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Risk factors of atherosclerosis in patients with history of breast cancer

Abstract: The aim of the present study was to evaluate the prevalence of the risk factor of atherosclerosis in patients with history of breast cancer. C-reactive protein, lipoprotein (a), cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triglycerides, homocysteine, fibrinogen, glucose, magnesium, uric acid, and urinary albumin were determined by routine methods in 61 patients with history of breast cancer and 74 control subjects. Urinary neopterin and creatinine concentrations were measured by high-performance liquid chromatography, and intima-media thickness was determined sonographically. Breast cancer patients were significantly older and also had higher systolic blood pressure, glycosylated hemoglobin, lipoprotein (a), serum neopterin, and intima-media thickness. Serum HDL cholesterol and magnesium concentrations were significantly higher in controls compared with patients. Differential patterns of associations between the risk factors of atherosclerosis were observed in patients with history of breast cancer compared with controls. In conclusion, the prevalence of risk factors of atherosclerosis is high in patients with history of breast cancer. Differential associations between risk factors suggest possible differences in the pathogenesis of atherosclerosis in breast cancer patients and controls.

Keywords: breast cancer; cholesterol; homocysteine; intima-media thickness; neopterin.

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Introduction

The progress accomplished in the treatment of breast cancer over the last decades resulted in significantly improved overall survival. In addition to early diagnosis, the amelioration of the outcome of women with breast cancer also likely results from the use of systemic treatment such as hormonal treatment and chemotherapy [1].

With improved survival, comorbidity represents an important issue in cancer patients, and frequently, comorbid disorders rather than cancer represent the ultimate cause of death [2]. Atherosclerosis and its complications are the most important cause of morbidity and mortality due to comorbid conditions in cancer patients. Age, smoking, obesity, or oxidative stress are associated with increased risk of atherosclerosis as well as cancer [3], and cardiovascular disorders currently represent an important issue in cancer survivors. There is now an increasing body of evidence indicating that the anticancer therapy may have a significant impact on the risk of atherosclerosis [4–6]. Although many retrospective series demonstrate in the long term an increased risk of cardiovascular events, e.g., myocardial infarction [7–9], or risk factors of

atherosclerosis [10], the reports on the prevalence of manifestations or risk factors of atherosclerosis in patients with common adult tumors are less numerous. Both an increase and a decrease of the incidence of complications of atherosclerosis have been reported in patients with history of breast cancer [11, 12].

The identification of risk factors or early diagnosis of atherosclerosis has been one of the most important medical advances of the past decades. A number of laboratory parameters, including cholesterol, homocysteine, or C-reactive protein [13] have been shown to be associated with atherosclerosis. An association with increased risk of atherosclerosis or its complications has also been demonstrated for neopterin, a heterocyclic compound produced by activated macrophages [14, 15]. The identification of risk factors opens the possibility of intervention(s) that could prevent later cardiovascular events. High serum cholesterol concentration is a well-established risk factor for the development of vascular lesions in atherosclerosis. Hypercholesterolemia is prevalent in the general population. Tamoxifen, a hormonal agent frequently used in the therapy of breast cancer, lowers serum cholesterol, but aromatase inhibitors, another class of drugs frequently used, probably have no effect on cholesterol concentrations [4]. The importance of disorders of lipid metabolism in cancer patients is becoming an even more important issue with the advent of targeted agents because some of these drugs that are now being used in the treatment of breast cancer, e.g., mammalian target of rapamycin inhibitors, may induce hyperlipidemia. The aim of the present study was to evaluate the prevalence of risk factor of atherosclerosis in patients with history of breast cancer compared with a control group of subjects followed up for benign breast disorders.

Patients and methods

Sixty-one female patients with histologically verified invasive breast cancer followed up at the Department of Oncology, Palacký University Medical School and Teaching Hospital, Olomouc, Czech Republic, were included in the present study. All patients, with the exception of 5 patients who presented with advanced or metastatic disease, had prior surgery. In addition to surgery, prior therapy included radiotherapy in 47 (77%) patients, hormonal therapy in 42 (69%) patients, chemotherapy in 29 (48%) patients, and targeted therapy in 4 (7%) patients. Twelve patients had active recurrent tumors, whereas the remaining 49 patients were breast cancer survivors without signs of disease activity. The control group consisted of 74 women followed up for benign breast disorders, including benign cysts or fibroadenoma, or because of family history of breast cancer followed up at the same institution. The investigations were

approved by the institutional ethics committee, and the patients signed informed consent.

The menopausal status, history of breast cancer, including the time from diagnosis, history of other tumors, history of smoking, hypertension, diabetes mellitus, ischemic heart disease, thyroid disease, or other comorbidity (including history of rheumatic fever, history of stroke, history of subarachnoid hemorrhage, history of seizures, tetany, varicose veins and chronic venous insufficiency, chronic pancreatitis, depression or other psychiatric disorders, peptic ulcer disease, hypercholesterolemia, diverticulosis, rheumatoid arthritis, Crohn disease, and hepatitis) were recorded. Height and weight were measured, and the body mass index (BMI) was calculated by the following formula: weight (in kilograms)/(height [in meters])². Systolic and diastolic blood pressure was measured using a digital upper-arm blood pressure monitor (Hartmann, Heidenheim, Germany) in sitting position after 5–10 min of rest.

Blood samples were drawn from a peripheral vein after an overnight fast. The samples were transferred immediately to the laboratory, centrifuged (1600 g for 8 min at 16°C); the serum and plasma were separated and analyzed immediately or frozen at –20°C until analysis. Potassium, magnesium, glucose, uric acid, C-reactive protein, total cholesterol, triglycerides, lipoprotein (a), high-density lipoprotein (HDL) cholesterol, and urinary albumin were determined using commercially available kits on Cobas c 8000 system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Low-density lipoprotein (LDL) cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Glycosylated hemoglobin was determined by high-performance ion-exchange liquid chromatography using Adams HA-8180V analyzer (Arkray, Kyoto, Japan). Fibrinogen was determined by Clauss clotting time method using commercially available reagents (Technoclone, Vienna, Austria).

Determination of total homocysteine in the plasma was performed by high-performance liquid chromatography (HPLC) with fluorescence detection (385 nm excitation, 515 nm emission) using HPLC H-P 1100 system (Hewlett-Packard, Waldbronn, Germany) as reported by Pfeiffer et al. [16]. Briefly, plasma homocysteine was reduced by Tris-([2-carboxyethyl)-phosphine. The sample was deproteinized by trichloroacetic acid and derivatized with 7-fluoroben-zofurazane-4-sulfonic acid in borate buffer (incubated for 60 min at 60°C). Separation of fluorescent products was performed with a H-P Hypersil ODS 125×4.0-mm column (Hewlett-Packard) with particles, 5 µm, protected with Agilent MetaGuard 4.6 mm Pursuit 5 µm C18 (Agilent Technologies, Santa Clara, CA, USA). The injection volume was 10 µL, and separation was performed using the mobile phase consisting of 0.1 mol/L acetate buffer (pH 5.5) containing 30 mL/L methanol. The flow rate was 0.7 mL/min, and the column temperature was 29°C. Serum neopterin was measured by Neopterin ELISA Kit (IBL International, Hamburg, Germany) using an automated microplate processor for enzyme immunoassays EVOLIS (Bio-Rad Laboratories, Hercules, CA, USA). Measurements were performed in ISO 15189-accredited laboratory.

Early morning urine samples were collected and stored at –20°C until analysis. Urinary neopterin was determined using a modification of the HPLC method described earlier [17]. Briefly, after centrifugation (5 min, 1300 g) and dilution of 100 µL of urine specimens with 1.0 mL of the mobile phase containing disodium EDTA (2 g/L), the samples were filtered using Microtiter, AcroPrep 96 Filter Plate 0.2 µm/350 µL (Pall Life Science, Ann Arbor, MI, USA) and Vacuum manifold (Pall Life Science) and then injected onto a column. Neopterin was determined using the Prominence LC20 HPLC

system (Shimadzu, Kyoto, Japan) composed of rack changer/C-special autosampler for microtitration plates, degasser DGU-20A5, two liquid chromatograph LC-20 AB pumps, auto sampler SIL-20 AC, column oven CTO-20 AC thermostat, fluorescence detector RF-10 AXL, diode array detector SPD-M20A, and communications bus module CBM-20A. Phosphate buffer, 15 mmol/L, pH 6.4, with a flow rate of 0.8 mL/min, was used as the mobile phase. Separation was performed using a hybrid analytical column, Gemini Twin 5 μ , C18, 150 $\times$ 3 mm (Phenomenex, Torrance, CA, USA), at 25°C; the injection volume was 1 μ L. Neopterin was identified by its native fluorescence (353 nm excitation wavelength, 438 nm emission wavelength). Creatinine was monitored simultaneously in the same urine specimen with a diode array detector at 235 nm. The time of analysis for urine neopterin and creatinine was 6 min, and the analytes were quantified by external standard calibration. The results were expressed as the ratio of the neopterin to creatinine (μ mol/mol creatinine).

For the measurement of intima-media thickness, carotid arteries were evaluated with two-dimensional imaging using an ultrasound scanner (Philips iE33; Philips, Bothell, WA, USA) with 3- to 10-MHz transducer as described earlier [6]. The intima-media thickness, defined as the distance between the echogenic lines representing the blood-intima interface and media-adventitia interface and measured on the posterior wall of the carotid artery in the longitudinal plane, was evaluated bilaterally at two levels. Three measurements were performed at both levels by the same observer, and the mean of these measurements was calculated. The presence of carotid plaque was noted. Sonographic signs of atherosclerosis were considered to be present if the intima-media thickness was equal or above 1 mm or if the presence of the plaques or calcifications was noted.

Differences between the patients and the control group or subgroups of subjects were analyzed by the Mann-Whitney U-test. Correlations were analyzed using Spearman's rank correlation coefficient (r_s). The differences in proportions were assessed using the Fisher's exact test. Differences in the intima-media thickness of the right and left carotid arteries were analyzed with Wilcoxon's signed rank test. The decision on statistical significance was based on $p=0.05$ level. The analyses were performed using NCSS software (Number Cruncher Statistical Systems, Kaysville, UT, USA).

Results

In patients with history of breast cancer, the median time from breast cancer diagnosis was 5.0 years (range 0.4–21.0 years). Compared with controls, significantly more breast cancer patients were postmenopausal, had history of hypertension, other primary tumors, or other comorbidity (Table 1). Second primary tumors, including endometrial carcinoma in two cases, non-melanoma skin cancer in one case, bladder carcinoma in one case, and renal cell carcinoma in one case, were noted only in patients with history of breast cancer. Significantly more controls were smokers, and the proportion of subjects with history of diabetes, ischemic heart disease, or thyroid disorder was comparable between patients and controls.

Breast cancer patients were significantly older, had higher systolic blood pressure, glycosylated hemoglobin,

Table 1 Subject history in patients and controls.

Parameter	Controls (n=74), n (%)	Patients (n=61), n (%)	p-Value
Menopausal status			
Premenopausal	20 (27)	4 (7)	0.003
Postmenopausal	54 (73)	57 (93)	
Smoking			
No	46 (62)	49 (80)	0.024
Yes	28 (38)	12 (20)	
Hypertension			
No	49 (66)	24 (39)	0.003
Yes	25 (34)	37 (61)	
Diabetes			
No	68 (92)	55 (90)	0.768
Yes	6 (8)	6 (10)	
Ischemic heart disease			
No	68 (92)	53 (87)	0.402
Yes	6 (8)	8 (13)	
Thyroid disorder			
No	54 (73)	45 (74)	1.000
Yes	20 (27)	16 (26)	
Malignancy other than breast cancer			
No	74 (100)	56 (92)	0.017
Yes	0 (0)	5 (8)	
Other comorbidity			
No	60 (81)	37 (61)	0.012
Yes	14 (19)	24 (39)	

p-Value indicates the results of Fisher's exact test.

lipoprotein (a), serum neopterin, and intima-media thickness compared with controls (Table 2). Serum HDL cholesterol and magnesium concentrations were significantly higher in controls compared with patients. The imbalance of age between patients and controls was partly due to higher proportion of premenopausal women in the control group. When only postmenopausal subjects were evaluated, the difference of age between controls and patients was less pronounced (62 ± 8 vs. 65 ± 8 years, $p=0.040$) but still statistically significant. The comparison between controls and patients was similar when the analysis was restricted to postmenopausal women (Table 3).

Significantly more patients with history of breast cancer had sonographic signs of atherosclerosis compared with the control group (8/44 patients, 18%, vs. 3/65 controls, 5%, $p=0.023$). Apart from the obvious difference in the intima-media thickness, no significant differences were noted in other parameters investigated between patients with and without sonographic signs of atherosclerosis (data not shown).

Differential associations between the intima-media thickness and the laboratory parameters were observed in patients and controls. In controls, a significant

Table 2 Distribution of clinical and laboratory parameters in patients and controls.

Parameter	Controls		Patients		p-Value
	n	Mean±SD (range)	n	Mean±SD (range)	
Age, years	74	58±11 (36–77)	61	64±10 (30–81)	0.001
Weight, kg	74	69±13 (46–101)	61	73±14 (47–103)	0.063
Height, cm	74	164±7 (150–178)	61	163±6 (148–175)	0.433
BMI, kg/m ²	74	25.57±4.07 (18.36–35.79)	61	27.09±4.69 (18.16–36.33)	0.079
Systolic BP, mm Hg	74	138±18 (90–178)	61	146±21 (100–197)	0.028
Diastolic BP, mm Hg	74	84±11 (58–117)	61	87±12 (60–111)	0.101
Fibrinogen, g/L	73	2.79±0.42 (1.76–3.80)	61	2.96±0.61 (1.75–4.90)	0.202
Total cholesterol, mmol/L	74	5.56±0.91 (2.90–7.31)	61	5.52±1.00 (3.45–8.19)	0.681
LDL cholesterol, mmol/L	74	3.14±0.89 (0.35–5.03)	61	3.22±0.99 (0.97–5.43)	0.654
HDL cholesterol, mmol/L	74	1.87±0.63 (0.91–5.28)	61	1.64±0.46 (0.56–3.27)	0.036
Triglycerides, mmol/L	74	1.44±0.95 (0.42–6.43)	61	1.51±0.77 (0.60–4.68)	0.271
Glucose, mmol/L	72	5.9±2.2 (4.5–20.1)	61	5.8±1.5 (3.9–13.5)	0.620
Glycosylated hemoglobin, %	74	4.0±1.2 (2.6–10.2)	61	4.2±0.7 (3.2–7.1)	0.008
Magnesium, mmol/L	74	0.84±0.09 (0.60–1.07)	61	0.79±0.07 (0.60–0.97)	0.0006
Potassium, mmol/L	74	4.22±0.31 (3.46–4.88)	61	4.15±0.31 (3.62–5.26)	0.053
Uric acid, µmol/L	74	259±67 (115–456)	61	278±73 (151–516)	0.080
Lipoprotein (a), g/L	74	0.30±0.33 (0.03–1.86)	61	0.39±0.40 (0.05–2.17)	0.049
Homocysteine, µmol/L	71	13.3±6.2 (4.5–44.4)	61	13.4±3.9 (5.8–26.9)	0.177
Albuminuria, mg/L	70	8.0±11.0 (0.6–62.8)	61	25.1±87.2 (0.3–666.7)	0.347
Albuminuria, g/mol creatinine	70	0.96±1.60 (0.05–10.26)	61	2.06±4.35 (0.02–23.64)	0.252
C-reactive protein, mg/L	73	2.4±2.4 (0.3–15.0)	59	3.7±5.8 (0.3–38.4)	0.255
Serum neopterin, nmol/L	68	7.27±2.53 (1.90–12.81)	61	8.62±3.32 (2.69–27.39)	0.017
Urinary neopterin, µmol/mol creatinine	74	169±67 (77–440)	61	162±59 (43–360)	0.988
IMT right carotid artery, mm	68	0.6±0.1 (0.4–1.1)	44	0.9±0.8 (0.5–4.0)	0.0008
IMT left carotid artery, mm	68	0.6±0.1 (0.4–1.1)	44	0.9±0.8 (0.4–4.1)	0.0009
Mean IMT, mm	68	0.6±0.1 (0.4–1.1)	68	0.9±0.8 (0.5–3.9)	0.0005

BP, blood pressure; IMT, intima-media thickness; SD, standard deviation. Parameters with statistically significant difference are in bold type.

positive correlation was evident between intima-media thickness and age, weight, BMI, glycosylated hemoglobin, and serum neopterin. In contrast, in breast cancer patients, a positive association was observed between intima-media thickness and albuminuria or C-reactive protein (Table 4).

Correlations with neopterin concentrations also differed between patients and controls, and different correlations were observed with urinary and serum neopterin concentrations. As expected, a significant correlation was noted between urinary and serum neopterin concentrations in both patients and controls (Table 5). In controls, serum neopterin concentrations exhibited, in addition to the correlation with intima-media thickness, a significant positive correlation with uric acid concentrations and a negative correlation with total cholesterol. Urinary neopterin in controls correlated positively with weight and triglycerides. In patients, serum neopterin had significant positive correlation with age, triglycerides, uric acid, homocysteine, albuminuria, and C-reactive protein, and a negative correlation with HDL cholesterol. Urinary neopterin in patients exhibited a significant negative

correlation with HDL cholesterol and a positive correlation with C-reactive protein (Table 5).

Thirty-seven patients (61%) with history of breast cancer and 25 controls (34%) had also a history of hypertension. As noted above, the proportion of subjects with history of hypertension was significantly ($p=0.003$) higher in patients compared with controls, but the proportion of subjects with hypertension among patients with active disease (8/12, 67%) and no signs of disease activity (29/49, 59%) was not significantly different. Despite the similar proportion of patients with history of hypertension, both systolic blood pressure (132 ± 17 vs. 148 ± 21 , $p=0.021$) and diastolic blood pressure (78 ± 11 vs. 89 ± 11 , $p=0.010$) were significantly lower in patients with active disease compared with patients with no signs of disease activity. Significant differences in clinical and laboratory parameters were noted in subjects with or without hypertension, but again, a differential pattern was observed in differences observed in patients and controls (Table 6). Both patients and controls with hypertension were significantly older, had higher BMI, and higher blood pressure, but significant differences between

Table 3 Distribution of clinical and laboratory parameters in postmenopausal patients and controls.

Parameter	Controls		Patients		p-Value
	n	Mean±SD (range)	n	Mean±SD (range)	
Age, years	54	62±8 (45–77)	57	65±8 (42–81)	0.040
Weight, kg	54	70±13 (47–101)	57	74±13 (47–103)	0.150
Height, cm	54	164±7 (150–178)	57	163±5 (148–175)	0.738
BMI, kg/m ²	54	26.40±3.96 (18.36–35.79)	57	27.40±4.63 (19.60–36.33)	0.324
Systolic BP, mm Hg	54	140±19 (90–178)	57	147±22 (100–197)	0.095
Diastolic BP, mm Hg	54	83±11 (58–117)	57	87±12 (60–111)	0.018
Fibrinogen, g/L	54	2.83±0.42 (1.76–3.80)	57	2.96±0.63 (1.75–4.90)	0.567
Total cholesterol, mmol/L	54	5.55±0.90 (2.90–7.19)	57	5.54±1.01 (3.45–8.19)	0.841
LDL cholesterol, mmol/L	54	3.15±0.95 (0.35–5.03)	57	3.23±0.99 (0.97–5.43)	0.768
HDL cholesterol, mmol/L	54	1.83±0.64 (0.91–5.28)	57	1.65±0.47 (0.56–3.27)	0.153
Triglycerides, mmol/L	54	1.47±0.96 (0.42–6.43)	57	1.54±0.79 (0.60–4.68)	0.503
Glucose, mmol/L	54	6.1±2.4 (4.5–20.1)	57	5.9±1.6 (3.9–13.5)	0.458
Glycosylated hemoglobin, %	54	4.2±1.3 (2.6–10.2)	57	4.2±0.7 (3.2–7.1)	0.146
Magnesium, mmol/L	54	0.82±0.09 (0.60–1.07)	57	0.79±0.07 (0.60–0.97)	0.032
Potassium, mmol/L	54	4.25±0.32 (3.50–4.88)	57	4.16±0.31 (3.62–5.26)	0.054
Uric acid, µmol/L	54	271±67 (115–456)	57	281±73 (151–516)	0.422
Lipoprotein (a), g/L	54	0.30±0.36 (0.03–1.86)	57	0.39±0.41 (0.05–2.17)	0.052
Homocysteine, µmol/L	54	13.7±6.5 (6.7–44.4)	57	13.6±3.8 (7.8–26.9)	0.236
Albuminuria, mg/L	52	7.6±11.7 (0.6–62.8)	57	26.8±90.0 (0.3–666.7)	0.064
Albuminuria, g/mol creatinine	52	0.84±1.21 (0.05–7.53)	61	2.19±4.47 (0.02–23.64)	0.145
C-reactive protein, mg/L	54	2.3±1.8 (0.3–10.2)	55	3.9±5.9 (0.3–38.4)	0.247
Serum neopterin, nmol/L	50	7.47±2.65 (1.90–12.81)	57	8.77±3.32 (2.69–27.39)	0.059
Urinary neopterin, µmol/mol creatinine	54	172±68 (77–440)	57	163±60 (43–360)	0.746
IMT right carotid artery, mm	48	0.7±0.1 (0.4–1.1)	42	1.0±0.8 (0.5–4.0)	0.009
IMT left carotid artery, mm	48	0.7±0.1 (0.4–1.1)	44	0.9±0.8 (0.5–4.1)	0.013
Mean IMT, mm	48	0.7±0.1 (0.4–1.1)	42	0.9±0.8 (0.5–3.9)	0.006

BP, blood pressure; IMT, intima-media thickness; SD, standard deviation. Parameters with statistically significant difference are in bold type.

subjects with or without hypertension in triglycerides, glucose, glycosylated hemoglobin, magnesium, uric acid, and homocysteine were noted only in controls. Significant differences between patients and controls in age, fibrinogen, HDL cholesterol, glycosylated hemoglobin, magnesium, lipoprotein (a), and intima-media thickness were observed in subjects without hypertension but not in subjects with hypertension (Table 6)

Forty-seven patients (77%) had prior radiotherapy. Among these patients, no significant differences in the intima-media thickness were observed between the left and the right carotid arteries of patients with left-sided tumors (1.1±1.0 vs. 1.1±0.9 mm, respectively), and similarly, no significant difference in the intima-media thickness were noted between the left and the right carotid arteries in patients with right-sided tumors (0.9±0.7 vs. 0.9±0.8 mm, respectively). Among 29 patients with history of chemotherapy, fibrinogen (3.13±0.67 vs. 2.80±0.52 g/L, $p=0.021$) and uric acid concentrations (292±68 vs. 264±75 µmol/L, $p=0.038$) were significantly higher compared with patients with no history of chemotherapy, but

no significant differences were observed based on prior chemotherapy in the other investigated parameters.

Discussion

Present data demonstrate a generally high prevalence of clinical and laboratory risk factors of atherosclerosis in patients with history of breast cancer and also in the control group of subjects followed up for benign breast disorders. This high prevalence in both patients and controls may be reflective of the general population of patients at risk for breast cancer. Importantly, intima-media thickness, an indicator of the presence of atherosclerosis [18], was significantly increased in patients with history of breast cancer. Meanwhile, no association was noted between prior radiotherapy and ipsilateral intima-media thickness. A correlation of intima-media thickness with well-known risk factors of atherosclerosis including age, weight, or glycosylated hemoglobin was evident only in the control group. In breast cancer patients,

Table 4 Correlation of mean intima-media thickness with clinical and laboratory parameters.

Parameter	r_s (p)	
	Controls	Patients
Age, years	0.419 (0.0004)	0.241 (0.115)
Weight, kg	0.280 (0.021)	0.005 (0.975)
Height, cm	-0.067 (0.585)	0.075 (0.629)
BMI, kg/m ²	0.340 (0.005)	-0.040 (0.797)
Systolic BP, mm Hg	0.136 (0.267)	0.021 (0.891)
Diastolic BP, mm Hg	0.186 (0.129)	-0.248 (0.105)
Fibrinogen, g/L	0.185 (0.131)	0.075 (0.628)
Total cholesterol, mmol/L	-0.001 (0.999)	-0.014 (0.930)
LDL cholesterol, mmol/L	0.124 (0.312)	-0.013 (0.934)
HDL cholesterol, mmol/L	-0.194 (0.111)	-0.286 (0.060)
Triglycerides, mmol/L	0.128 (0.297)	0.259 (0.089)
Glucose, mmol/L	0.142 (0.254)	0.071 (0.649)
Glycosylated hemoglobin, %	0.266 (0.028)	0.238 (0.120)
Magnesium, mmol/L	-0.002 (0.986)	0.078 (0.615)
Potassium, mmol/L	0.085 (0.491)	0.052 (0.740)
Uric acid, μ mol/L	0.193 (0.114)	0.053 (0.735)
Lipoprotein (a), g/L	0.057 (0.643)	0.039 (0.803)
Homocysteine, μ mol/L	0.169 (0.179)	0.233 (0.128)
Albuminuria, mg/L	-0.195 (0.123)	0.297 (0.051)
Albuminuria, g/mol creatinine	-0.227 (0.071)	0.311 (0.040)
C-reactive protein, mg/L	-0.132 (0.286)	0.308 (0.047)
Serum neopterin, nmol/L	0.262 (0.040)	0.088 (0.569)
Urinary neopterin, μ mol/mol creatinine	0.139 (0.258)	0.058 (0.707)

Values are Spearman's rank correlation coefficient (r_s), with the p-value in parentheses. Statistically significant correlations are in bold type.

intima-media thickness correlated only with albuminuria and C-reactive protein, indicating that atherosclerosis in the breast cancer patient population (consisting mostly of survivors) is associated with renal dysfunction, which is prevalent in this population [19] and with the acute phase response.

In general, different associations, reflected in correlations, were observed between neopterin concentrations and clinical or laboratory parameters of atherosclerosis risk in patients and controls. A significant correlation of intima-media thickness with serum neopterin was observed only in the control group, but a number of significant correlations of biomarkers of lipid metabolism associated with atherosclerosis with parameters of inflammatory response were observed in both patients and controls. Thus, present data indicate that in the general population as well as in patients with breast cancer, some of the well-known risk factors of atherosclerosis are linked to inflammatory response. It is difficult to determine whether these associations reflect the inflammatory response associated with atherosclerosis, or vice

versa, these changes are caused by the inflammatory response elicited by breast cancer. Among the laboratory parameters investigated in the present study, systemic inflammatory response is reflected in increased C-reactive protein and neopterin concentrations. Neopterin is produced by activated macrophages also in association with systemic inflammatory response. In patients with tumors of different primary locations, increased serum or urinary neopterin concentrations were associated with poor prognosis [20, 21]. As expected, a significant correlation was observed between serum and urinary neopterin concentrations. Among other factors, serum neopterin concentrations depend on renal function. Although urinary neopterin concentration is assessed as the neopterin/creatinine ratio, the correction for creatinine concentrations is not performed routinely as a part of serum neopterin measurement. Consequently, the correlation between urinary and serum neopterin concentrations was relatively weak in the present study, which included patients with laboratory evidence of renal dysfunction. In fact, most of the other correlations observed were also relatively weak. High concentrations of neopterin in the serum or urine were associated with low HDL cholesterol in patients with history of breast cancer but not in controls. Serum neopterin concentrations in patients correlated with the triglyceride levels, homocysteine, and albuminuria, and serum uric acid correlated with serum neopterin in both patients and controls. The correlation between serum neopterin and uric acid concentrations that was evident in both patients and controls may be explained by the similar effect of subclinical renal dysfunction on serum concentrations of both substances. The association between neopterin and homocysteine concentrations in cancer patients has been reported before [22]. Moreover, serum homocysteine concentrations may, similarly to neopterin, reflect renal dysfunction [23].

In patients with breast carcinoma, urinary neopterin concentrations are increased in only approximately 20% of patients [24, 25], but similarly to tumors of other primary location, increased neopterin is associated with poor prognosis [24, 26]. Thus, breast cancer results in increased neopterin concentrations only in a minority of affected patients. Moreover, most patients in the present cohort were breast cancer survivors with no evidence of disease activity. It has been previously demonstrated that disease control results in lower neopterin concentrations and that neopterin concentrations may be transiently increased during therapy. Therefore, we can speculate that a correlation was observed between intima-media thickness only in the control group because neopterin was increased as a result of chronic disorders in the

Table 5 Correlation of neopterin concentration with clinical and laboratory parameters.

Parameter	r_s (p)			
	Serum neopterin, nmol/L		Urinary neopterin, μ mol/mol creatinine	
	Controls	Patients	Controls	Patients
Age, years	0.232 (0.056)	0.275 (0.032)	0.094 (0.426)	0.031 (0.812)
Weight, kg	0.192 (0.118)	0.065 (0.621)	0.266 (0.022)	0.046 (0.724)
Height, cm	0.045 (0.717)	0.088 (0.501)	0.130 (0.269)	0.157 (0.228)
BMI, kg/m ²	0.132 (0.282)	-0.032 (0.807)	0.186 (0.113)	-0.055 (0.675)
Systolic BP, mm Hg	0.116 (0.348)	-0.117 (0.370)	0.060 (0.613)	-0.020 (0.880)
Diastolic BP, mm Hg	0.039 (0.753)	-0.049 (0.707)	-0.019 (0.871)	-0.050 (0.699)
Fibrinogen, g/L	0.081 (0.514)	0.194 (0.134)	-0.078 (0.510)	0.180 (0.166)
Total cholesterol, mmol/L	-0.255 (0.036)	0.022 (0.868)	-0.142 (0.226)	0.098 (0.450)
LDL cholesterol, mmol/L	-0.196 (0.109)	0.047 (0.719)	-0.128 (0.276)	0.133 (0.306)
HDL cholesterol, mmol/L	-0.046 (0.707)	-0.274 (0.033)	-0.140 (0.233)	-0.353 (0.005)
Triglycerides, mmol/L	0.074 (0.550)	0.366 (0.004)	0.232 (0.047)	0.230 (0.075)
Glucose, mmol/L	0.104 (0.403)	0.144 (0.270)	-0.053 (0.659)	-0.015 (0.906)
Glycosylated hemoglobin, %	0.060 (0.628)	0.085 (0.515)	0.144 (0.221)	-0.011 (0.931)
Magnesium, mmol/L	-0.196 (0.110)	-0.114 (0.381)	-0.068 (0.564)	-0.196 (0.130)
Potassium, mmol/L	-0.098 (0.424)	0.230 (0.075)	-0.022 (0.849)	0.164 (0.206)
Uric acid, μ mol/L	0.259 (0.033)	0.354 (0.005)	0.127 (0.279)	0.163 (0.208)
Lipoprotein (a), g/L	-0.109 (0.377)	0.011 (0.931)	-0.112 (0.341)	0.207 (0.110)
Homocysteine, μ mol/L	0.226 (0.070)	0.369 (0.003)	0.195 (0.103)	0.037 (0.779)
Albuminuria, mg/L	0.078 (0.534)	0.293 (0.022)	-0.054 (0.658)	0.018 (0.891)
Albuminuria, g/mol creatinine	0.077 (0.540)	0.223 (0.085)	0.013 (0.912)	0.082 (0.530)
C-reactive protein, mg/L	-0.003 (0.981)	0.304 (0.019)	0.172 (0.147)	0.298 (0.022)
Serum neopterin, nmol/L	–	–	0.446 (0.0001)	0.338 (0.008)

Values are Spearman's rank correlation coefficient (r_s), with the p-value in parentheses. Statistically significant correlations are in bold type.

control group, whereas in patients with history of breast cancer, the relation between systemic immune activation reflected in neopterin concentrations and intima-media thickness might have been obscured because previous therapeutic interventions resulted in an apparent cure in the majority of patients. Interestingly, C-reactive protein correlated significantly with intima-media thickness in breast cancer patients but not in controls, indicating that the two biomarkers of risk of atherosclerosis associated with host response, neopterin and C-reactive protein, may be dissociated in patients with history of cancer and in the general population. Of note, a significant correlation between serum or urinary neopterin and C-reactive protein was evident only in breast cancer patients.

The differential associations between intima-media thickness and the risk factors of atherosclerosis indicate that the pathogenic mechanisms responsible for atherosclerosis may be different in patients with breast cancer compared with subjects with no cancer history. The relation among history of cancer, antitumor therapy, and increased risk of atherosclerosis is best characterized in patients with germ cell or pediatric tumors [7–10], whereas data on the risk of atherosclerosis in patients with breast cancer are more limited [11, 12]. In an earlier study in

patients with breast cancer, the probability of the presence of sonographic signs of carotid atherosclerosis was, in a logistic regression analysis, associated only with age, whereas intima-media thickness was significantly associated in multiple regression analysis models with age, glucose, HDL cholesterol, and time from chemotherapy start [5]. Moreover, intima-media thickness was reported to be increased in patients with distant metastases [5]. Present data indicating that factors that may cause the progression of atherosclerosis are present with higher frequency in patients with history of breast cancer are in agreement with these earlier observations, further supporting the hypothesis that systemic response associated with the tumor or anticancer therapy may accelerate atherosclerosis in the survivors of breast cancer.

Hypertension was the most prevalent risk factor of atherosclerosis in the present study. A significant proportion of both patients and controls had hypertension, but hypertension was almost twice more common in patients. The association of hypertension with age, disorders of glucose or lipid metabolism, and hyperuricemia is well known. A higher prevalence of hypertension has been previously reported in cancer survivors [27, 28]. Hypertension in patients with history of breast cancer was not

Table 6 Clinical and laboratory parameters in subjects with or without the history of hypertension.

Parameter	Controls		Patients		p ^a	p ^b	p ^c	p ^d
	No hypertension (n=49), mean±SD (range)	Hypertension (n=25), mean±SD (range)	No hypertension (n=24), mean±SD (range)	Hypertension (n=37), mean±SD (range)				
Age, years	54±10 (36–77)	64±9 (45–76)	59±13 (30–71)	67±8 (47–81)	0.0002	0.0069	0.048	0.565
Weight, kg	67±12 (47–98)	72±14 (46–101)	70±13 (47–96)	75±14 (49–103)	0.079	0.287	0.224	0.625
Height, cm	164±7 (150–178)	164±7 (150–176)	164±5 (148–173)	163±6 (152–175)	0.913	0.321	0.804	0.585
BMI, kg/m ²	24.65±3.60 (18.36–31.88)	27.38±4.38 (19.65–35.79)	25.61±4.67 (18.16–36.33)	28.05±4.51 (20.55–35.64)	0.011	0.036	0.435	0.796
Systolic BP, mm Hg	134±17 (90–170)	146±17 (120–178)	138±18 (100–177)	151±22 (110–197)	0.010	0.019	0.228	0.455
Diastolic BP, mm Hg	85±11 (60–117)	82±9 (58–99)	82±10 (60–99)	89±12 (64–111)	0.664	0.016	0.581	0.015
Fibrinogen, g/L	2.73±0.41 (1.76–3.50)	2.89±0.43 (2.30–3.80)	3.12±0.72 (2.20–4.90)	2.85±0.51 (1.75–4.00)	0.215	0.261	0.044	0.796
Total cholesterol, mmol/L	5.64±0.99 (2.90–7.31)	5.39±0.71 (4.35–6.88)	5.54±1.27 (3.45–8.19)	5.50±0.80 (4.07–7.18)	0.124	0.994	0.569	0.576
LDL cholesterol, mmol/L	3.19±0.96 (0.35–5.03)	3.03±0.74 (1.78–4.56)	3.32±1.17 (0.97–5.43)	3.15±0.86 (1.73–4.95)	0.370	0.530	0.618	0.576
HDL cholesterol, mmol/L	1.91±0.51 (0.91–3.35)	1.79±0.81 (1.03–5.28)	1.60±0.48 (0.56–2.45)	1.67±0.44 (0.94–3.27)	0.124	0.669	0.024	0.892
Triglycerides, mmol/L	1.32±0.98 (0.42–6.43)	1.67±0.86 (0.48–3.92)	1.49±0.88 (0.71–4.68)	1.52±0.71 (0.60–3.19)	0.026	0.540	0.188	0.576
Glucose, mmol/L	5.4±0.9 (4.5–10.6)	6.8±3.3 (4.4–20.1)	5.9±1.4 (4.4–10.1)	5.7±1.6 (3.9–13.5)	0.004	0.399	0.286	0.026
Glycosylated hemoglobin, %	3.8±0.8 (2.8–8.3)	4.5±1.6 (2.6–10.2)	4.3±0.8 (3.4–7.1)	4.1±0.6 (3.2–6.4)	0.003	0.599	0.008	0.454
Magnesium, mmol/L	0.86±0.07 (0.70–1.07)	0.80±0.09 (0.60–0.95)	0.81±0.07 (0.72–0.97)	0.78±0.08 (0.60–0.94)	0.019	0.162	0.014	0.256
Potassium, mmol/L	4.18±0.30 (3.46–4.86)	4.30±0.32 (3.69–4.88)	4.15±0.30 (3.63–4.86)	4.15±0.32 (3.62–5.26)	0.241	0.773	0.442	0.034
Uric acid, µmol/L	242±55 (115–389)	292±78 (175–456)	274±81 (155–516)	280±68 (151–484)	0.006	0.734	0.082	0.672
Lipoprotein (a), g/L	0.28±0.36 (0.03–1.86)	0.33±0.29 (0.03–1.03)	0.40±0.43 (0.06–2.17)	0.39±0.39 (0.05–1.47)	0.168	0.497	0.012	0.813
Homocysteine, µmol/L	11.9±4.4 (4.5–27.6)	15.9±8.0 (6.2–44.4)	12.6±3.2 (5.8–18.6)	13.9±4.3 (8.4–26.9)	0.006	0.575	0.131	0.447
Albuminuria, mg/L	7.1±10.8 (0.6–62.8)	9.6±11.3 (1.0–48.5)	42.4±136.3 (0.4–666.7)	13.9±21.9 (0.3–114.8)	0.299	0.590	0.734	0.674
Albuminuria, g/mol creatinine	0.89±1.64 (0.07–10.26)	1.08±1.54 (0.05–7.53)	2.09±5.25 (0.04–23.64)	2.04±3.73 (0.02–20.14)	0.183	0.384	0.465	0.723
C-reactive protein, mg/L	2.6±2.7 (0.3–15.0)	2.1±1.5 (0.3–6.0)	4.7±8.6 (0.3–38.4)	3.1±3.1 (0.3–14.1)	0.884	0.906	0.498	0.405
Serum neopterin, nmol/L	6.96±2.37 (1.90–11.46)	7.91±2.69 (2.13–12.81)	8.62±2.25 (4.62–13.44)	8.58±3.87 (2.69–27.39)	0.080	0.421	0.006	0.941
Urinary neopterin, µmol/mol creatinine	168±75 (77–440)	171±50 (86–267)	169±44 (106–279)	159±67 (43–360)	0.251	0.376	0.288	0.333
IMT right carotid artery, mm	0.6±0.1 (0.4–0.8)	0.7±0.2 (0.4–1.1)	0.7±0.1 (0.5–0.9)	1.1±1.0 (0.5–4.0)	0.238	0.511	0.020	0.090
IMT left carotid artery, mm	0.6±0.1 (0.4–1.0)	0.7±0.2 (0.4–1.1)	0.7±0.2 (0.4–1.0)	1.1±1.0 (0.5–4.1)	0.282	0.521	0.026	0.072
Mean IMT, mm	0.6±0.1 (0.4–0.8)	0.7±0.2 (0.4–1.1)	0.7±0.1 (0.5–0.9)	1.1±1.0 (0.5–3.9)	0.242	0.539	0.022	0.053

^aControls without history of hypertension vs. controls with history of hypertension. ^bPatients without history of hypertension vs. patients with history of hypertension. ^cControls without history of hypertension vs. patients without history of hypertension. ^dControls with history of hypertension vs. patients with history of hypertension. p-Values with statistically significant difference are in bold type.

associated with metabolic risk factors of atherosclerosis, in contrast to the control group. Meanwhile, even among subjects with no history of hypertension in the present study, many risk factors of atherosclerosis, including age, fibrinogen, glycosylated hemoglobin, HDL, cholesterol, magnesium, lipoprotein (a), or serum neopterin, were unfavorable in patients with history of breast cancer compared with the control group.

The high prevalence of hypertension in breast cancer patients has to be taken into account when planning studies with agents that negatively impact the parameters of risk of atherosclerosis. Specifically, the high prevalence of hypertension in this population may represent an impediment in the introduction of agents targeting vascular endothelial growth factor (VEGF) [29]. From this point of view of potential targeted therapy, lower blood pressure values in patients with active disease that were present despite a comparable incidence of history of hypertension are an interesting observation for planning future trials of anti-VEGF therapy in patients with metastatic breast cancer.

The present study has obvious limitations. One of the most important limitations is the size of the cohort of patients and controls that did not allow for a multivariable analysis. The Bonferroni correction for multiple analyses was not performed because of the pilot nature of the study and limited number of subjects studied. Moreover, patients with history of breast cancer were older than the control group, and some of the observed differences may be linked to age. A selection bias resulting from referral of patients with more comorbidities to the tertiary center cannot also be excluded. The findings of the present pilot study should be confirmed in larger patient cohorts in

future studies. Meanwhile, present data demonstrating the presence of one or more risk factors of atherosclerosis in patients with history of breast cancer may serve as a basis of an interventional study aimed at the prevention of atherosclerosis, which is by far the most important competitive cause of death in this population. Data on the prevalence of atherosclerosis or prevalence of risk factors of atherosclerosis in patients with breast cancer are of great practical significance, especially in view of the data demonstrating that therapy may further increase the risk of atherosclerosis [6]. Moreover, the control group in the present study was not defined as healthy individuals, and the control subjects also had significant comorbidities, further supporting the hypothesis that some of the differences observed between patients with history of breast cancer and controls in the present study may be linked to the breast cancer or anticancer therapy.

In conclusion, the prevalence of risk factors of atherosclerosis is high in patients with history of breast cancer, resulting in an increase of intima-media thickness, an indicator of the presence of atherosclerosis. Among other factors, the risk of atherosclerosis was associated with inflammatory response. Differential associations between risk factors suggest possible differences in the pathogenesis of atherosclerosis in breast cancer patients and controls.

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