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Relationship between arterial vascular calcifications seen on screening mammograms and biochemical markers of endothelial injury

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Abstract

To assess whether breast arterial calcifications (BAC) are associated with altered serum markers of cardiovascular risk, mammograms and records from 1759 women (age range: 45–65 years) screened for breast cancer were revised. One hundred and forty seven (8.36%) women showed BAC. A total of 136 women with BAC and controls (mean age: 57 and 55 years, respectively) accepted entering the study. There were no significant differences in serum levels of urea, glucose, uric acid, creatinine, total cholesterol, HDL-C, LDL-C, folic acid, vitamin B_{12}, TSH or cysteine, between both groups of patients. However, women with BAC showed higher serum levels of triglycerides ($p = 0.006$), homocysteine ($p = 0.002$) and hs-CRP ($p = 0.003$) than women without BAC. Likewise, we found a significantly higher percentage of cases with an elevated LDL-C/HDL-C ratio (coronary risk index >2) amongst women with BAC than in women without BAC (56.7 and 38.2%, respectively; $p = 0.04$). Our results indicate that the finding of BAC identify women showing altered serum markers of cardiovascular risk.

Keywords: Cardiovascular risk; Mammography; Breast cancer screening; Atherosclerosis; Coronary risk index; Cholesterol; Homocysteine; C-reactive protein

1. Introduction

Greater awareness about breast cancer and a better compliance with screening programs have resulted in the increment on the absolute number of women undergoing mammography, making it a very attractive tool to detect potential markers for increased risk of cardiovascular disease. In addition, improvements in the resolution of modern mammography to more accurately detect markers of breast cancer, such as breast tissue microcalcifications, has led to the discovery of readily identifiable calcium deposits within the wall of breast arteries.

The presence of breast arterial calcifications (BAC) is a common finding on screening mammograms, having been described in 8–12% of women undergoing these studies [1–3]. BAC is currently an unreported finding because it is commonly thought to be of no clinical significance. However, accumulating evidence

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suggests that BAC may be important as a marker for generalized vascular disease. Significant associations have been found between BAC and the presence of atherosclerosis-associated morbidity, such as diabetes, hypertension, albuminuria, transient ischemic attack (TIA)/stroke, thrombosis and myocardial infarction [1,2]. More specifically, it has been reported that finding BAC on a mammogram may indicate unsuspected diabetes or hypertension, particularly in women over 59 years of age [4], as well as being an additional risk factor for coronary artery disease, particularly in diabetic patients over age 50 [2,5,6]. Thus, all these relationships suggest that BAC detected on breast cancer screening mammograms is associated with disorders related to increased or accelerated atherosclerosis. Consequently, this finding might be of special interest given the importance of early detection of coronary heart disease since nearly 40% of initial cardiovascular events in women are fatal [7]. Additionally, the majority of women who die from sudden cardiac death had no previously recognized symptoms of disease, emphasizing the need to develop novel methods to identify women at increased risk [8].

The aim of the present study was to assess whether the presence of BAC in mammograms from a population-based breast cancer-screening program carried out in the North of Spain is associated with altered circulating levels of several markers of increased cardiovascular risk.

2. Materials and methods

This study was performed in the framework of a population-based program for the early detection of breast cancer in women aged 45–65 years, carried out at Hospital de Jove in Gijón (Spain). We revised mammograms and records from 1759 women who had been screened for breast cancer and undergone diagnostic mammography between June 1998 and June 2003.

BAC was regarded as being present if the characteristic pattern of two parallel calcific lines or a calcific ring configuration was observed on the mammogram of either the right, left, or both breasts (Fig. 1). According to this definition, a total of 147 women (8.36%) presented BAC. We invited as many patients as possible ($n = 92$), as soon as a similar number of women without BAC as controls was available, to participate in the study in prevision to obtain an adequate number of patients meeting all the inclusion criteria. Women with co-morbid inflammatory diseases known to be associated with elevation of C-reactive protein (CRP), such as malignancy, rheumatoid arthritis, inflammatory bowel disease or infection, as well as those undergoing treatment with statins, were excluded from the study. Finally, 68 women with BAC and 68 of those without BAC accepted to enter the study. None of these patients showed changes in the BAC status with regard to the latest mammogram, performed either 1 or 2 years earlier.

Written informed consent was obtained from each participant prior to inclusion into the study. The study protocol was approved by our institution's Ethics and Investigation Committee, and was conducted according to the 1975 Helsinki Declaration.

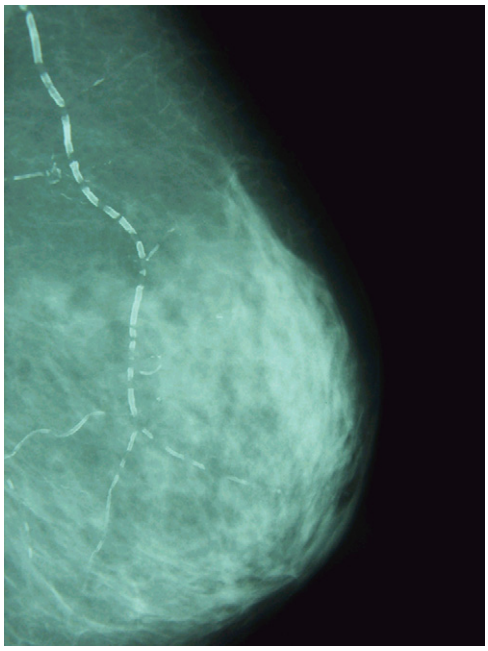

Fig. 1. Mammogram showing arterial vascular calcifications.

2.1. Clinical data

Women's baseline characteristics, including demographic and clinical data, were obtained at enrolment. Participants completed questionnaires that covered family, medical and reproductive history, history of cardiovascular diseases, risk factors for cardiovascular disease, and use of medication. Ongoing smokers and those who had stopped smoking less than 6 weeks prior to the study were considered as current smokers. All others were grouped as non-smokers for this study. Diabetes was defined as the use of oral hypoglycemic agents and/or insulin. All participants underwent an electrocardiogram (ECG) irrespectively of their blood pressure level. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, use of antihypertensive medication, or a combination of these features. A history of cardiovascular diseases was considered positive if a woman had been treated for thrombosis, transient ischemic attack/stroke, or myocardial infarction up to 5 years before the visit or if she had used drugs for the treatment of cardiovascular diseases. These drugs consisted of antiarrhythmic drugs, and nitrates or other antianginal drugs. Classic angina was defined as retrosternal chest discomfort exacerbated by exertion and relieved by rest or nitroglycerin. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (BMI = weight/height^2, kg/m^2) and we applied the classification system recommended by the World Health Organization (WHO), where overweight individuals are those with a BMI between 25 and 29 kg/m^2, and obese individuals those with a BMI equal to or greater than 30 kg/m^2.

In this study, two radiologists assessed the presence of vascular calcifications by "blind" rereading of the mammograms. When there was a disagreement in their interpretation (present

vs. absent), they revised the conflictive mammogram together in order to reach a consensus.

2.2. Blood sampling and biochemical analysis

Peripheral blood was obtained early in the morning after more than 12 h of fasting, using Vacutainer™ tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey). After centrifugation, serum samples were either analyzed fresh for most of the tests or stored frozen at −20 °C until being evaluated for vitamins and high-sensitivity C-reactive protein (hs-CRP). EDTA-anticoagulated blood samples were immediately refrigerated and centrifuged within 1 h at 4 °C; EDTA-plasma samples were then kept at −20 °C until determinations for total homocysteine (tHcy) were performed.

Plasma tHcy was measured by HPLC with fluorescence detection, according to the method described by Pfeiffer et al., with minor modifications. Hs-CRP was determined in serum by particle-enhanced immunological agglutination (Tinaquant, Roche Diagnostics, Basel, Switzerland) in a Hitachi 917 system (Hitachi Ltd., Ochanomizu, Tokyo). Serum folic acid and vitamin B12 were analyzed by electrochemiluminescence immunoassay on a Modular Analytics SWA DPEE (Roche Diagnostics). Likewise, other analytes were determined enzymatically on the same apparatus following the procedures recommended by the manufacturer.

2.3. Statistical analysis

Women were subdivided into groups based on different clinical parameters and as a function of the presence or absence of BAC. Continuous variables were presented as median (range). We analyzed the distribution of these variables by the Kolmogorov–Smirnov test. On the base of this analysis, parametric methods (unpaired Student's test) or non-parametric rank methods (Mann–Whitney test) were used for comparison between groups of women with or without BAC. Categorical variables were analyzed using a Chi-square test. Complementary, we performed a multiple logistic regression models. All analyses were performed using SPSS v11.5 software (SPSS Inc., Chicago, Illinois). Statistical significance was considered at 5% probability level ($p \leq 0.05$).

3. Results

Of the 68 women with BAC included in the present study, 51 (75%) had BAC in one breast and 17 (25%) in both breast. Intensity of arterial calcifications was light in 45 (66.1%),

Table 1
Characteristics of study population

Characteristics of study population	Patients with BAC ($n = 68$) n (%)	Patients without BAC ($n = 68$) n (%)	p^*
Age (years)	Mean: 57.1 (range: 44–65)	Mean: 55.1 (range: 44–65)	
Menopausal status			N.S
Premenopausal	17 (25)	27 (39.7)	
Postmenopausal	51 (75)	41 (60.3)	
Breast feeding			N.S.
No	24 (35.3)	29 (42.6)	
Yes	44 (64.7)	39 (57.1)	
Parity			N.S.
Multiparous	64 (94.1)	63 (92.6)	
Nulliparous	4 (5.9)	5 (7.4)	
Obesity			N.S.
BMI <25	19 (27.9)	30 (44.1)	
BMI 25–30	31 (45.5)	28 (41.2)	
BMI >30	18 (26.4)	10 (14.7)	
Blood pressure			
DBP >90 mmHg	9 (13.2)	4 (5.8)	N.S.
SBP >140 mmHg	21 (30.8)	7 (10.3)	
Current smoker	4 (5.9)	14 (20.6)	0.023
Diabetes	7 (10.3)	3 (4.4)	N.S.
Hypertension	27 (39.7)	18 (26.5)	N.S.
Dislypemia	32 (47.1)	16 (23.5)	0.007
Coronary syndromes	4 (5.9)	1 (1.5)	N.S.
Stroke	1 (1.5)	1 (1.5)	N.S.
Cardiovascular diseases			N.S.
Absent	63 (92.6)	67 (98.5)	
Present	5 (7.47)	1 (1.5)	

Abbreviations: BAC, breast arterial calcification; N.S. not significant.
Data are reported as number of cases (%).
* Chi-squared test.

Table 2
Relationship between serum marker levels and breast arterial calcifications

Serum levels	Women with BAC $n=68$ median (range)	Women without BAC $n=68$ median (range)	p
Triglycerides (mg/dL)	98 (47–664)	80 (35–242)	**0.006**$^{\beta}$
Total cholesterol (mg/dL)	226 (20–302)	212 (132–293)	N.S.$^{\alpha}$
HDL-C (mg/dL)	67 (32–108)	68 (31–122)	N.S.$^{\alpha}$
LDL-C (mg/dL)	135 (66–212)	128.5 (41–196)	N.S.$^{\alpha}$
Homocysteine (μmol/L)	9.8 (3.7–119.9)	8.5 (5–18.5)	**0.002**$^{\beta}$
Folic acid (μg/L)	8.5 (4.1–17.6)	8.25 (4.3–20)	N.S.$^{\alpha}$
Vitamin B_{12} (ng/L)	502 (69–2000)	503 (158–1128)	N.S.$^{\beta}$
hs-CRP (mg/L)	0.31 (0.04–5.24)	0.18 (0.00–3.14)	**0.003**$^{\beta}$
TSH (μU/mL)	2.1 (0.23–20.8)	2.5 (0.43–6.26)	N.S.$^{\beta}$
Cysteine (μmol/L)	262 (183–421)	242 (156–336)	N.S.
Glucose (mg/dL)	96 (77–315)	97 (83–156)	N.S.$^{\beta}$
Urea (mg/dL)	40 (22–62)	38 (21–57)	N.S.$^{\alpha}$
Creatinine (mg/dL)	0.8 (0.4–1.3)	0.8 (0.6–1.4)	N.S.$^{\beta}$
Uric acid (mg/dL)	4.6 (2.4–8.3)	4.2 (2.5–9.4)	N.S.$^{\alpha}$

Abbreviations: BAC, breast arterial calcification; hs-CRP, high sensitivity C-reactive protein; N.S., not significant.
Data are reported as median (range).
Note: after to analyze the distribution of the variables by the Kolmogorov–Smirnov test, unpaired Student's test $^{\alpha}$ or non-parametric rank method (Mann–Whitney test $^{\beta}$) were used.

moderate in 18 (26.4%) and intense in 5 (7.3%) women. A total of 46 (67.6%) women had one BAC, 16 (23.5%) had two BACs, 4 (5.8%) had three, and 2 (2.9%) had four BACs.

Baseline characteristics of women with and without BAC are shown in Table 1. This table shows the relationship between BAC and a variety of clinical characteristics, including age, menopausal status, lactation, parity, obesity, blood pressure and smoking, as well as any history of diabetes, hypertension, dislypemia, coronary syndromes or stroke. As that table shows, the percentage of women with dislypemia was significantly higher among those with BAC ($p=0.007$); however, the percentage of smokers was significantly higher among women without BAC ($p=0.023$).

Table 2 summarizes the biochemical features at the time of the study as continuous variables. There were not significant differences in serum levels of urea, glucose, uric acid, creatinine, total cholesterol, HDL-C, LDL-C, folic acid, vitamin B_{12}, TSH, T_3, T_4, glutathione or cysteine, between the groups of women with or without BAC. However, our results did show significant differences in the levels of triglycerides ($p=0.006$), homocysteine ($p=0.002$) and hs-CRP ($p=0.003$). Women with BAC showed higher serum levels of triglycerides, homocysteine and hs-CRP than those without them.

Table 3 shows the serum levels of the different markers with regard to the several current reference levels derived predominantly from European populations. In this context, women with

Table 3
Cut-off serum marker levels and breast arterial calcifications

Serum levels	Women with BAC $n=68$	Women without BAC $n=68$	p**
Triglycerides >170 mg/dL	8 (11.8)	5 (7.4)	N.S.
Total cholesterol >220 mg/dL	38 (55.9)	27 (39.7)	**0.04**
HDL-C <35 mg/dL	2 (2.9)	1 (1.5)	N.S.
LDL-C >130 mg/dL	36 (53.7)	32 (47.1)	N.S.
Coronary risk index* >2	38 (55.9)	26 (38.2)	**0.04**
Homocysteine <5.2 μmol/L	2 (2.9)	1 (1.5)	N.S.
Homocysteine >14.0 μmol/L	4 (6)	2 (3)	N.S.
Vitamin B_{12} < 200 ng/L	4 (6)	1 (1.5)	N.S.
hs-CRP levels			N.S.
hs-CPR <1 mg/L	61 (89.7)	66 (97.1)	
hs-CPR 1–3 mg/L	4 (6)	1 (1.5)	
hs-CRP >3 mg/L	2 (3)	1 (1.5)	
TSH <0.27 mU/L	1(1.5)	0(0)	N.S.
TSH >4.2 mU/L	12 (17.6)	6 (8.8)	N.S.
Glucose >120 mg/dL	8 (11.8)	5 (7.3)	N.S.
Urea >50 mg/dL	4 (5.9)	8 (11.8)	N.S.
Creatinine >1.1 mg/dL	1 (1.5)	1 (1.5)	N.S.

Abbreviations: BAC, breast arterial calcification; hs-CRP, high sensitivity C-reactive protein; N.S., not significant.
Data are reported as number of cases (%).
Values of serum markers were dichotomized according to the high or low range as a function of their clinical interest.
* Coronary risk index: LDL-C level/HDL-C level.
** Chi-squared test.

BAC also showed a significantly higher percentage of cases with an elevated total cholesterol ($p = 0.04$) as well as an elevated LDL-C/HDL-C ratio (coronary risk index >2) (55.9%) than women without BAC (38.2%) ($p = 0.04$). With regard to the hs-CRP, we assessed our results according to three risk categories: low (<1.0 mg/L), moderate (1.0–3.0 mg/L) and high (>3.0 mg/L), based on guidelines from the American Heart Association and the Centers for Disease Control and Prevention. Thus, as it can be seen in Table 3, we found a higher percentage of women with elevated serum levels of hs-CRP in the group with BAC, but without statistically significant differences.

On the other hand, it was also remarkable that our analyses did not show any statistically significant difference when comparing the several biochemical markers with the semiquantitative subgroups of vascular calcifications, such as bilaterally, number, and intensity (data not shown). On the other hand, using a multiple logistic regression model that included homocisteyne, triglycerides, hs-CRP and coronary risk index (which reach significance in the univariate analysis) as covariables, only homocysteine showed as independent factor to estimate the risk of BAC (OR: 1.22; confidence interval: 1.04–1.43; $p = 0.01$).

4. Discussion

Our results confirm previous studies reporting a significant percentage of women, ranging from 8 to 12%, showing BAC in population-based breast cancer screening programs [1,2,9], although it has been recently reported that the incidence of BAC varies by age, and differs substantially among racial/ethnic subpopulations [10]. For that reason, it is important to design studies able to evaluate the clinical utility of mammography as a potential screening tool for coronary heart disease among different women populations. Our results show that women with BAC included in our study population present higher serum levels of cardiovascular risk markers than women without BAC.

BAC has been attributed to medial sclerosis of the breast arteries. This process is comparable to Mönckeberg's medial sclerosis, often seen in the smaller peripheral vessels of older diabetic patients [1,5]. It has been reported that arterial calcifications seen on mammograms are associated with increasing age, pregnancy, and lactation but not with various cardiovascular risk factors, being these permanent BAC attributed to physiologic changes in the calcium metabolism of pregnant women in order to meet the high requirements needed for fetal growth and breast-milk production [9]. However, in the present study, differences in parity or in the history of lactation in women with or without BAC were not identified. In addition, in a recently published 25-year prospective study of 12,761 ethnically diverse women, Iribarren et al. [11] reported that after adjustment for multiple confounders including but not limited to age, race, hypertension, diabetes and use of hormone replacement therapy, BAC was significantly associated with an increased risk of coronary heart disease, ischemic stroke and heart failure. In fact, there are several available evidences demonstrating that vascular arterial calcification is not just a degenerative, end-stage passive process associated with aging, but a dynamic and regulated event that serves as a marker of endothelial injury and occurs early in the natural history of atherosclerosis [12]. In this line, it is felt that coronary artery calcification is not an end-stage process, but a widespread event in the early, positive, outward remodelling phase, being predictive of cardiac events prior to the development of coronary stenotic lesions [12].

It is worthy to mention that our results show that women with BAC presented more frequently a history of dislypemia, when compared with women without BAC, being a clinical condition associated with a higher cardiovascular risk. All these associations are in accordance with previous studies in women from a population-based breast cancer screening program, showing that women with BAC frequently have several recognized clinical conditions associated with cardiovascular risk, such as obesity, diabetes, hypertension, dislypemia, or cardiovascular events [1,2]. Nevertheless, similarly to two previous reports, smoking was apparently inversely related to BAC [9], although the reason for the lower prevalence of BAC found in current smokers is at present unclear.

As our results demonstrate, it is remarkable the existence of significant differences in serum levels of several markers of cardiovascular risk between these two groups of patients. Thus, women with BAC had significantly higher triglyceride levels when compared with women without BAC, which is a well-recognized marker of cardiovascular risk. In addition, women with BAC showed a significantly higher percentage of cases with an elevated total cholesterol and an elevated coronary risk index (LDL-C/HDL-C >2; 55.9%) than women without BAC (38.2%). Consequently, the results in our study population lead us to consider that women with BAC are candidates for preventive strategies for cardiovascular disease based on modifications of their serum lipid profile. Nevertheless, in a prior investigation, it was reported that the existence of no relationship between BAC and the lipid profile or a high risk for cardiovascular disease [13]. However, the population of women included in this study by Maas et al. was not comparable to the one in ours, because they included women at high risk of cardiovascular disease into the high-risk cohort of the Raloxifene Use for the Heart (RUTH) Study, with inclusion criteria such age ≥55 year, ≥1 year postmenopausal with established coronary heart disease, being these majority of patients with BAC older than 65 years.

It is remarkable our finding of significantly higher hs-CRP levels in women with BAC than in women without BAC. We consider that this result is in accordance with data reported by other authors showing that some mediators of inflammation such as oxidation, carbonyl stress, cytokines or the CRP itself, may directly stimulate the development of vascular calcifications [14]. Although in a recent report the presence of BAC was not associated with serum levels of hs-CRP [3], the age range of patients included in that study (49–70 years) was higher than ours, and the criteria for patient selection were not specified as they are in the present study.

Another marker of cardiovascular risk is homocysteine. Accumulating data from epidemiological studies indicate that individuals with even moderately elevated levels of homocysteine have an increased risk of cardiovascular disease [15]. Our results also show higher homocysteine serum levels in women with BAC than in women without BAC. Plausible mechanisms

for high homocysteine levels to mediate a deleterious effect include vascular endothelial dysfunction [16], promotion of oxidation of LDL-C [17], vascular smooth cell proliferation [18] and coagulation abnormalities [19]. Thus, our finding may be of clinical interest, because women with BAC could be candidates to the beneficial effects of folic acid and other B-vitamins in preventing cardiovascular disease [20].

In summary, despite the relatively small number of women included in the present study, it is of note that our results showed significant associations of BAC with both clinical and biochemical factors associated with cardiovascular risk. In addition, our results did not show significant differences with the several parameters when semiquantitative subgroups were performed based on these arterial imaging peculiarities, such as bilaterally, number, and intensity of the arterial calcifications. Therefore, we think that the single finding of BAC in a population-based breast cancer screening program carried out in the North of Spain, and with an age range of 45–65 years, may help to identify women showing altered serum markers of cardiovascular risk. In consequence, we consider that our study opens the possibility of new investigations on preventive strategies for cardiovascular diseases. Whether or not vascular calcification can be reversed is not yet known, but exciting new studies might assess this possibility in the future.

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