Int J Clin Exp Pathol 2016;9(3):3094-3104
www.ijcep.com /ISSN:1936-2625/IJCEP0021426

Original Article

Medial arterial calcification in breast cancer: clinicopathological features and its impact on tumor behavior

Mu Yang[1,2*], Li Zhang[1,2*], Fangfang Liu[1], Chengying Jiang[1], Yang Lin[1], Xiaolong Feng[1], Feng Lv[1], Xiaoyan Li[1], Yiqian Zhang[1], Beibei Shen[1], Xinmin Zhang[3], Li Fu[1], Zhongsheng Tong[2], Xiaojing Guo[1]

Departments of [1]Breast Cancer Pathology and Research Lab, [2]Breast Oncology, Key Laboratory of Breast Cancer Prevention and Therapy, National Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China; [3]Department of Pathology, Cooper Medical School of Rowan University, Camden, New Jersey, USA. [*]Equal contributors.

Received December 8, 2015; Accepted February 17, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Purpose: Medial arterial calcification (MAC) reduces arterial wall compliance, resulting in impaired vasodilation and tissue ischemia. MAC is frequently observed at mammography where it may mimic malignancy. However, its relationship to breast cancer has not yet been investigated. This study is to explore clinicopathological features of breast MAC and its association with breast cancer. Methods: 452 cases of MAC were identified from 2953 mastectomy specimens (15.3%) with invasive carcinomas diagnosed by whole breast examination from January 2011 to January 2012. Silver nitrate staining and immunohistochemistry were performed. Results: The presence and severity of MAC were significantly associated with advanced age and menopausal status. Less frequent lymphovascular invasion (LVI) and lower Ki-67 labelling were identified in cancer with MAC than in control cases (P=0.01; P=0.002). The severity of MAC was negatively associated with Ki-67 labelling (P=0.045) of tumors. A trend was noted that patients in MAC group manifested a better disease free survival (DFS) than that in non-MAC group after 30 months of follow-up. And the inert biological behavior of cancer might be associated with poor tumor microenvironment created by MAC. Conclusions: MAC was identified in a significant portion of mastectomy specimens with breast cancer. Although not a driving factor for patient's prognosis, the accompanying MAC does slow tumor proliferation and reduces LVI, which may offer patients additional window time for clinical interventions that could result in more favorable outcomes. Further exploration of the impact of MAC on breast cancer patients in large scale studies is justified.

Keywords: Breast cancer, medial arterial calcification, invasion and metastasis, proliferation, tumor microenvironment

Introduction

Cancer, cardiovascular disease and diabetes are the three major threats to human health. Research on interaction of these diseases has recently become a hotspot and has brought up new views in disease management. Concurrent chronic non-neoplastic diseases can complicate the management of cancer patients and may impact on the clinical courses. Breast cancer is one of the most common female malignant tumors, and its incidence increases remarkably in recent decades in postmenopausal women [1, 2]. Coincidently, cardiovascular disease and diabetes are also prevalent in aged population. They theoretically may change the microenvironments of cancers and modify their behaviors.

Medial arterial calcification (MAC) is a form of arteriosclerosis where calcium deposits are found in the tunica media of intermediate to small arteries. It is a non-obstruction condition leading to reduced arterial compliance, and is commonly seen in patient with diabetes mellitus, particularly in association with distal symmetrical neuropathy, chronic renal failure and coronary artery disease [3-6]. Breast MAC is regularly observed at mammography where it may mimic malignancy, though no evidence has been identified to indicate its association with the development of breast cancer. In our prac-

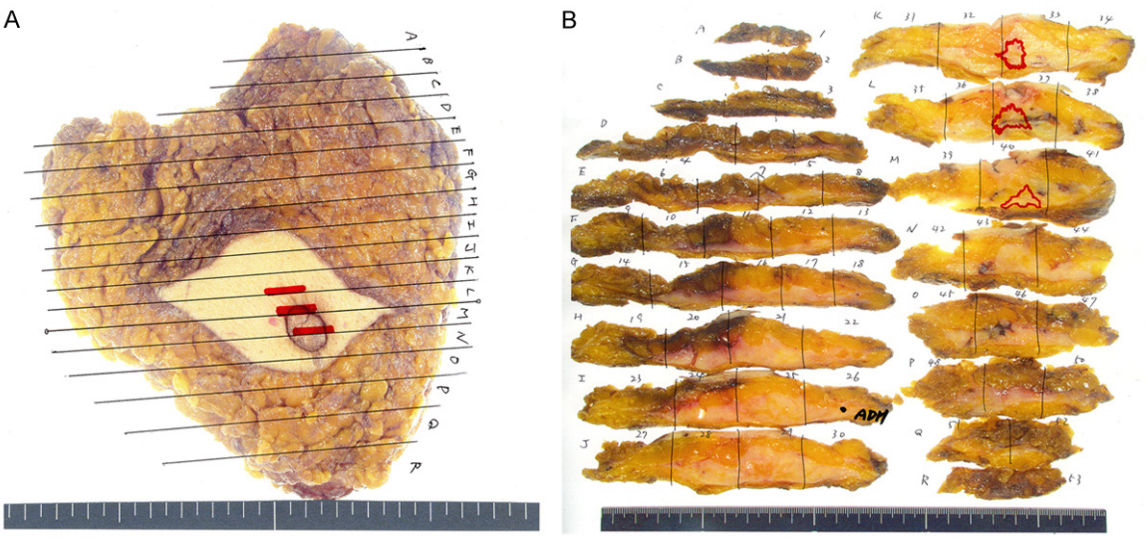

Figure 1. Stereo positioning and whole breast examination. The specimen was serially sectioned at 5 mm interval, in the ABC order (A); The breast tissue in each section was recorded by drawing guided by gross microscopic observation, and the areas circled by red color are breast cancer tissue (B).

tice, we have noted that breast cancer patients with MAC intend to present with smaller tumor size, infrequent lymphovascular invasion (LVI) and low rate of lymph node metastasis (LNM), suggesting that MAC may have impact on pathologic features and biologic behavior of breast cancer. However, the relationship has not yet been investigated. We retrospectively identified 452 cases of MAC in mastectomy specimens processed by whole breast examination for evaluation of invasive carcinoma in our department, and assessed their clinicopathological features and their associations with biologic behavior of breast cancer.

Materials and methods

Case selection and histological evaluation

All 2953 modified radical mastectomy (MRM) specimens from patients with invasive carcinoma performed between January 2011 and January 2012 at the Department of Breast Cancer Pathology and Research Laboratory, Tianjin Medical University Cancer Hospital, Tianjin, China, were reviewed and 452 cases of MAC were identified. All patients with MAC were female, with a mean age of 51 years (range 20 to 89 years). The specimens were evaluated by stereo positioning and whole breast examination (**Figure 1**). In brief description, the specimen was serially sectioned at 5 mm interval followed by 10% formalin fixation. After a diagram being drawn to document the gross features and the position of tumors, the specimen was entirely embedded in paraffin blocks. The specimen was then routinely processed for tissue sectioning and HE staining. The histologic diagnosis of breast cancer was made using the criteria described in 2012 World Health Organization histologic classification of tumors of the breast [7].

Pathological diagnosis of MAC

MAC was first identified on HE stained tissue sections followed by assessment using tissue sections with silver nitrate (Von Kossa) staining. It was graded into I to III, based up the severity of arterial calcification in the medial layers of arteries. Grade I was defined as arteries with muscle fiber fracture, hyaline degeneration and calcium salt deposition (**Figure 2**), grade II with arterial media calcification on a background of grade I lesion (**Figure 3**), and grade III with separation of the arterial media by calcification (**Figure 4**). The cases were first evaluated independently by 3 pathologists (LF, RL and XG) and consensus was reached by group discussion in all the cases with initial discordance.

Histological assessment of breast cancer and MAC

The following histologic changes were observed and recorded: (1) The patterns of MAC and can-

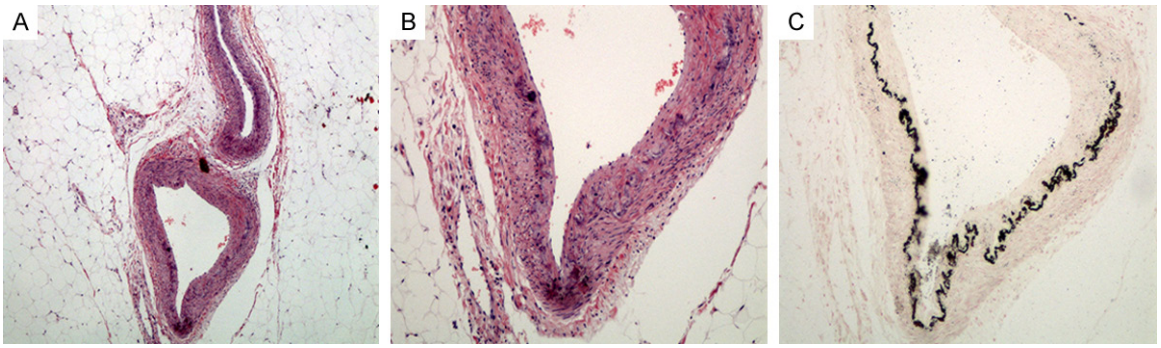

Figure 2. Pathological diagnosis and grading of MAC-Grade I. Hematoxylin and eosin stained tissue sections showing arteries with muscle fiber fracture, hyaline degeneration and calcium salt deposition, and MAC away from carcinoma nests (A. ×40 magnification; B. ×100 magnification), and silver nitrate (Von Kossa) stained section showing MAC grade I of the same case (C. ×100 magnification).

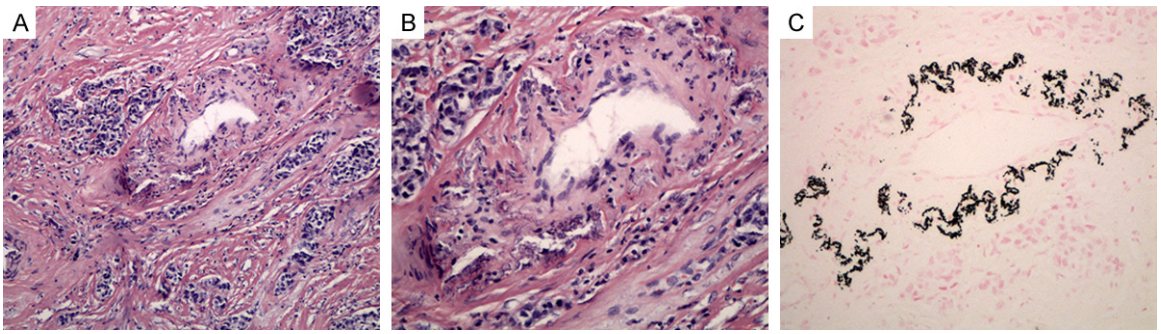

Figure 3. Pathological diagnosis and grading of MAC-Grade II. Hematoxylin and eosin stained tissue sections showing arterial media calcification on a background of grade I lesion, and MAC within carcinoma nests (A. ×40 magnification; B. ×100 magnification), and silver nitrate (Von Kossa) stained section showing MAC grade II of the same case (C. ×100 magnification).

cer geographic locations: MAC away from carcinoma nests (**Figure 2**), MAC within carcinoma nests (**Figure 3**), or MAC at the junction of carcinoma nests and stroma (**Figure 4**). (2) The ratio of tumor stroma and cancer cell nests.

Immunohistochemistry

Immunohistochemistry was performed using the avidin-biotin-immunoperoxidase technique for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki-67 and p53 in the formalin-fixed paraffin embedded representative tumor sections of each case. All primary antibodies were purchased from Abcam (Cambridge, MA, USA). Briefly, 4 μm tissue sections on coated slides were heated for antigen retrieval, pretreated with a 3% solution of hydrogen peroxide for 5-10 minutes, and incubated with 10% normal goat serum as the blocking agent. The sections were then incubated sequentially with the primary antibodies followed by a biotinylated secondary antibody and avidin-peroxidase conjugate (obtained from Santa Cruz Biotechnology). All steps were preceded by rinsing of sections with PBS (pH 7.6). The chromogen was 3,3'-diaminobenzidine (DAB). The sections were counterstained with hematoxylin, dehydrated and mounted.

The ER, PR and HER2 status was determined using the criteria of American Society of Clinical Oncology/College of American Pathologists [8, 9]. For ER and PR, nuclear staining in ≥1% of the tumor cells was considered positive. HER2 immunoreactivity was evaluated on a standardized scale from 0-3 based on the intensity of membranous staining and the proportion of tumor cells stained, with strong complete membranous staining in >10% of tumor cells (3+) was considered positive. Ki-67 and p53 immunoreaction presented with nuclear staining. Ki-67 labelling index was calculated and high

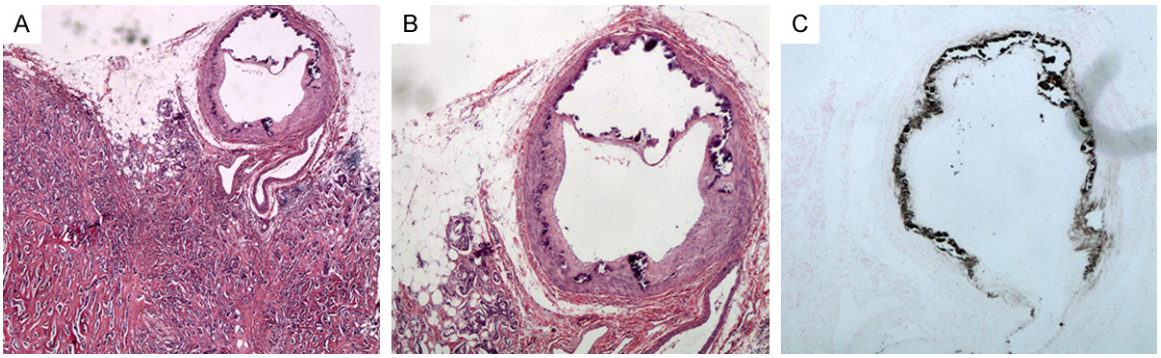

Figure 4. Pathological diagnosis and grading of MAC-Grade III. Hematoxylin and eosin stained tissue sections showing separation of the arterial media by calcification, and MAC at the junction of carcinoma nests and stroma (A. ×40 magnification; B. ×100 magnification), and silver nitrate (Von Kossa) stained section showing MAC grade III of the same case (C. ×100 magnification).

Table 1. Clinicopathological Characteristics of patients with MAC and without MAC

Characteristics	MAC group (n=452)	Non-MAC group (n=2501)	*P* value
Age (%, $\bar{x}\pm s$)	55.98±11.334	50.35±10.088	<0.001*
Menopausal status (%)			<0.001#
Premenopause	190 (42.0)	1413 (56.5)	
Postmenopause	262 (58.0)	1088 (43.5)	
Side (%)			0.378#
Left	215 (47.6)	1278 (51.1)	
Right	235 (52.0)	1213 (48.5)	
Both	2 (0.4)	10 (0.4)	
Pathologic types (%)			0.382#
IDC-NOS	368 (81.4)	2091 (83.6)	
IC-ST	49 (10.9)	220 (8.8)	
NIC/MIC	35 (7.5)	190 (7.6)	
T (%)			0.667#
0	14 (3.0)	108 (4.3)	
1	203 (44.9)	1048 (41.9)	
2	205 (45.4)	1165 (46.6)	
3	26 (5.8)	160 (6.4)	
4	4 (0.9)	20 (0.8)	
N (%)			0.227#
0	248 (54.8)	1450 (58.0)	
1	119 (26.3)	545 (21.8)	
2	45 (10.0)	273 (10.9)	
3	40 (8.9)	233 (9.3)	
LVI (%)			0.010#
Yes	47 (10.4)	375 (15.0)	
No	405 (89.6)	2126 (85.0)	
Pathological stage (%)			0.389#
0	16 (3.5)	128 (5.1)	
1	126 (28.0)	700 (28.0)	
2	219 (48.4)	1123 (44.9)	
3	91 (20.2)	550 (22.0)	

Histological grading (%)			0.083#
1 (Nottingham I)	37 (8.2)	210 (8.4)	
2 (Nottingham II)	345 (76.3)	1778 (71.1)	
3 (Nottingham III)	70 (15.5)	513 (20.5)	
ER (%)			0.324#
Positive	338 (74.8)	1811 (72.4)	
Negative	114 (25.2)	690 (27.6)	
PR (%)			1.000#
Positive	297 (65.8)	1646 (65.8)	
Negative	155 (34.2)	855 (34.2)	
HER2 (%)			0.391#
0	155 (34.3)	875 (35.0)	
1	135 (29.8)	673 (26.9)	
2	122 (27.1)	680 (27.2)	
3	40 (8.8)	273 (10.9)	
Molecular Classification			0.117#
Luminal A	250 (55.3)	1248 (49.9)	
Luminal B	98 (21.6)	608 (24.3)	
HER2 positive	63 (14.0)	348 (13.9)	
Triple negative	41 (9.0)	297 (12.0)	
Ki-67 (%)			0.002#
<14	150 (33.2)	648 (25.9)	
≥14	302 (66.8)	1853 (74.1)	
(x ± s)	27.66±22.64	32.01±23.45	<0.001*
P53 (%)			0.124#
<25	344 (76.1)	1786 (71.4)	
25-50	20 (4.4)	135 (5.4)	
>50	88 (19.5)	580 (23.2)	
Surgery (%)			0.435#
Modified radical	407 (90.0)	2286 (91.4)	
Others	45 (10.0)	215 (8.6)	
Chemotherapy (%)			0.847#
Yes	400 (88.5)	2236 (89.4)	
No	52 (11.5)	265 (10.6)	
Radiotherapy (%)			0.524#
Yes	107 (23.7)	625 (25.0)	
No	345 (76.3)	1876 (75.0)	
Endocrine therapy (%)			0.124#
Yes	353 (78.1)	1836 (73.4)	
No	99 (21.9)	665 (26.6)	
Targeted therapy (%)			0.958#
Yes	59 (13.1)	370 (14.8)	
No	393 (86.9)	2131 (85.2)	

*T test #χ^2 test. IDC-NST: Invasive carcinoma of no specific type; IC-ST: Invasive carcinoma, specific types; NIC: Non-invasive carcinoma; MIC: Microinvasive carcinoma; T: Primary Tumor; N: Regional lymph nodes; LVI: Lymphovascular invasion; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; MAC: Medial arterial calcification.

tumor proliferation was defined as a labelling index ≥14% [10]. Immunoreactivity for p53 was semi-quantitatively assessed bases upon the percentage of tumor nuclei stained. Molecular classification of tumor was performed using the established criterial [11].

Table 2. MAC grades and clinicopathological features of breast cancer

Characteristics	Grade of MAC			*P* value
	I (n=339)	II (n=69)	III (n=44)	
Age (age, x±s)	53.47±10.66	60.75±10.56	67.81±10.77	<0.001*
Menopausal status (%)				<0.001#
Premenopause	49.0	33.3	2.3	
Postmenopause	51.0	66.7	97.7	
LVI (%)				0.907#
Yes	10.3	10.1	11.4	
No	89.7	89.9	88.6	
T				0.164#
0	3.1	4.8	0.0	
1	46.7	36.5	45.2	
2	44.7	49.2	45.2	
3	4.8	7.9	9.5	
4	0.7	1.6	0.0	
Ki67 (%)				0.240#
<14	31.8	35.3	40.9	
≥14	68.2	64.7	59.1	
(x±s)	29.26±23.42	23.71±20.76	20.15±14.30	0.045*
p53 (%)				0.706#
<25	76.7	71.0	76.1	
25-50	4.5	4.3	4.5	
≥50	18.8	24.6	19.4	

*Pearson's correlation; #Spearman's correlation. T: Primary Tumor; LVI: Lymphovascular invasion; MAC: Medial arterial calcification.

Patient survival analysis

Patients were managed postoperatively by standard protocols, including chemotherapy, radiotherapy, anti-HER2 targeted therapy, and/or endocrine therapy. The entire group of patients was followed up regularly for 9-42 months (median follow up of 38 months). The follow-up information included the date of recurrence or metastasis and survival status. Patients were censored from the date of last follow-up visit or death from causes other than breast cancer, local or regional recurrences, or the development of a second primary carcinoma, including contralateral breast cancer. If a patient was confirmed metastasis during follow-up without recurrence, the last follow-up visit date was used. Age, time to first recurrence, and survival time were calculated based upon the data of primary tumor diagnosis.

Statistical analysis

The SPSS 18.0 software was used for statistical analyses. T test and χ^2 test were performed for group comparisons. Patient's disease free survival (DFS) and overall survival (OS) was analyzed. Correlations were studied by Pearson's correlation or the analysis of Variance. All statistical tests were two-sided, and a *P* value of <0.05 was considered significant.

Results

MAC incidence

Four hundred and fifty-two cases of MAC were identified in 2953 mastectomy specimens (15.3%) from patients with breast cancer, in which grade I, II and III of MAC were found in 339 cases (75.0%), 69 cases (15.3%), and 44 cases (9.7%) respectively.

Age and menopausal status of patients

In comparing with the non-MAC patients, MAC presented more frequently in older patients (56 versus 50; P<0.001), and in postmenopausal women (58.0% versus 43.5%; P<0.001) (**Table 1**). The grade of MAC was also positively correlated with the age of patients and the status of menopause, with severe lesions more common identified in older and in postmenopausal women (P<0.001; P<0.001) (**Table 2**).

Pathologic characteristics of MAC and the associated breast carcinomas

LVI was less frequently identified in MAC-associated carcinomas (10.4% versus 15.0%; P=0.01) and the tumors showed less proliferation measured by Ki-67 labelling index (average labelling 27.7% vs 32.0%, P<0.001; high labelling 66.8% vs 74.1%, P=0.002) in comparing to those without accompanying MAC (**Table 1**). The severity of MAC was negatively associated with the Ki-67 labelling index of breast cancer (P=0.045; **Table 2**). No significant difference was found between the MAC and control groups in tumor size, LNM, histologic types and grades,

Table 3. Age sub-stratification of patients with MAC and without MAC

Characteristics	<50 age			50-60 age			61-70 age			≥70 age		
	MAC	Non-MAC	*P*	MAC	Non-MAC	*P*	MAC	Non-MAC	*P*	MAC	Non-MAC	*P*
Side (%)			0.098			0.439			0.187			0.870
Left	65 (43.3)	634 (52.0)		56 (43.1)	420 (49.1)		60 (55.6)	178 (53.3)		34 (53.1)	46 (50.5)	
Right	85 (56.7)	582 (47.7)		73 (56.1)	431 (50.3)		47 (43.5)	156 (46.7)		30 (46.9)	45 (49.5)	
Both	0	4 (0.3)		1 (0.8)	5 (0.6)		1 (0.9)	0		0	0	
Pathologic types (%)			0.279			0.466			0.860			0.173
IDC-NOS	118 (79.7)	972 (84.4)		110 (85.9)	693 (83.6)		84 (82.3)	268 (83.5)		49 (76.6)	64 (74.4)	
IC-ST	17 (11.5)	91 (7.9)		12 (9.4)	72 (8.7)		11 (10.8)	29 (9.0)		8 (12.5)	18 (20.9)	
NIC/MIC	13 (8.8)	89 (7.7)		6 (4.7)	64 (7.7)		7 (6.9)	24 (7.5)		7 (10.9)	4 (4.7)	
T (%)			0.684			0.023			0.673			0.705
0	8 (6.7)	41 (4.4)		0	36 (5.0)		3 (3.0)	10 (3.3)		1 (1.6)	2 (2.5)	
1	48 (40)	409 (43.4)		60 (52.2)	287 (39.7)		46 (46.5)	130 (43.0)		24 (38.7)	32 (40.0)	
2	55 (45.8)	424 (45.0)		50 (43.5)	347 (48.0)		47 (47.5)	142 (47.0)		28 (45.2)	40 (50.0)	
3	9 (7.5)	63 (6.7)		4 (3.5)	47 (6.5)		3 (3.0)	16 (5.3)		7 (11.3)	4 (5.0)	
4	0	5 (0.5)		1 (0.9)	6 (0.8)		0	4 (1.3)		2 (3.2)	2 (2.5)	
N (%)			0.834			0.408			0.765			0.223
0	86 (57.3)	705 (58.0)		65 (50.0)	495 (57.8)		61 (56.5)	196 (58.7)		34 (55.7)	51 (56.0)	
1	37 (24.7)	273 (22.5)		34 (26.2)	184 (21.5)		27 (25.0)	68 (20.4)		20 (32.8)	20 (22.0)	
2	13 (8.7)	130 (10.7)		17 (13.1)	94 (11.0)		11 (10.2)	40 (12.0)		4 (6.6)	8 (8.8)	
3	14 (9.3)	107 (8.8)		14 (10.8)	83 (9.7)		9 (8.3)	30 (9.0)		3 (4.9)	12 (13.2)	
LVI (%)			0.333			0.158			0.548			0.700
Yes	132 (88.0)	1031 (84.5)		115 (88.5)	713 (83.3)		96 (88.9)	296 (88.6)		62 (96.9)	86 (94.5)	
No	18 (12.0)	189 (15.5)		15 (11.5)	143 (16.7)		12 (11.1)	38 (11.4)		2 (3.1)	5 (5.5)	
Pathological stage (%)			0.872			0.058			0.398			0.632
0	8 (6.5)	46 (4.9)		0	45 (6.2)		3 (3.1)	11 (3.7)		3 (4.8)	2 (2.5)	
1	35 (28.5)	272 (28.7)		31 (27.2)	187 (25.8)		30 (30.6)	95 (31.6)		15 (24.2)	22 (27.5)	
2	56 (45.5)	431 (45.5)		56 (49.1)	333 (45.9)		49 (50.0)	125 (41.5)		31 (50.0)	34 (42.5)	
3	24 (19.5)	199 (21.0)		27 (23.7)	161 (22.2)		16 (16.3)	70 (23.3)		13 (21.0)	22 (27.5)	
Histological grade (%)			0.320			0.202			0.110			0.953
1	6 (5.4)	83 (9.1)		7 (6.3)	52 (7.5)		13 (14.0)	23 (8.4)		4 (7.7)	5 (7.7)	
2	87 (78.4)	660 (72.5)		85 (76.6)	474 (68.3)		70 (75.3)	203 (73.8)		38 (73.1)	46 (70.8)	
3	18 (16.2)	167 (18.4)		19 (17.1)	168 (24.2)		10 (10.8)	49 (17.8)		10 (19.2)	14 (21.5)	
ER (%)			0.600			0.273			0.622			0.424
Positive	115 (79.3)	902 (77.0)		91 (70.5)	539 (65.5)		80 (74.1)	232 (71.2)		46 (74.2)	71 (80.7)	

Negative	30 (20.7)	270 (23.0)		38 (29.5)	284 (34.5)		28 (25.9)	94 (28.8)		16 (25.8)	17 (19.3)	
PR (%)			0.319			0.704			0.359			0.585
Positive	112 (77.2)	857 (73.2)		74 (57.4)	455 (55.4)		63 (58.3)	207 (63.7)		43 (69.4)	64 (73.6)	
Negative	33 (22.8)	313 (26.8)		55 (42.6)	367 (44.6)		45 (41.7)	118 (36.3)		19 (30.6)	23 (26.4)	
HER2 (%)			0.387			0.680			0.666			0.809
0	48 (37.2)	416 (37.4)		38 (29.9)	252 (32.4)		34 (32.4)	92 (30.0)		27 (45.0)	36 (42.4)	
1	41 (31.8)	287 (25.8)		30 (23.6)	202 (26.0)		38 (36.2)	99 (32.2)		15 (25.0)	27 (31.8)	
2	31 (24.0)	295 (26.6)		43 (33.9)	222 (28.6)		26 (24.8)	87 (28.3)		14 (23.3)	16 (18.8)	
3	9 (7.0)	113 (10.2)		16 (12.6)	101 (13.0)		7 (6.7)	29 (9.4)		4 (6.7)	6 (7.1)	
Molecular Classification			0.144			0.273			0.698			0.899
Lunimal A	81 (62.8)	589 (53.0)		57 (44.9)	339 (43.6)		58 (55.2)	153 (49.8)		37 (61.7)	56 (65.9)	
Lunimal B	26 (20.2)	286 (25.7)		35 (27.6)	180 (23.2)		20 (19.0)	73 (23.8)		10 (16.7)	14 (16.5)	
HER2 positive	14 (10.9)	122 (11.0)		24 (18.9)	143 (18.4)		13 (12.4)	43 (14.0)		8 (13.3)	8 (9.4)	
Triple negative	8 (6.2)	114 (10.3)		11 (8.7)	115 (14.8)		14 (13.3)	38 (12.4)		5 (8.3)	7 (8.2)	
Ki67 (%)			0.048			0.267			0.143			0.864
<14	50 (34.5)	310 (26.6)		36 (27.9)	189 (23.3)		38 (35.2)	88 (27.4)		24 (38.1)	31 (36.0)	
≥14	95 (65.5)	857 (73.4)		93 (72.1)	621 (76.7)		70 (64.8)	233 (72.6)		39 (61.9)	55 (64.0)	
p53 (%)			0.431			0.635			0.390			0.281
<25	113 (75.8)	826 (70.8)		95 (73.6)	571 (69.6)		87 (80.6)	243 (74.8)		46 (74.2)	73 (83.9)	
25-50	7 (4.7)	62 (5.3)		7 (5.4)	47 (5.7)		3 (2.8)	17 (5.2)		3 (4.8)	4 (4.6)	
≥50	29 (19.5)	279 (23.9)		27 (20.9)	202 (24.6)		18 (16.7)	65 (20.0)		13 (21.0)	10 (11.5)	

IDC-NST: Invasive carcinoma of no specific type; IC-ST: Invasive carcinoma, specific types; NIC: Non-invasive carcinoma; MIC: Microinvasive carcinoma; T: Primary Tumor; N: Regional lymph nodes; LVI: Lymphovascular invasion; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; MAC: Medial arterial calcification.

Table 4. Histological features of breast cancer in patients with MAC

Characteristics	Cases	%
Grade of MAC		
I	339	75.00
II	69	15.27
III	44	9.73
Location of MAC and cancer nests		
Within cancer nests	50	11.57
At edge of cancer nests	248	57.41
Away from cancer nests	134	31.02
Proportion of stroma and cancer		
Stroma>cancer nests	173	43.80
Stroma=cancer nests	149	37.72
Stroma<cancer nests	73	18.48

pathological stage, biomarker status (ER, PR and HER2), and p53 expression (**Table 1**).

When patients were stratified by age, high Ki-67 tumor labelling index in the MAC group was found significantly lower than that in the non-MAC group in patients younger than 50 years (65.5% vs 73.4%; P=0.048). In the age group between 50 to 60 years, the tumor size in MAC group was significantly smaller than that in non-MAC group (T1 tumor 52.2% vs 39.7%; P=0.023). No significant difference was found in other pathologic features (**Table 3**).

The MAC was most commonly located at the junction of carcinoma nests and fibrous stroma (57.4%) followed by away from carcinoma nests (31.0%), and it was least commonly found within the tumor nests (11.6%). Tumor stroma constituting >50% of the mass was identified in 43.8% of the cases, and only in 18.5% of the tumors carcinoma component was more than the volume of stroma (**Table 4**).

Clinical management and survival analysis

Patients with MAC and without MAC were postoperatively managed similarly according to the guidelines (P>0.05; **Table 1**). A trend was noted that patients in MAC group manifested a better DFS than that in the non-MAC group after 30 months of follow-up (**Figure 5A**).

Discussion

MAC was first described by the German pathologist, J. G. Mönckeberg, in 1903 [12], and therefore is also known as Mönckeberg's medial sclerosis. It primarily involves the muscular arteries for unknown reasons, and starts at middle age with increase in aggravation along the aging process. The pathological changes include muscle fiber fracture in arterial medium, glass-like degeneration, necrosis, and gradual disappearance of elastic tissue that is replaced by calcinosis, leading to hardening and flexion extension of the blood vessels [13]. MAC at early stage does not result in lumen stenosis or rupture, and is not clinically detectable. It is the traditional perception that MAC is usually not clinically significant, and its role in cancer progression is therefore completely ignored. To our knowledge, this is the first study on the significance of MAC in breast cancer patients.

By carefully setting the diagnostic criteria and grading system of MAC, we retrospectively reviewed 2953 mastectomy specimens with breast cancer by whole breast examination, and identified 452 cases with MAC (15.3%). The whole breast examination allowed thorough evaluation of the entire specimen and ensured the accuracy of the observations. The incidence of MAC in breast cancer patients is remarkable and deserves our appropriate attention.

MAC in this study was identified prevailingly in older and postmenopausal patients comparing with breast cancer patients without MAC (56 years vs 50 years, P<0.001). In addition, the severity of MAC was positively correlated with the age of patients and the status of menopause. Blumenthal reported that only 4% of patients aged 20-30 years had significant calcification, while this value increased to 98% in individuals above 50 years [14]. These are not surprising since MAC in general intends to link with aging, although so far there is no report in literature of the trend of MAC in breast cancer specimens. However, it is worthy of mentioning that breast cancer is also peaked in old patients, particularly those with indolent clinical courses.

What also identified were the significantly less LVI and lower Ki-67 labelling of carcinoma in the MAC group in contrast to those without MAC (P=0.01; P=0.002). When patients were sub-stratified based upon age, those younger than 50 years demonstrated a significant less portion of high Ki-67 labeling (>14%) of tumors in the MAC group than in the non-MAC group

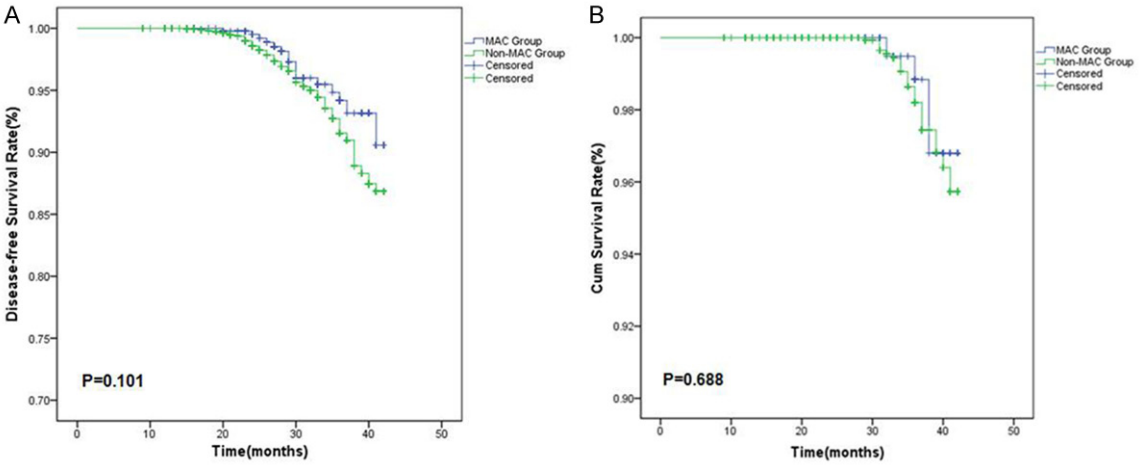

Figure 5. Patient's survival curves of MAC and non-MAC. A trend was noted that patients in MAC group manifested a better DFS than that in the non-MAC group after 30 months of follow-up (A), although no significant difference in DFS and OS (P=0.101, P=0.688; A and B) was identified between the groups of patients with MAC and non-MAC.

(P=0.048). In the group at ages between 50 to 60 years old, patients with MAC presented with significant smaller tumor size than that in non-MAC group (P=0.023). The results indicated MAC could slow down the tumor proliferation and migration. These inert behaviors are likely associated with the deficiency of nutritional supply in the tumor microenvironment that could result from the functional vessel damage in MAC. Studies have concluded that MAC is a degenerative vascular lesion with decreased permeability and elasticity [15-17]. This hypothesis was supported by the fact that majority of the MAC (57.4%) were located at the boundary of cancer cell nests and the tumor stroma. This geographic location provided the optimal opportunity for MAC to effectively limit the blood supply to tumors and to create the poor microenvironment for tumor growth. The hypo-oxygenic microenvironment may trigger fibrosis that could explain why over 80% of tumors with accompanying MAC in this cohort manifested significant fibrosis with tumor hypo-cellularity. In the disadvantageous microenvironment, tumor cell motility and growth may be limited, resulting in the inert biological behavior.

A trend was observed that patients with MAC demonstrated a better DFS than that in the non-MAC group after 30 months of follow-up. The finding requires further validation by studies with large cohort of patients and prolonged follow-up. Even though MAC may not serve as the driving factor for patient's prognosis, the reduced tumor proliferation and migration may offer additional window time to patients for clinical interventions that may result in more favorable outcomes.

In summary, this study identifies a significant incidence of MAC in mastectomy specimens with breast cancer, particularly in those of older and postmenopausal patients. MAC in breast seems able to create an adverse microenvironment to reduce tumor proliferation and migration. The inert tumor growth may provide a prolonged window time to patients for additional clinical intervention with the hope that would improve patient's outcome. MAC could become a considerable factor in postoperative adjuvant therapy and therefore an appropriate attention to its presence in breast cancer patients by clinician and pathologists is advisable. Further exploration of the impact of MAC on breast cancer patients in large scale studies is justified.

Acknowledgements

We thank the grant support from National Natural Science Foundation of China (Grant No. 81172531) to Xiaojing Guo, National Science & Technology Pillar Program of China (2015BAI12B15) and Anticancer Key Technologies R & D Program of Tianjin (12ZCDZSY16200) to Zhongsheng Tong.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaojing Guo, Department of Breast Cancer Pathology and

Research Lab, Tianjin Medical University Cancer Institute and Hospital, West Huanhu Road, Tianjin 300060, China. Tel: 86-22-23340123 (6119); Fax: 86-22-23340123 (6119); E-mail: guoxiaojing0728@126.com; Dr. Zhongsheng Tong, Department of Breast Oncology, Tianjin Medical University Cancer Institute and Hospital, West Huanhu Road, Tianjin 300060, China. Tel: 86-22-23340123 (2131); Fax: 86-22-23340123 (2131); E-mail: 18622221181@163.com

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