computed tomography (MSCT). With logistic regression analysis the independent effect of BAC and CAC on various risk factors was measured.

RESULTS: BAC was present in 58 of 499 women (11.6%) and CAC score >0 was present in 262 of 499 women (52.5%). Age was the strongest determinant for both BAC and CAC. BAC was strongly associated with CAC (OR 3.2, 95% CI 1.71−6.04) and this remained significant after adjustment for age at baseline and the duration of follow-up (OR 2.1, 95% CI 1.10−4.23). Most CV risk factors were associated with CAC but not with BAC. Only parity was significantly associated with both increased CAC (OR 2.1, 95% CI 1.21−3.60) and increased BAC (OR 5.3, 95% CI 1.23−22.43). Breastfeeding was associated with BAC (OR 3.4, 95% CI 1.40−8.23) but not with CAC (OR 1.3, 95% CI 0.84−1.93).

CONCLUSION: Breast arterial calcifications are predictive of subsequent development of calcifications in the coronary arteries. However, the absence of common risk factors for BAC and CAC suggests a different aetiology between both types of vascular calcification.

Introduction

Calcium in the coronary vascular wall is a known marker of the presence of atherosclerotic disease in the coronary arteries. The amount of calcium, detected by electron beam computed tomography (EBCT) or multi slice computed tomography scanning (MSCT), is strongly related to the risk of coronary heart disease (CHD) events.[1,2] Disadvantages of using these new radiology techniques in risk assessment are the costs and feasibility for its widespread application, the impaired additional diagnostic value in low-risk and high-risk patients and the risks involved in radiation exposure.[3-5] Calcium deposits are also present in other parts of the vascular tree as a sign of subclinical atherosclerosis. Imaging of calcifications in the thoracic and abdominal aorta on conventional x-rays for example has also been associated with an increased CHD event risk, but its clinical application for screening purposes has not been established.[6-8].

Calcifications in the arteries of the breasts can be seen in 3−29% of women undergoing mammography, with the advantage that mammograms are often made in already running breast cancer screening programs.[9-13] The simultaneous use of mammograms for screening purposes on breast cancer and CHD could be very cost effective. The clinical significance of breast arterial calcifications however is controversial and thus far merely been associated with aging, diabetes, parity and lactation.[14-16] It is yet undetermined whether breast arterial calcifications (BAC) and coronary artery calcifications (CAC) are related. Furthermore, it is not known whether both types of vascular calcifications share common CHD risk factors. Moreover, it has not been studied whether women with BAC are at greater risk to develop CAC as a sign of atherosclerotic disease. In the current study we investigated the relation between the prevalence of BAC at baseline and CAC scores at 6−11 years follow-up in 499 women participating in a breast-cancer screening program.

Methods

Population

Participating women were recruited from the PROSPECT study, one of the Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC). Characteristics of the population have been described before.[17] In brief, PROSPECT consists of 17,357 healthy women, aged 49−70 years, from a breast cancer screening program in Utrecht and its surroundings, enrolled between 1993 and 1997. The purpose of EPIC is to assess the relation between nutrition and cancer and other chronic diseases. Women were asked to participate along with their invitation for a routine mammography.

At baseline women filled in a general questionnaire and a medical examination was performed. All women signed an informed consent prior to study inclusion with permission to use personal data and biologic material in substudies.

The present substudy was performed in a random sample of 573 women from the original total population of 17,357 women. Women were selected from 5844 eligible women from the original cohort, based on the following criteria: no participation in other studies, valid written informed consent, postmenopausal, and currently no use of oral contraceptives (OC) or hormone therapy (HT). A random selection of 1996 women was invited by a personal letter of whom 1000 were willing to participate. Out of this group, a random selection of 573 women were chosen for CAC measurement. In 5 women no calcium scores could be obtained, and of 69 women the baseline mammograms could not be retrieved. Thus, the current study population includes 499 women. The study was approved by the Institutional Review Board of the University Medical Center Utrecht and written informed consent was obtained from all participants. Data collection with regard to calcium scores took place between November 2003 and February 2005.

Baseline measurements

The baseline questionnaire was obtained at the time of the baseline mammograms and contained information on date of birth, cardiovascular disease history, and established risk factors for cardiovascular disease. Smoking was defined as current, past, or never smoking.

Systolic and diastolic blood pressures were measured in duplicate at the left arm with the subjects in sitting position after 10 minutes of rest with an automated and calibrated oscillomat (Bosch & Son, Jungingen, Germany). Subsequently, the mean systolic and diastolic blood pressures were calculated. Body height was measured to the nearest 0.5 cm with a wall mounted stadiometer (Lameris, Utrecht, The Netherlands). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg with a floor scale (Seca, Atlanta, GA, USA). Body mass index was calculated as weight divided by height squared (kg/m²). Hypertension was defined as present when women reported that a physician diagnosed this, and/or when they had a measured systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg. Hypercholesterolemia and diabetes mellitus were defined as present when women reported that a physician diagnosed this. Parity was defined as the total number of live born or still born children. Breastfeeding was defined as having breastfed after at least one pregnancy.

Mammograms

All baseline mammograms (mediolateral-oblique and craniocaudal views of both breasts) of the 499 eligible women were retrieved from the archives and analysed for the presence of calcium in the breast arteries by 2 experienced radiologists, who were blinded to the clinical data. Breast arterial calcium was defined as present if calcium deposits were seen along the contours of the arterial wall(s) in one or both breasts. If there was disagreement in analysis, occurring in 5% of cases, the mammograms were reviewed by both observers to reach consensus.

Coronary calcification

The amount of calcium in the coronary arteries was assessed with a MSCT scanner (Mx 8000 IDT 16, Philips Medical Systems, Best, The Netherlands). Subjects were positioned within the gantry of the MSCT scanner in supine position. A 16-slice scanner with 0.42 seconds rotation time was used to obtain 1.5 mm thick sections. During a single breath hold, images of the heart, from the level of the tracheal bifurcation to below the base of the heart, were acquired using prospective ECG triggering at 50−80% of the RR-interval,

depending on the heart rate. Scan duration was approximately 10 seconds, depending on heart rate and patient size. From the acquired raw data, 3 mm thick sections were reconstructed. Quantification of CAC was performed on a separate workstation with software for calcium scoring (Heartbeat-CS, EBW, Philips Medical Systems, Best, The Netherlands). All regions with a density over 130 Hounsfield units are identified as potential calcifications. A trained scan reader manually selects only the calcifications within one of the coronary arteries (left main, left anterior descending, left circumflex, right coronary artery, and PDA). To reduce the influence of noise, the minimum size of a calcified lesion was set at 0.5 mm^2. The peak density in Hounsfield units and the area in mm^2 of each selected region were calculated. The Agatston calcium score was obtained by multiplying the area by a weighting factor that is dependent on the peak signal anywhere in the lesion.[18] The scores of individual lesions were added to obtain the Agatston calcium score for the entire coronary tree. Reproducibility of the MSCT was assessed by having 199 scans read by two independent observers and by having 58 subjects undergoing a second scan within 3 months. The inter-reader reproducibility and the inter-scan reproducibility were excellent with intraclass correlation coefficients above 0.95.

Table 1. Distribution of baseline characteristics in the study population of 499 women.

Variables	
Age, yr (mean ± SD)	57.9 ± 5.2
Body mass index, mean ± SD (kg/m^2)	25.6 ± 4.0
Mean systolic BP, mmHg	130 ± 19
Mean diastolic BP, mmHg	78 ± 10
Waist/hip ratio	0.78 ± 0.05
Smoking,	
current, n (%)	92 (18.4)
previous, n (%)	187 (37.5)
Never, n (%)	220 (44.1)
Diabetes, n (%)	6 (1.2)
Hypertension, n (%)	90 (18.1)
Hypercholesterolemia, n (%)	37 (7.4)
Previous CVD, n (%)	20 (4)
OC use, n (%)	312 (62.5)
Previous HT use, %	128 (25.7)

OC = oral contraceptive; HT = hormone therapy.

Table 2. Distribution of categories of Agatston scores in the population of 499 women.

Agatston score	Number	%
0	237	47.5
0−10	45	9.1
10−99	114	22.8
100−400	69	13.8
>400	34	6.8

Data analyses

CAC scores were obtained at 1.5 and 3.0 mm slice thickness and yielded similar results. Therefore results from 3.0 mm slices are presented which are most commonly used in the literature. The Agatston score was categorized as 'no CAC' (Agatston = 0) and 'CAC' (Agatston >0). Baseline characteristics are calculated as means with standard deviations (SD) for continuous normally distributed variables and as a percentage for categorical variables. Logistic regression analysis was used to analyze the association of the dichotomized Agatston score with BAC, with adjustment for age. Multivariate logistic regression analysis with adjustment for age was done to determine the associations of individual cardiovascular risk factors with BAC as well as CAC

Results

Baseline characteristics of the participating women are shown in Table 1. BAC was present in 58 of 499 women (11.6%) at baseline. CAC score >0 was present in 262 of 499 women (52.5%) at follow-up. Table 2 represents the distribution of the Agatston scores in the study population. Forty-four (76%) women with mammographic arterial calcium at baseline had coronary artery calcifications after a mean period of 9 (SD 1.3) years, whereas 218 (49%) women without BAC had coronary calcifications at follow up. In all age groups CAC was more prevalent than BAC (Table 3). BAC at baseline was significantly associated with CAC (OR 3.2, 95% CI 1.71−6.04). When adjusted for age at baseline this association

Table 3. Prevalence of breast arterial calcium (BAC) and coronary artery calcium (CAC) scores >0, according to groups of age at baseline (n = 499).

Age (yrs)	BAC+		CAC >0	
	n	%	n	%
49−54	3	1.9	52	32.7
55−59	21	12.5	85	50.6
60−64	18	15.4	78	66.7
65−70	16	29.1	47	85.5

remained significant (p = 0.04, OR 2.0, 95% CI 1.03−3.86). Further adjustment for the duration of follow-up did not change these results.

Age was the most important determinant of both BAC and CAC with ORs per year of 1.17 (95% CI 1.11−1.24) and 1.16 (95% CI 1.11−1.20) for BAC and CAC, respectively. As expected, other classical cardiovascular risk factors were strongly associated with the occurrence of CAC (Table 4). Previous CHD events (OR 4.5, 95% CI 1.40−14.53), current smoking (OR 3.8, 95% CI 2.14−6.65) and hypercholesterolemia (OR 4.0, 95% CI 1.66−9.78) were significantly associated with CAC. In contrast, none of these risk factors were significantly related with the presence of BAC. The low number of diabetics in this population (n = 6) prohibited further analysis with diabetes.

Of the reproductive parameters, parity was statistically significantly associated with the presence of BAC as well as the occurrence of CAC, although the risk was more pronounced for BAC (age-adjusted ORs 5.3 [95% CI 1.23−22.43] for BAC and 2.1 [95% CI 1.21−3.60] for CAC, respectively). Breastfeeding after pregnancy was also associated with BAC (OR 3.4, 95% CI 1.40−8.23) but not with CAC (OR 1.3, 95% CI 0.84−1.93). Previous use of oral contraceptives (OC) or postmenopausal hormone therapy (HT) was neither associated with BAC nor with CAC.

Table 4. Associations of cardiovascular risk factors* with BAC and CAC (n = 499).

Cardiovascular risk factor	BAC		CAC	
	OR	**95% CI**	**OR**	**95% CI**
Age (years)	1.17	1.11−1.24	1.16	1.11−1.20
Smoking				
Previous	0.75	0.40−1.40	1.20	0.79−1.83
Current	0.60	0.25−1.39	3.77	2.14−6.65
Previous CVD	1.88	0.56−6.32	4.51	1.40−14.53
Hypertension	1.33	0.68−2.63	1.60	0.97−2.63
Hypercholesterolemia	1.42	0.56−3.60	4.03	1.66−9.78
Systolic BP (mmHg)	0.999	0.983−1.014	1.009	0.998−1.020
Diastolic BP (mmHg)	1.006	0.997−1.035	1.027	1.006−1.048
Body mass index (kg/m^2)	0.959	0.888−1.035	1.011	0.964−1.060
Parity	5.3	1.23−22.43	2.1	1.21−3.60
Breastfeeding	3.4	1.40−8.23	1.3	0.84−1.93
Oral contraceptive	1.6	0.85−2.95	1.0	0.67−1.53
Hormone therapy	0.8	0.37−1.65	1.2	0.78−1.88

*Adjusted for age at baseline.

Discussion

We found that calcifications in the breast arteries are associated with the subsequent development of coronary artery calcifications. In both types of vascular calcification aging was found to be a major determinant. Most CHD risk factors were associated with CAC but not with BAC. Parity and breastfeeding were the strongest determinants for BAC.

Some issues need to be addressed to better understand our findings. First, we used MSCT scans to measure coronary calcium scores. Although electron beam computed tomography (EBCT) has been considered the gold standard for the assessment of calcified plaques, comparable results are provided by MSCT scanning.[19-21] We used a dichotomized CAC scoring into yes/no calcium, as the negative predictive value of a zero calcium score is reported to be 99%.[22] Second, the mammograms were scored visually by 2 separate radiologists with more than 18 years of experience in mammography reading. The inter-

reader correlation was very good, with disagreement in only 5% of cases, which could be resolved in discussion.

Coronary artery calcification reflects 'chronic plaque burden'. It can be present in more advanced atherosclerotic lesions, but it may also be present as small deposits of calcium earlier in the course of lesion development.[19] Age, race and gender are the most important determinants of coronary calcification and its impact is greater than the other cardiovascular risk factors.[23-26] In women calcifications in the vascular tree are less prevalent in all age groups compared to men. The average age for the appearance of calcium is <40 years for men and 55−60 years for women.[27,28] The correlation of calcifications across different vascular beds has a similar pattern in men and women.[28] In the Healthy Women Study, in participants aged 58 years, calcification in the in the coronary arteries (37%) were less frequent compared to calcifications in the aorta (71%), with a strong correlation ($r = 0.44$, $p< 0.001$).[29] Others found a smaller correlation between aortic and coronary calcium.[30] In one previous small study involving 74 women, the presence of BAC was also found to be associated with CAC at MSCT.[31]

While CAC screening has shown to be a useful additional tool in risk prediction of CHD, our present study does not support the use of screening for arterial calcium in mammograms for the detection of subclinical atherosclerosis. Although we did find an association between CAC and BAC, the lack of association between cardiovascular risk factors and BAC does not make it feasible as a risk marker for atherosclerosis.

A major explanation for the differences in aetiology between the two types of calcification is that CAC is merely located in the intima of the vessel wall, while BAC reflects calcification in the media without signs of inflammation.[32,33] The inverse association we found between BAC and smoking is another indication for the absence of inflammatory effects on calcification in the breast arteries[34,35] Although various other CHD risk factors have been described in relation with BAC, only diabetes has been shown to be independently related to BAC and CHD risk.[9-11,13,14] In diabetics, prolonged exposure to hyperglycaemia has been recognized as a major factor in the pathogenesis of both medial and intimal sclerosis, indicating that similar mechanisms in glucose metabolism are

involved in both types of calcification[36,37] Due to the low number of diabetics in our data, we were not able to reproduce this finding here.

In women, arterial calcifications in the coronary arteries are reported to be related to their estrogen status[38,39] We found no differences in previous OC use and use of HT in women with and without BAC or in women with and without CAC. Thus far, data on chronic HT use and CAC scores are conflicting and fail to explain what mechanisms are involved in the protective effects of sex hormones on vascular calcification.[29,36,39-41] The much stronger association of BAC than CAC with reproductive parameters suggests that other hormonal factors related to pregnancy and lactation are important in the calcification process in the breast arteries.

A common causative finding in the medial sclerosis that is present in breast arteries and the medial sclerosis that is found in patients with end-stage renal disease (ESRD) is the alterated mineral metabolism.[42,43] Pregnant women and patients with ESRD have an activated calcium metabolism that induces vascular smooth muscle cells (VSMC) into a more chondrogenic phenotype that enhances the uptake of calcium (hydroxyapatite). Similar mineralization-regulating proteins of bone resorption and formation (e.g. osteocalcin, bone morphogenic protein) are upregulated in pregnancy and lactation and in patients with ESRD.[37,43-46] An older study on BAC confirms that these calcifications are more prevalent in women with chronic renal failure, compared to patients with a normal renal function.[47]

In our present study we found that calcifications in breast arteries are related to subsequent development of calcifications in the coronary arteries, but differences in aetiology indicate that mammograms may be not a useful tool in CHD risk assessment in women.

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CHAPTER 9

Progression of calcifications in breast arteries in women at high risk of coronary heart disease events

Angela H.E.M. Maas, Yvonne T. van der Schouw
Willem P.Th.M. Mali, Yolanda van der Graaf

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Abstract

OBJECTIVE: To investigate to what extent breast artery calcifications (BAC) progress or regress over time and whether changes in BAC severity are related to coronary heart disease (CHD) risk factors.

METHODS: We studied the severity of breast arterial calcifications (BAC) at baseline and after 4−6 years of follow-up in 453 postmenopausal women at high risk of cardiovascular events, participating in the Raloxifene Use for The Heart (RUTH) study. Baseline mammograms and follow-up mammograms were scored by 2 independent observers on the presence of BAC, according to a grading scale with 3 categories. Logistic regression analysis was performed to investigate the independent effect of risk factors on the progression of BAC.

RESULTS: BAC was present in 94 of 453 (21%) women at baseline and in 116 of 453 (26%) women after a mean follow-up duration of 5.0±1.04 years. Progression in the severity of BAC was seen in 44 of 453 (10%) women of whom both mammograms were available. In 22 participants (5%) BAC was not present at baseline, while in 22 participants (5%) the severity of BAC merely changed from grade 1 to grade 2 calcification. Regression was seen in 3 women. Age was significantly associated with progression of BAC (OR 1.09 per each year increase in age, 95% CI 1.03−1.14). Multivariate regression analysis with adjustment for age and the duration of follow up revealed no association between CHD risk factors and the progression of BAC. Lipid-lowering drugs protected for progression (adjusted OR for age and the duration of follow-up 0.5, 95% CI 0.22−0.98). The strongest determinant in the progression of BAC was the presence of BAC at baseline (adjusted OR 4.2, 95% CI 2.10−8.27).

CONCLUSION: Progression of BAC is not associated with CHD risk factors, but with increasing age and the presence of BAC at baseline. Lipid lowering drugs protect for progression of BAC.

Introduction

Imaging of vascular calcification has become a promising tool in risk assessment for subclinical atherosclerosis. Thus far, age, race and gender are the most potent determinants of coronary calcification.[1] Limited information is available on the rate of progression of calcified lesions over time. The rate of progression can vary among different vascular beds and is also related to coronary heart disease (CHD) risk factors, ethnic factors and genetic factors.[2-4] For coronary artery calcium in women, the most potent determinants of progression are baseline calcium scores.[5] Progression of vascular calcification in both the intima and media of the vessel wall is a strong predictor of CHD mortality in diabetics and in patients with end-stage renal disease.[6,7]

Calcifications in the arteries of the breasts are also more often present in diabetic women and have been associated with an increased mortality risk.[8,9] Although the aetiology of breast arterial calcifications (BAC) is merely different from calcifications elsewhere in the vascular tree, common mechanisms are involved in the calcifying process.[10]

Thus far, it is unknown to which extent BAC changes over time and whether CHD risk factors may interfere with its progression. Therefore, we studied the prevalence of BAC in baseline mammograms and in mammograms after 4−6 years follow-up, in 453 women participating in the Raloxifene Use for The Heart (RUTH) study .

Methods

Population

The Dutch participants of the RUTH study were included in this follow-up study. RUTH was started in 1998 and is a multi-center, randomized, double-blind, placebo-controlled clinical trial involving women from 26 countries. Its aim is to evaluate treatment with 60 mg raloxifene versus placebo in 10,101 postmenopausal women at high risk of major cardiovascular events. The two separate primary endpoints are coronary events

(coronary death, nonfatal myocardial infarction (MI) or hospitalization for acute coronary syndromes other than MI) and invasive breast cancer. The trial design, methods, and participant characteristics at baseline have been described in detail elsewhere.[11,12]

Participants were classified according to a points risk score related to the presence of risk factors and previous CHD events. A minimum of 4 points was required for inclusion in RUTH. Briefly, inclusion criteria were age ≥55 years, ≥1 year postmenopausal with established coronary heart disease (i.e., prior myocardial infarction (MI), coronary artery bypass graft surgery (CABG), percutaneous coronary interventions (PCI), or angiographic evidence of a 50% occlusion of one or more major coronary arteries) or an increased risk of a major coronary event, based on the presence of multiple cardiovascular risk factors. An inclusion criterion for women without established CHD was the presence of multiple factors (minimum score 4) that increase the risk of MI and coronary death, such as smoking, hypertension, hyperlipidemia, and diabetes. The baseline data on cardiovascular risk factors were recorded at randomization before study entry. All participants gave written informed consent prior to inclusion and the study was approved by the local medical ethics committees of the participating hospitals. The Dutch part of the RUTH study consists of 610 women and of 453 participants two-sided views of follow-up mammograms after 4−6 years were available for analysis.

Risk factors

Body mass index was calculated as the ratio of body weight (in kg) to height (in meters) squared (kg/m²). Current smoking was defined as smoking an average of 10 or more cigarettes a day in the 6 months before randomization. Hypertension was present in patients taking antihypertensive medications or in patients with a systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg on at least two measurements prior to randomization. Hyperlipidemia was present in patients with lipid-lowering medications, or in patients with a fasting LDL-cholesterol >160 mg/dL (4.14 mmol/L) or in patients with fasting HDL-cholesterol <45 mg/dL (1.16 mmol/L) with fasting triglycerides >250 mg/dL (2.82 mmol/L). Diabetes mellitus was defined as taking oral antidiabetic medication or

insulin or as having fasting serum glucose >140 mg/dL (7.8 mmol/L) within 3 months prior to randomization.

Parity was defined as the total number of live born and still born children after pregnancy.

Mammograms

Standard mammograms at baseline (cranio-caudal and lateral views) were made for all women before entry in the RUTH trial. Every 2 years follow-up mammograms were made according to the study protocol. Of 453 patients, who were still participating in the study, both baseline and follow-up mammograms after 4−6 years were available for analysis.

All mammograms were read by two experienced, independent observers, who were blinded to the clinical data of the patients. BAC was characterized by the presence of calcium deposits along the wall of one or more arteries in the right, the left, or both breasts.

The intensity of BAC was graded as follows:

A. grade 1 = mild BAC, the artery was faintly outlined by calcium,
B. grade 2 = moderate BAC, the artery was distinctly outlined by calcium,
C. grade 3 = severe BAC, the artery was distinctly outlined by thick columns of calcium.

Progression or regression of BAC was characterized by a visual change in at least 1 degree in the extent of calcium deposition along the border of the vascular wall, taking into account differences in breast imaging techniques and consensus agreement of both observers. Calcifications that were seen in the follow-up mammograms that were not present at baseline were also graded as progression.

Statistical analysis

Baseline characteristics are calculated as means with standard deviations (SD) for continuous normally distributed variables and as a percentage for categorical variables.

Determinants of change in BAC were examined with logistic regression, with adjustment for age and the duration of follow-up. Odds ratios (ORs) and corresponding

95% confidence intervals were calculated as an approximation of relative risk. Analyses were carried out using SPSS 12.0 package.

Results

BAC was present in 94 of 453 (21%) women at baseline and in 116 of 453 (26%) women after a mean follow-up duration of 5.0±1.04 years. Progression of BAC was seen in 44 of 453 (10%) women of whom both mammograms were available. In 22 (5%) participants BAC was not present at baseline, while in the other 22 (5%) women the severity of BAC mostly changed from grade 1 to grade 2 calcification. In 3 patients a one-graded regression in the intensity of BAC was seen. In 69 of 453 (15%) women the presence of BAC was unchanged in severity. In Table 1 the baseline CHD risk factors are represented in patients with progression of BAC and in patients with unchanged mammograms at follow-up.

Table 1. Baseline characteristics of 453 women with progression of BAC and unchanged mammograms at 5 years follow-up.

	Progression BAC (n = 44)	**Mammograms unchanged (n = 409)**
Mean age, yrs	70 ± 6	66 ± 6
BMI, kg/m^2	27 ± 4	28 ± 4
Systolic BP, mmHg	155 ± 25	151 ± 22
Diastolic BP, mmHg	85 ± 11	85 ± 10
Total cholesterol, mmol/L (mg/dl)	5.4 ± 1.2 (210 ± 47)	5.1 ± 0.9 (198 ± 36)
HDL-cholesterol, mmol/L (mg/dl)	1.4 ± 0.3 (54 ± 12)	1.3 ± 0.3 (50 ± 12)
LDL-cholesterol, mmol/L (mg/dl)	3.2 ± 1.0 (122 ± 39)	2.9 ± 0.8 (111 ± 32)
Lipid lowering medication, %	73	84
Current smoking, %	9	20
Hypertension medicine use, %	58	62
Diabetes mellitus, %	24	24
Mean number of children	3.3 ± 1.4	3.1 ± 1.6
BAC positive at baseline, n (%)	22 (50)	72 (18)
Mean duration follow up, months	65 ± 12	60 ± 13

Table 2. Crude and adjusted relations between cardiovascular risk factors and progression of BAC.

Variables	Crude OR (95% CI)	Adjusted for age OR (95% CI)	Adjusted for age and duration follow-up OR (95% CI)
Age	1.09 (1.03−1.14)		
Hypertension	0.8 (0.43−1.51)	0.7 (0.39−1.41)	0.8 (0.40−1.46)
Diabetes	1.1 (0.52−2.20)	1.2 (0.57−2.45)	1.3 (0.61−2.71)
Lipid lowering therapy	0.5 (0.25−1.04)	0.5 (0.26−1.12)	0.5 (0.22−0.98)
Current smoking	0.4 (0.14−1.20)	0.5 (0.18−1.56)	0.6 (0.20−1.74)
Parity	1.1 (0.88−1.30)	1.0 (0.84−1.24)	1.0 (0.85−1.27)
BAC at baseline	4.7 (2.45−8.86)	3.8 (1.94−7.46)	4.2 (2.10−8.27)

Multivariate analysis showed that none of the CHD risk factors was associated with the progression of BAC (Table 2). Only age was significantly associated with progression of BAC (OR 1.09 per each year increase in age, 95% CI 1.03−1.14). The duration of follow up was significantly ($p<0.001$) longer in women with progression (65±12 months) than in women with unchanged mammograms (60±13 months). Participants using lipid-lowering therapy had less progression of BAC, when adjusted for age and the duration of follow-up (OR 0.5, 95% CI 0.22−0.98). Parity was not associated with progression of BAC. The presence of BAC at baseline was the strongest determinant for progression of BAC (adjusted OR 4.2, 95% 2.10−8.27).

Discussion

We found a 10% progressive change in the severity of BAC in mammograms, after a mean follow-up duration of 5 years. Previous calcifications in the breast arteries and increasing age, were the only independent predictors of progression. Our study underscores that BAC is not a suitable marker of CHD risk. Although we have previously found an independent association of diabetes with the presence of BAC, we currently failed to observe an association of diabetes at baseline with further progression of BAC.[8]

A limitation of our study is that unblinding of the RUTH study medication was not yet allowed and therefore we could not investigate whether raloxifene may have had any effect on the progression of BAC. However, in previous studies the prevalence of BAC was not associated with the use of oral contraceptive (OC) or hormone therapy (HT) and in other types of vascular calcification the role of HT is still controversial.[10,13-16] Another limitation of our study may be the improvements in breast imaging techniques over the past several years. However, all follow up mammograms were reviewed simultaneously by 2 experienced observers who were familiar with different mammography techniques. The diagnostic accuracy of digital and film mammography has been shown to be similar.[17]

Thus far, limited data are published on the changes in vascular calcification that occur over time. For coronary calcium it was shown in two separate studies that the strongest determinant of progression of coronary calcium was the baseline calcium score and not separate CHD risk factors.[5,18] We found that women using statin treatment had less progression of BAC. In some studies on coronary calcifications statin use has been shown to reduce the progression of calcifications,[5,19] while in other more recent studies statin use had no effect on the progression of arterial- and valvular calcifications.[20-23] The complexity of progression of vascular calcification is further illustrated in in-vitro studies demonstrating that statin therapy can even paradoxically stimulate bone cell calcification.[24, 25]

Although vascular calcifications in the coronary arteries are related to CHD risk factors and calcifications in the breast arteries are more related to reproductive factors, both types of calcifications are correlated and show progression over time.[10] The initiating mechanisms of calcifications in various vascular beds and the factors that are involved with progression may not be similar. Smoking for instance promotes vascular calcification in atherosclerotic lesions and is independently associated with its progression thereafter, while in all studies on BAC smoking seems to protect against these calcifications.[8,26-28]. In our present study we even observed a non-significant lower rate of progression of BAC in current smokers. In all manifestations of vascular calcification older age apparently leads to more dysfunction of cellular proteins that may further enhance the calcifying process once it is present, independently of initially causative factors.[29,30] Gender- and genetic related polymorphisms in the variability of different types of vascular calcifications (intimal,

medial, valvular) may further account for the inter-individual differences in the susceptibility for calcification and its progression thereafter[1,31-33]

In conclusion, we found that progression of vascular calcification in the breast arteries is related to age and the presence of calcifications at baseline, but not to CHD risk factors.

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CHAPTER 10

General discussion

Calcification is nowadays widely used as a clinical indicator of atherosclerosis. Imaging of vascular calcification has become an important step forward in early preventive strategies of coronary heart disease (CHD). Calcium deposits of the aorta and the coronary arteries predispose to increased risks of morbidity and mortality from CHD.[1-5] The currently most used radiographic methods for plaque burden screening are coronary artery calcium (CAC) measurements with electron beam computed tomography (EBCT) or multi-slice computed tomography (MSCT). Screening for arterial calcium through CAC measurements is especially useful in individuals at intermediate risk for coronary artery disease.[6]

In women, imaging of arterial calcium in the breasts is another possible radiographic modality. The simultaneous use of mammograms for breast cancer screening and screening for atherosclerotic disease could be very cost effective and has a lower radiation exposure compared tot CAC screening. In this thesis we have investigated whether breast arterial calcifications (BAC) can predict cardiovascular risk in two different populations of women. Based on our findings we conclude that these calcium deposits are fundamentally different from calcifications elsewhere in the vascular tree, although overlapping mechanisms are involved.

Previous studies on calcification in breast arteries

Breast arterial calcifications were first described by Hall in 1977.[7] Baum et al. found a significant higher prevalence of BAC in women with diabetes mellitus compared to non-diabetics.[8] Others confirmed these findings, but emphasized the predominant importance of age on the occurrence of BAC.[9] Advancing age was also considered to be the major determinant in some small population-based mammographic studies, that disputed the relevance of other risk factors like diabetes and hypertension.[10,11] In the study of Leinster et al. it was also noted that the prevalence of BAC was related to hormonal factors.[11] The occurrence was lower in women on hormone therapy (HT) and in women using oral contraceptives (OC) and it was higher in women who had been pregnant. Cox et al. also

found a lower prevalence of BAC in current or former users of HT.[12] Sommer et al, reported that BAC was more prevalent in women with chronic renal failure compared to women with a normal renal function.[13]

Most recent studies in women from breast cancer screening populations found a prevalence of BAC between 3 to 35%, independently associated with age, hypertension and diabetes.[14-16] Smoking was inversely associated with BAC in all studies. Reddy et al. also described racial differences with the highest prevalence of BAC in Hispanics, compared to whites and Africans.[16] In a large population-based breast cancer screening population of 12.239 women, the DOM-cohort, we previously found a prevalence of BAC of 9% in women aged 50−69 years, with a significantly higher presence (15.4%) in diabetics.[17] Age ≥60 years was the strongest determinant of BAC. We also noticed that parity was positively associated with BAC. Cardiovascular mortality after 16−19 years follow-up was significantly higher in diabetic women with BAC compared to non-diabetic women with BAC.[18]

In a relative small study Moshyedi et al. observed no association between BAC and angiographic coronary artery disease in women aged 59 years and older but they suggested that BAC may be an additional risk indicator in younger women.[19] In a recent larger study in 319 women, the occurrence of BAC was not found to be associated with coronary artery disease.[20] The discrepancies that arise from these data dispute the usefulness of mammograms as a tool in CHD risk assessment in women. Therefore, we studied breast arterial calcifications into more detail in a high risk population of women and in women participating in a breast cancer screening program.

Determinants of BAC

In the high risk women participating in the Raloxifene Use for The Heart (RUTH) study we found a prevalence of BAC of 23%, increasing with age. Only diabetes mellitus was found to be independently associated with BAC (Chapter 3). The lack of association with other separate CHD risk factors was also seen in our study in participants from the

Prospect-EPIC study (Chapter 4). We further investigated the relation between BAC and the novel CHD risk markers fibrinogen, high-sensitivity CRP and microalbuminuria and failed to find any association (Chapters 5 and 6). Interestingly, we observed a statistically significant association of BAC with CAC, indicating that there are similarities in the calcification process across different vascular beds (Chapter 8). There was no association between BAC and vitamin K intake (Chapter 7). In both populations studied, we found that previous pregnancies and lactation were the strongest determinants of BAC. In contrast to reports from older studies, we observed no association between the prevalence of BAC and previous use of OC or HT .

In mammograms after 5 years follow-up, we found that age and the presence of BAC at baseline were the strongest determinants of progression of BAC (Chapter 9). The use of statins reduced the progression of BAC.

Calcium uptake in the arterial wall

The major mineral component in vascular calcification is hydroxyapatite (calcium phosphate), similar to that observed in bone. Bonelike tissues have been found in a variety of vascular locations such as the vascular wall, the myocardium and cardiac valves.[21-23] Ectopic mineralization is currently considered not a degenerative disease but a regulated process with similar mechanisms as in bone formation. Vascular smooth muscle cells (VSMC) are considered to be responsible for the calcification process.[24] VSCM can migrate and dedifferentiate in response to calcifying and atherosclerotic stimuli into cells with osteocytic and chondrogenic changes in gene expression.[25,26] The so-called calcifying vascular cells (CVC) originate from mesenchymal stem cells that have the ability to undergo osteoblastic differentiation and mineralization[27,28] Smooth muscle cells in several parts of the vascular tree have their origin in different embryonic lineages, that may explain differences in responses to various stimuli.[29] A variety of osteogenic factors that play a role in the vascular calcification process have been found in VSMC, such as osteopontin (OPN), bone morphogenic protein (BMP), osteoprotegerin (OPG) and matrix Gla-protein (MGP).[30]

Medial versus intimal calcification

Vascular calcification can be present in the intima or media of the arterial wall. Intimal calcification is predominantly present in atherosclerotic lesions while medial calcification, also called Mönckeberg's medial sclerosis, is mostly described in the peripheral arteries in patients with diabetes.[31-35] The combination of both types of vascular calcification is often seen in patients with diabetes and in patients with end-stage-renal disease (ESRD).

Medial calcification is different from intimal calcification by the absence of signs of inflammation.[33-35] It is mostly present in arteries that are less prone to develop atherosclerosis, such as the abdominal visceral arteries, the peripheral arteries and it is also often seen in the aorta as a sign of vascular stiffening in elderly. On x-rays it is not possible to differentiate with certainty between intimal and medial calcifications. From pathologic studies it is known that calcifications in the breast arteries are also located in the media.[36] In general, calcifications of the media are more fine and diffuse and located in smaller vessels compared to the large and discontinuous calcifications of the intima in greater and medium-sized arteries. At mammography, the more severe forms of medial artery calcifications in the arteries of the breast can appear as linear, parallel calcifications in a 'railroad-track' configuration.

The concept of calcification in the breast arteries

Based on our findings we can now conclude that the major determinants of medial calcification in the breast arteries are age, diabetes, previous pregnancies and lactation after pregnancy. The mechanisms of BAC are comparable to the medial calcification that is seen in the peripheral vessels of diabetics and in patients with ESRD. In diabetics, hyperglycaemia is an important determinant for medial sclerosis and not the classic CHD risk factors.[33,34,37] High glucose can induce proliferation of VSMC into calcifying cells, but other metabolic stimuli in patients with diabetes, like hyperinsulinemia and glycoxidation

products, can also promote the calcifying process.[38-40] In patients with ESRD the uptake of calcium by VSMC is multifactorial and enhanced by elevated extracellular calcium- and phosphate levels, secondary hyperparathyreoidism and uremic toxins.[41-43] The wide-spread use of calcium containing oral phosphate binders, to prevent uremic dystrophy, further promote the calcifying process.[44]

The alterated mineral metabolism during pregnancy and lactation is presumably the most important causative factor in the occurrence of BAC. Pregnancy is associated with major changes in the calcium metabolism to meet the high requirements for foetal growth and for breast-milk production.[45] Some biochemical proteins of bone resorption and formation (e.g. osteocalcin, bone morphogenic protein) are elevated in the first months of lactation and are also found in calcified vascular tissues.[25,46] We assume that when calcium is more available in the (breast) circulation, like during pregnancy and lactation, vascular smooth muscle cells can become activated and promote mineralization. Another common factor in the enhancement of vascular calcification in pregnant women and patients with ESRD may be caused by elevated levels of parathyroid hormone-related protein (PTHrP).[43,45,47] PTHrP is expressed in a variety of bone and vascular tissues and causes hypercalcaemia. The production of PTHrP by the lactating mammary gland may be an explanation for the association of BAC with breastfeeding.

Directions for future research

The association we found between BAC and CAC indicates that there is overlap in the calcification process within various parts and layers of the vascular tree, although the clinical significance is different. The severity of calcification is often comparable across different vascular areas in individuals with atherosclerotic disease and in individuals with combined vascular and valvular calcification.[48-50] One major determinant in studies on the progression of calcification is the degree of calcification at baseline.[51-53] Some people are apparently more prone to calcification than others and this may have implications for its clinical importance. Cumulative evidence indicates that racial, genetic and gender related

variations in the expression of genes that promote calcification play a role in the inter-individual variation in the severity of vascular calcification.[16,54-56] At least 40% of the variability in coronary calcium is not related to CHD risk factors but attributed to largely unknown genetic factors.[57,58] Thus far, there is still limited understanding in the genetic components that are linked to vascular calcification.[31,59]

The strong overlapping mechanisms that are involved in bone- and vascular metabolism raises further questions on the association between osteoporosis and vascular calcification. In women, progression of aortic calcification and mitral annular calcification have been associated with loss of bone mineral density in postmenopausal women.[60-62] An association of BAC with a low bone mineral density has also been reported.[63] As we now know that BAC is not a marker of atherosclerotic disease it is yet undetermined whether it may serve as a marker for osteoporosis in postmenopausal women. The contributing factors that BAC shares with the elevated bone metabolism in ESRD and pregnancy may indicate a closer relation to osteoporosis than to atherosclerosis.

Conclusion

Arterial calcifications in the breasts are merely a marker of previous pregnancies and exclude mammograms as a possible diagnostic tool in CHD risk assessment.

Future research should aim at genetic factors in the variability of vascular calcification and a possible association of BAC with osteoporosis.

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CHAPTER 11

Summary
&
Samenvatting

Imaging of vascular calcification is increasingly used for cardiovascular screening purposes in asymptomatic patients. Coronary and aortic calcium deposits in the vascular wall have been shown to be related to atherosclerotic plaque burden. New imaging techniques with electron beam computed tomography (EBCT) and multi-slice computed tomography (MSCT), to measure the calcium content in the coronary arteries, are promising methods for clinical risk assessment. However, the rising costs of these emerging cardiac imaging techniques and the radiation exposure involved demand a more critical evaluation of already existing and less expensive technology.

In this thesis we have investigated whether arterial calcifications that are frequently seen on mammograms may serve as a screening tool for atherosclerotic risk assessment in women. The simultaneous use of mammograms for screening on breast cancer and atherosclerotic disease could be very cost-effective.

Sex-hormone related factors are important in the biological differences that exist in atherosclerotic disease among men and women. In **Chapter 2** we have evaluated the literature on the role of endogenous and exogenous estrogens in atherosclerotic disease in women. During the fertile period of life estrogens have a protective role against atherosclerosis, predominantly through their effects on lipid metabolism and vascular reactivity. Several observational studies have shown a reduction in the occurrence of coronary heart disease (CHD) in postmenopausal women using hormone therapy (HT). However, all prospective randomized clinical trials have failed to show a beneficial effect of HT on atherosclerotic disease and even demonstrated an increase in CHD event rate. Cumulative evidence supports the hypothesis that HT is important in maintaining vascular health and that it has detrimental effects on the vascular wall once endothelial dysfunction or more extensive atherosclerotic disease is established. The interval between the onset of menopause and the initiation of HT is apparently crucial in the effectiveness of HT on the vascular system.

In **Chapter 3** we have investigated the prevalence of risk factors for breast arterial calcifications (BAC) in mammograms of postmenopausal women who were at risk for

major CHD events due to the presence of risk factors or to previous CHD events. In 600 women with a mean age of 68 years, participating in the Raloxifene Use for The Heart (RUTH) study, we investigated whether classic CHD risk factors were independently related to these calcifications. BAC was present in 23% of women, and the prevalence increased with age and the total number of risk factors, but after adjustment for age only diabetes mellitus was found to be independently associated with BAC. The strongest risk factor for BAC was the total number of previous pregnancies resulting in live born or stillborn children. Women with ≥6 children had a 6.1 times higher risk of BAC (95% CI of odds ratio (OR) 2.26−16.39) than women with 0 or 1 child. We conclude that in the aetiology of BAC gestational factors may be more important than CHD risk factors.

Chapter 4 describes the occurrence of BAC in 1,699 women, aged 49−70 years, participating in the Prospect-EPIC cohort, one of the two Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC). We found a prevalence of BAC of 11%, rising to 20% in the highest quartile of age. When adjusted for age, no separate CHD risk factors were found to be associated with BAC. In contrast, we found a significant inverse association of smoking with BAC (OR 0.6, 95% CI 0.4−0.9). We confirmed our previous observation that parity was the strongest determinant for BAC with an OR of 5.3 (95% CI 2.2−13.2) in parous versus nonparous women. We also showed an independent association of BAC with lactation after pregnancy (OR 2.2, 95% CI 1.4−3.6). We hypothesize that when calcium is more present in the breast circulation, like during pregnancy and lactation, vascular smooth muscle cells that are located in the wall of the breast arteries, can become activated and promote the uptake of calcium. We found no association between the prevalence of BAC and previous use of oral contraceptives (OC) or HT.

Chronic low-grade inflammation is considered to be an important aspect of atherosclerosis. In **Chapter 5** we describe our study in 1726 women from Prospect-EPIC that investigated the relation of BAC with high-sensitivity C-reactive protein (hs-CRP) and fibrinogen. We found no differences in serum levels of both markers of inflammation in

women with and without BAC. A major cause for the lack of signs of vascular inflammation in women with BAC, may be that these calcifications are located in the media and not in the intima of the vessel wall. The inverse association we previously observed in smokers and the absent association with separate CHD risk factors are in agreement with this finding.

In **Chapter 6** we focused on a possible association between microalbuminuria and BAC as it has been described that in diabetics the presence of both microalbuminuria and coronary vascular calcification implicate a higher CHD risk. As BAC is associated with diabetes, it may also be associated with microalbuminuria. In 509 participants from RUTH we found that microalbuminuria (20−200 mg/L) was present in 10% of women. The occurrence of BAC was not related to microalbuminuria (OR 1.1, 95% CI 0.52−2.29) and equally distributed among tertiles of urinary albumin concentration (UAC). Although we observed no association between microalbuminuria and BAC, both risk markers were significantly more prevalent in diabetics compared to non-diabetics, suggesting overlapping etiologic mechanisms. Prolonged exposure to hyperglycaemia is an important factor in the pathogenesis of endothelial dysfunction and medial and intimal sclerosis in diabetics and this may be a common causative pathway in the aetiology of microalbuminuria and BAC.

Vitamin K metabolism plays an important role in preventing vascular calcification, by promoting the carboxylation of matrix-Gla-protein (MGP), that inhibits calcification in the vascular wall and in bone tissue. In some population-based studies, CHD risk has been associated with lower intakes of vitamin K1 and K2. In **Chapter 7** we have investigated in 1689 women from Prospect-EPIC whether the presence of BAC is related with nutritional vitamin K intake. Vitamin K1 and K2 intake was assessed with a validated food questionnaire. BAC was less common in the highest (9%) quartile of vitamin K2 intake, compared to the lowest (13%) (OR 0.7, 95% CI 0.5−1.1) and not different across quartiles of vitamin K1 intake. However, after adjustment for confounders and dietary factors, only vitamin K2 subtype MK5 remained significantly associated with BAC. The lack of association of BAC with CHD risk may be an explanation of our findings, whereas it may

also be possible that nutritional vitamin K status is closer related to osteoporosis than to atherosclerosis.

In **Chapter 8** we have studied whether the presence of BAC can predict future development of coronary artery calcifications (CAC) in 499 women from the Prospect-EPIC cohort.

Baseline mammograms were reviewed for the presence of BAC. After a mean follow-up duration of 9 years, CAC was assessed by multi-slice computed tomography (MSCT) and quantified with the Agatston score. BAC was present in 58 of 499 women (11.6%) and CAC score >0 was present in 262 of 499 women (52.5%). The presence of BAC at baseline was strongly related to future presence of CAC (OR 3.2, 95% CI 1.71−6.04) and this remained significant after adjustment for age and the duration of follow-up (OR 2.1, 95% CI 1.10−4.23). Most CHD risk factors were associated with CAC but not with BAC. Parity was associated with both BAC (OR 5.3, 95% CI 1.23−22.43) and CAC (OR 2.1, 95% CI 1.21−3.60). Breastfeeding after pregnancy was associated with BAC (OR 3.4, 95% CI 1.40−8.23), but not with CAC. Our findings underscore that different causative factors are involved with both types of vascular calcification.

In **Chapter 9** we describe the progression of BAC in mammograms of 453 participants in the RUTH study. All mammograms were reviewed according to a grading scale. Progression was defined as the presence of new calcifications and/or a change in the severity of BAC. After a mean follow-up of 5 years BAC was present in 26% of participants, compared with 21% at baseline. In 22 (5%) patients new calcifications were seen, whereas in 22 (5%) patients the severity of BAC merely changed from grade 1 (mild) to grade 2 (moderate) calcification. In 3 patients a regression in the severity of BAC was seen. None of the CHD risk factors were associated with the progression of BAC. Statin therapy protected for the progression of BAC. Age and the presence of BAC at baseline were the only independent determinants of progression. As in other types of vascular- and valvular calcification the progression of the calcifications is also frequently unrelated to

specific risk factors, we assume that the initiating mechanisms of calcifications in various vascular beds and the factors that are involved with progression may be different.

In **Chapter 10** we discuss the clinical significance of BAC, based on the literature and the findings of our studies. The lack of association of BAC with cardiovascular risk factors excludes this modality as a screening tool for CHD risk assessment in women.

Het afbeelden van kalk in de vaatwand wordt in toenemende mate gebruikt als screeningstest voor hart- en vaatziekten. Kalkafzetting in de kransslagaderen en in de aorta is gerelateerd aan de mate van aanwezige slagaderverkalking. Nieuwe beeldvormende technieken, zoals de electron beam computer tomografie (EBCT) en multislice computer tomografie (MSCT), die in staat zijn de hoeveelheid kalk in de kransslagaderen te bepalen, zijn veelbelovend voor toepassing in de praktijk. De hoge kosten die deze geavanceerde technologie met zich meebrengt en de bijkomende stralingsbelasting zijn echter een goede reden om de klinische toepasbaarheid voor screening van al bestaande en minder dure technieken kritisch te beoordelen.

In dit proefschrift hebben wij onderzocht in hoeverre kalkafzettingen in de slagaderen van de borsten, zoals deze vaak gezien worden op mammogrammen (borstfoto's), gebruikt kunnen worden voor het screenen van vrouwen met een verhoogd risico op hart- en vaatziekten. Het gelijktijdige gebruik van mammogrammen voor het screenen op borstkanker en op aderverkalking zou zeer kosten-effectief kunnen zijn.

Veel van de biologische verschillen in aderverkalking tussen mannen en vrouwen, hebben te maken met de geslachtshormonen. In **Hoofdstuk 2** geven wij een samenvatting van de rol van oestrogenen op de gezonde- en door aderverkalking aangedane vaatwand. Gedurende de vruchtbare levensfase hebben oestrogenen een beschermend effect op aderverkalking, met name door gunstige effecten op de vetstofwisseling en de vaatwand zelf. In diverse observationele onderzoeken is vastgesteld dat vrouwen die na de menopauze hormonen gebruiken, een lager risico hebben op het krijgen van hart- en vaatziekten. De prospectieve, gerandomiseerde studies hebben echter geen gunstig effect van hormoontherapie na de menopauze kunnen aantonen, maar lieten eerder een toename zien in vaatcomplicaties. Naarmate het inzicht uit onderzoek toeneemt, wordt steeds duidelijker dat hormonen waarschijnlijk gunstig zijn voor de vaatwand zolang deze gezond is, maar schadelijk wanneer deze door aderverkalking is aangetast. Het interval waarin de hormoontherapie na de menopauze wordt gestart lijkt daarbij belangrijk voor het effect op de vaatwand.

In **Hoofdstuk 3** hebben wij de relatie onderzocht tussen risicofactoren voor hart- en vaatziekten en het voorkomen van vaatkalk in de borsten, bij vrouwen met een verhoogd cardiovasculair risico. Mammogrammen van 600 vrouwen uit de Raloxifene Use for The Heart (RUTH) studie werden beoordeeld op de aanwezigheid van vaatkalk. Bij 23% van de vrouwen, met een gemiddelde leeftijd van 68 jaar, werd vaatkalk gevonden en dit percentage nam toe met de leeftijd en het totale aantal risicofactoren. Na correctie voor leeftijd bleek alleen diabetes mellitus een onafhankelijke voorspeller te zijn voor de aanwezigheid van vaatkalk. Het aantal voldragen zwangerschappen was het sterkst geassocieerd met vaatkalk, waarbij vrouwen met 6 of meer kinderen een ruim 6x zo grote kans hadden op kalk dan vrouwen zonder kinderen of met maar één kind. Wij concluderen daaruit dat factoren gerelateerd aan de zwangerschap waarschijnlijk belangrijker zijn in het ontstaan van deze vaatkalk dan de risicofactoren voor hart- en vaatziekten.

Hoofdstuk 4 beschrijft het voorkomen van vaatkalk in mammogrammen bij 1.699 vrouwen met een gemiddelde leeftijd van 49−70 jaar, die deelnemen in het Prospect-EPIC cohort, een screeningspopulatie voor borstkanker. Vaatkalk was aanwezig bij 11% van de vrouwen, oplopend naar 20% in het hoogste leeftijdskwartiel. Er werden geen afzonderlijke risicofactoren gevonden die geassocieerd waren met vaatkalk. Rooksters hadden significant minder vaatkalk dan niet-rooksters (odds ratio [OR] 0.6, 95% betrouwbaarheids-interval [BI] 0.4−0.9). Ook in dit cohort vrouwen vonden wij dat het hebben gehad van kinderen (ja/nee) de belangrijkste voorspeller was van vaatkalk (OR 5.3, 95% BI 2.2−13.2). Bij de vrouwen met kinderen bleek borstvoeding na een of meerdere zwangerschappen ook significant geassocieerd te zijn met vaatkalk (OR 2.2, 95% BI 1.4−3.6). Mogelijk zijn de hogere calcium(kalk)spiegels in het bloed tijdens de zwangerschap en de lactatieperiode een prikkel voor de gladde spiercellen in de vaatwand om calcium op te nemen en vast te houden. Wij vonden geen relatie tussen het gebruik van de anticonceptie-pil of hormoontherapie na de menopauze en de aanwezigheid van vaatkalk in mammogrammen.

Tegenwoordig beschouwt men chronische ontstekingsverschijnselen als een belangrijk onderdeel van aderverkalking. In **Hoofdstuk 5** hebben wij bij 1726 vrouwen

binnen Prospect-EPIC onderzocht in hoeverre vaatkalk in de borsten geassocieerd is met de ontstekingseiwitten C-reactive protein (CRP) en fibrinogeen. Er bleek geen verschil te zijn in de bloedspiegels van deze ontstekingseiwitten tussen vrouwen met- en zonder vaatkalk. Een mogelijke verklaring hiervoor is dat de vaatkalk in de borsten niet gelocaliseerd is in de intima- maar in de media van de vaatwand, waarin ontsteking geen belangrijke rol speelt.

Hoofdstuk 6 beschrijft ons onderzoek naar een mogelijke associatie tussen vaatkalk in mammogrammen en microalbuminurie (eiwituitscheiding in de urine), aangezien eerder is aangetoond dat zowel kalkafzetting in de kransslagaderen als eiwit in de urine een aanwijzing zijn van een verhoogd risico op hart- en vaatziekten. Bij 509 deelnemers uit de RUTH- studie vonden wij bij 10% van de vrouwen microalbuminurie (20−200 mg/L), maar dit bleek niet geassocieerd met de aanwezigheid van vaatkalk in de borsten. Bij diabeten kwamen zowel micoalbuminurie als vaatkalk vaker voor. Ofschoon wij geen onderlinge relatie hebben gevonden, zijn verhoogde bloedsuikerspiegels bij beide processen een belangrijke oorzakelijke factor.

Vitamine K speelt een belangrijke rol in het voorkomen van vaatkalk, door het activeren van het matrix-Gla-eiwit, dat een remmend effect heeft op kalkafzetting in de vaatwand en kalkopname in botweefsel. In enkele populatie onderzoeken is beschreven dat het risico op hart- en vaatziekten gerelateerd is aan een lagere inname van vitamine K1 en vitamine K2.

In **Hoofdstuk 7** hebben wij onderzocht bij 1689 vrouwen binnen Prospect-EPIC in hoeverre de aanwezigheid van vaatkalk in mammogrammen geassocieerd is met de voedselinname van vitamine K. Door middel van een gevalideerde vragenlijst werd bij alle deelnemers de inname van vitamine K1 en K2 vastgesteld. Er was minder vaatkalk in de borsten in het hoogste kwartiel van vitamine K2 inname ten opzichte van het laagste kwartiel, maar dit was niet significant na correctie voor risico- en voedingsfactoren (OR 0.7, 95% BI 0.5−1.1). De inname van vitamine K1 was niet geassocieerd met de aanwezigheid van vaatkalk. Mogelijk is het ontbreken van een relatie tussen vaatkalk en cardiovasculaire risicofactoren een verklaring voor onze negatieve bevindingen, maar het

zou ook kunnen zijn dat de vitamine K status meer informatie geeft over de botstofwisseling dan over aderverkalking.

In **Hoofdstuk 8** hebben wij onderzocht in hoeverre de aanwezigheid van vaatkalk in borstslagaders, gezien op mammogrammen, een voorspellende waarde heeft voor het ontstaan van vaatkalk in de kransslagaderen, by 499 vrouwen uit het Prospect-EPIC cohort. Baseline mammogrammen werden beoordeeld op de aanwezigheid van vaatkalk en na een gemiddelde follow-up duur van 9 jaar werd de kalkscore in de kransslagaderen bepaald met multi-slice computed tomography (MSCT). De aanwezigheid van vaatkalk in mammogrammen bleek een significante voorspeller te zijn voor de latere ontwikkeling van coronaire kalk, ook na correctie voor leeftijd en de duur van de follow-up (OR 2.1, 95% BI 1.10−4.23). De bekende cardiovasculaire risicofactoren waren wel geassocieerd met coronaire vaatkalk, maar niet met vaatkalk in de borsten. Zwangerschappen in het verleden waren geassocieerd met coronaire kalk (OR 2.1, 95% BI 1.21−3.60), maar veel sterker met vaatkalk in de borsten (OR 5.3, 95% BI 1.23−22.43). Borstvoeding was niet geassocieerd met coronaire kalk, maar wel met vaatkalk in de borsten. Deze bevindingen bevestigen dat de oorzakelijke factoren van kalk in de slagaderen van de borsten en van kalk in de kransslagaderen verschillend zijn.

In **Hoofdstuk 9** beschrijven wij de progressie van vaatkalk in mammogrammen bij 453 vrouwen in de RUTH studie. Progressie werd gedefinieerd als de aanwezigheid van nieuwe kalkafzettingen of toename in ernst van reeds aanwezige vaatkalk. De mate van kalk werd in 3 gradaties gescoord. Na een gemiddelde follow-up duur van 5 jaar werd een toename in vaatkalk gezien bij 10% van de vrouwen, van wie bij 5% nieuwe kalk en bij 5% een toename in kalk. Er was geen relatie tussen de cardiovasculaire risicofactoren en de progressie van kalk. Het gebruik van cholesterol-verlagende medicijnen had een remmend effect op de progressie van vaatkalk in de borsten. De aanwezigheid van kalk op het eerste mammogram en leeftijd waren de enige voorspellers van progressie.

Omdat ook bij toename van vaatverkalkingen elders in het vaatstelsel en bij verkalkingen van hartkleppen de traditionele risicofactoren veelal geen rol lijken te spelen,

veronderstellen wij dat de oorzaken van kalkafzettingen en de factoren die betrokken zijn bij de progressie hiervan niet obligaat dezelfde hoeven te zijn.

In **Hoofdstuk 10** bespreken wij de klinische relevantie van kalkafzettingen in de slagaderen van de borsten, op grond van de gegevens uit de literatuur en onze eigen onderzoeksresultaten. Het ontbreken van een associatie tussen cardiovasculaire risico-factoren en deze vorm van vaatkalk, impliceert dat mammogrammen niet geschikt zijn voor risicoscreening op hart- en vaatziekten bij vrouwen.

DANKWOORD

Met de afronding van dit proefschrift gaat een lang gekoesterde wens in vervulling. Zonder mijn fantastische en onverstoorbare (co-)promotores waren mijn plannen ongetwijfeld gestrand in ijdelheid en illusie. Het heeft mij veel energie gegeven om met deze ervaren onderzoekers te werken, als afwisseling op de dagelijkse cardiologische praktijk.

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CURRICULUM VITAE

The author was born on August 9th 1956 in Utrecht, the Netherlands.

She graduated secondary school in 1974 at the Stedelijk Gymnasium in Utrecht and started Medical School in the same year at the University of Groningen. The in-hospital training during the last 2 years of medical study (1980−1981) was done at the St. Elisabeth Hospital in Curaçao, Netherlands Antilles. She obtained her medical degree in December 1981.

In 1982 she worked for one year at the department of Thoracic Surgery at the St. Antonius Hospital in Utrecht (drs. F.E.E.Vermeulen). In 1983 she continued her residency in Internal Medicine at the St. Antonius Hospital in Nieuwegein (dr. C.E.M. de Maat), followed by her residency in Cardiology (dr. C.A.P.L. Ascoop). In January 1988 she was registered as a cardiologist and worked for nearly 5 years at the department of Cardiology in Arnhem/Velp. In September 1992 she joined the department of Cardiology at the Isala klinieken in Zwolle, were she has been a staff member since 1994. Over the last decade she has developed a main interest in heart disease and its prevention in women.

Angela is married to Ernst Faber and they have two sons, Arthur (1989) and Eric (1990).

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