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.

References

1. Pedrinelli R, Dell'Omo G, Penno G, Mariani M. Non-diabetic microalbuminuria, endothelial dysfunction and cardiovascular disease. *Vasc Med* 2001; 6: 257-264.
2. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002; 106: 1777-1782.
3. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B et al. Albuminuria and risk of cardiovascular events, death and heart failure in diabetic and non-diabetic individuals. *JAMA* 2001; 286: 421-426.
4. Roest M, Banga JD, Janssen WMT, Grobbee DE, Sixma JJ, de Jong PE, de Zeeuw D, van der Schouw YT. Excessive urinary albumin levels are associated with future cardiovascular mortality in postmenopausal women. *Circulation* 2001; 103: 3057-3061.
5. Wong ND, Sciammarella MG, Polk D, Gallagher A, Miranda-Peats L, Whitcomb B, Hachamovitch R, Friedman JD, Hayes S, Berman DS. The metabolic syndrome, diabetes, and subclinical atherosclerosis assessed by coronary calcium. *J Am Coll Cardiol* 2003; 41: 1547-1553.
6. Lehto S, Niskanen L, Suhonen M, Rönnemaa T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1996; 16: 978-983.
7. Mehrotra R, Budoff M, Christenson P, Ipp E, Takasu J, Gupta A, Norris K, Adler S. Determinants of coronary artery calcification in diabetics with and without nephropathy. *Kidney Intern* 2004; 66: 2022-2031.
8. Speer MY, Giachelli CM. Regulation of vascular calcification. *Cardiovasc Pathol* 2004; 13: 63-70.
9. Baum JK, Comstock CH, Joseph L. Intramammary arterial calcifications associated with diabetes. *Radiology* 1980; 136: 61-62.
10. Moshyedi AC, Puthawala AH, Kurland RJ, O'Leary DH. Breast arterial calcification: association with coronary artery disease. *Radiology* 1995; 194: 181-183.
12. Iribarren C, Go AS, Tolstykh I, Sidney S, Johnston SC, Spring DB. Breast vascular calcification and risk of coronary heart disease, stroke and heart failure. *J Women's Health* 2004; 13: 381-389.
13. Maas AH, van der Schouw YT, Mali WP, van der Graaf Y. Prevalence and determinants of breast arterial calcium in women at high risk of cardiovascular disease. *Am J Cardiol* 2004; 94: 655-659.
14. Kemmeren JM, Beijerinck D, van Noord PAH, Banga JD, Deurenberg JJM, Pameijer FA, van der Graaf Y. Breast arterial calcifications: association with diabetes mellitus and cardiovascular mortality. *Radiology* 1996; 201: 75-78.
15. Mosca L, Barrett-Connor E, Wenger NK, Collins P, Grady D, Kornitzer M, Moscarelli E, Paul S, Wright TJ, Helterbrand JD, Anderson PW. Design and methods of the Raloxifene Use for The Heart (RUTH) study. *Am J Cardiol* 2001; 88: 392-395.
16. Wenger NK, Barrett-Connor E, Collins P, Grady D, Kornitzer M, Mosca L, Sashegyi A, Baygani SK, Anderson PW, Moscarelli E. Baseline characteristics of participants in the Raloxifene Use for The Heart (RUTH) Trial. *Am J Cardiol* 2002; 90: 1204- 1210.
17. Hadjadj S, Gourdy P, Zaoui P, Guerci B, Roudaut N, Gautier J-F, et al. Effect of raloxifene on urinary albumin excretion in post-menopausal type 2 diabetic women: a randomized, placebo-controlled rial. Abstract , 41st EASD annual meeting 2005.
18. Blumenthal RS, Baranowski B, Dowsett SA. Cardiovascular effects of raloxifene: the arterial and venous systems. *Am Heart J* 2004; 147: 783-789.

19. Kramer H, Jacobs DR, Bild D, Post W, Saad MF, Detrano R, Tracy R, Cooper R, Liu K. Urine Albumin Excretion and subclinical cardiovascular disease. The multi-ethnic study of atherosclerosis. *Hypertension* 2005; 46: 38-43.
20. Raggi P, Shaw LJ, Berman DS, Callister TQ. Prognostic value of coronary artery calcium screening in subjects with and without diabetes. *J Am Coll Cardiol* 2004; 43: 1663-1669.
21. Freedman BI, Langefeld CD, Lohman KK, Bowden DW, Carr JJ, Rich SS, Wagenknecht LE. Relationship between albuminuria and cardiovascular disease in type 2 diabetes. *J Am Soc Nephrol* 2005; 16: 2156-2161.
22. Psyrogiannis A, Kyriazopoulou V, Vagenakis AG. Medial arterial calcification is frequently found in patients with microalbuminuria. *Angiology* 1999; 50: 971-975.
23. Aronson D, Rayfield EJ. How hyperglycaemia promotes atherosclerosis: molecular mechanisms. *Cardiovasc Diabetol* 2002; 1: 1.
24. Mori S, Takemoto M, Yokote K, Asaumi S, Saito Y. Hyperglycemia induced alteration of vascular smooth muscle phenotype. *J Diabetes Complications* 2002; 16: 65-68.
25. Sakata N, Takeuchi K, Noda K, Saku K, Tachikawa Y, Tashiro T, Nagai R, Horiuchi S. Calcification of the medial layer of the internal thoracic artery in diabetic patients: relevance of glycoxidation. *J Vasc Res* 2003; 40: 567-574.
26. Sodhi CP, Phadke SA, Batlle D, Sahai A. Hypoxia stimulates osteopontin expression and proliferation of cultured vascular smooth muscle cells. *Diabetes* 2001; 50: 1482-1490.
27. Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures and mortality in elderly women. *J Clin Endocrinol Metab* 2001; 86: 631-637.
28. Knudsen ST, Foss CH, Poulsen PL, Andersen NH, Mogensen CE, Rasmussen LM. Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. *Eur J Endocrinol* 2003; 149: 39-42.
29. Schoppet M, Al-Fakhiri N , Franke FE, Katz N, Barth PJ, Maisch B, Preissner KT, Hofbauer LC. Localization of osteoprotegerin, tumor necrosis factor related apoptosis-inducing ligand, and receptor activator of nuclear factor-κB ligand in Mönckeberg's sclerosis and atherosclerosis. *J Clin Endocrinol Metab* 2004; 89: 4104-4112.
30. Cucina A, Sapienza P, Corvino V, Borrelli V, Randone B, Santoro-D'Angelo L, Cavallaro A. Nicotine induces platelet-derived growth factor release and cytoskeletal alteration in aortic smooth muscle cells. *Surgery* 2000;127: 72-78.
31. Ross R. Atherosclerosis-An inflammatory disease. N Engl J Med 1999; 340: 115-126.
32. Tintut Y, Alfonso Z, Saini T, Radcliff K, Watson K, Boström K, Demer LL. Multilineage potential of cells from the artery wall. *Circulation* 2003; 108: 2505-2510.