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Original Article

Is macroscopic tumor stiffness on strain elastography related to the axillary nodal status in T1 stage ductal invasive breast cancer?

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Abstract: This study was to investigate the association of tumor stiffness on strain elastography with axillary nodal status in T1 stage ductal invasive breast cancer, and to correlate the results with pathological findings. Conventional ultrasonography and strain elastography were performed in 102 T1 stage ductal invasive breast cancers (median diameter: 15.5 mm; range: 6.1-20 mm on US) of 102 women with clinically lymph node-negative breast cancer. The relationship between tumor elasticity score and axillary nodal status was assessed by using a logistic regression model. The collagen content of 95 tumors was measured by using a specific stain and image processing software. Of 102 patients, 21 (20.6%) had axillary nodal metastasis. On univariate analysis, the lymphovascular invasion, tumor grade, and tumor elasticity score were significantly associated with the axillary nodal status of T1 stage ductal invasive breast cancers. On multivariate analysis, lymphovascular invasion, tumor grade, and tumor elasticity score remained associated with the axillary nodal status. Tumors with an elasticity score of ≥4 had an odds ratio (OR) of 5.68 for axillary lymph node metastasis as compared to those with elasticity score of <4 (P=0.045). The tumor collagen content was not only correlated significantly with tumor elasticity score (P<0.001), but with lymph node metastases in T1 stage ductal invasive breast cancer (P=0.047). Tumor macroscopic stiffness on strain elastography is an independent predictor of axillary lymph node metastasis in T1 stage ductal invasive breast cancer without clinically axillary nodal metastasis.

Keywords: Strain elastography, ultrasound, breast cancer, axillary lymph node metastasis, collagen

Introduction

With the improvement of breast cancer screening techniques and the development of health consciousness, increasing malignant breast tumors are diagnosed at an early stage. However, some of them still have poor outcomes. Among prognostic factors of breast cancer (such as tumor size, histological grade, axillary nodal status, expression of hormonal receptors and human epidermal growth factor receptor 2 [HER2]) [1-3], axillary nodal status is the most powerful one and crucial for the breast cancer management [3-5].

The gold standard for evaluating axillary metastasis is the pathological findings of lymph nodes dissected. Axillary lymph node dissection (ALND) has been a standard step in the treatment of invasive breast cancer. However, some studies have shown the incidence of axillary nodal metastasis in early breast tumors is low (5-19% in T1a, b and 15-30% in T1c cancers) [6-9]. For most patients with early breast cancer, ALND may not exert therapeutic effect, but can increase the postoperative complications [7]. In recent years, the sentinel lymph node biopsy (SLNB) technique is increasingly used and gradually integrates into the guideline for the early breast cancer treatment. However, it is expensive and invasive and has a risk of stain allergy and false negative results. Thereby, noninvasive, accurate and feasible detection system of axillary metastasis is still needed to investigate the breast cancer, especially the small invasive, clinically lymph node-negative breast cancers.

Real-time elastography (RTE) is widely applied in routine clinical practice and can improve the differential diagnosis of breast masses according to mass stiffness. Recently, several studies have reported the relationship between tumor stiffness and axillary nodal metastasis, though the conclusions are conflicting. Chang et al [10] and Evans et al [11] found that the breast tumors' stiffness determined by shear-wave elastography (SWE) in women was associated with axillary nodal metastasis at univariate analysis. Multivariate analysis showed the mean stiffness at SWE was an independent predictor of axillary nodal metastasis in women with invasive cancer [12]. Therefore, the present study was to investigate the relationship between macroscopic stiffness of breast tumors and axillary nodal involvement in T1 stage ductal invasive, clinically lymph node-negative breast cancer.

Materials and methods

Patients

Between September 2013 and January 2015, 851 breast tumor women (920 lesions) were consecutively received ultrasonography in the Breast Surgery Department of the First Affiliated Hospital of Guangxi Medical University. Histopathology was obtained through sonographically guided core needle biopsy or surgery and 575 ductal invasive breast cancers were confirmed. Of these invasive tumors, 24% (138/575) had the diameter of less than 20 mm. Breast cancers with missing data on key variables-dissatisfactory or substandard strain elastographic images (n=5) and those without inadequate immunohistochemical results (n=4), or with deep involvement (n=6) were excluded from the present study. In addition, 4 patients were excluded because they received radiotherapy and chemotherapy due to contralateral breast cancer. In addition, 17 tumors were excluded, because lymph node metastasis was diagnosed by physical examination or ultrasonography. Finally, 102 women with T1 stage ductal invasive breast carcinoma were included for the final analysis and mastectomy or breast-conserving therapy was performed in all the patients. The mean age was 50.6 ± 11.3 years (age range: 27-83 years).

This retrospective study was approved by the Hospital Institutional Review Board and informed consent was obtained before study.

US examination

Conventional B-mode US and elastographic images were obtained by using an ultrasound machine (GE, Logiq e9, 6.0-15.0 MHz linear transducer, USA) and examinations were performed by one of 4 radiologists who had 8-25 years' experience in breast US and 3-5 years' experience in ultrasound elastography. During the ultrasonography, conventional images, Color Doppler images and elastographic images of breast tumors were stored and used for following analysis. The baseline information, such as the location, maximal diameter and posterior echoes of breast tumors was also recorded and analyzed.

Elastographic images were generated by using a manual freehand compression technique. The ultrasonic probe was oriented perpendicular to the chest wall, and paralleled to the pectoralis muscle. The region of interest (ROI) was determined and used for obtaining elasticity images: the top of ROI included the subcutaneous fat, the bottom of ROI included the pectoral muscle and the lateral borders of ROI were more than 5 mm away from the lesions [14].

According to the elasticity scoring system described by Itoh et al [14], each lesion was evaluated and scored. The scoring criteria were described in detail as follows: An elasticity score of 1, which represents a very soft tumor, indicates strain in the entire hypoechoic lesion (i.e., the entire lesion is evenly shaded in green); an elasticity score of 2, which represents a soft tumor, indicates strain in most of the hypoechoic lesions and no strain in several areas (i.e., hypoechoic lesions have a mosaic pattern of green and blue); an elasticity score of 3, which represents the intermediate soft tumor, indicates strain at the periphery of the hypoechoic lesion, sparing the center of the lesion (i.e., peripheral part of the lesion is green, and central part is blue); an elasticity score of 4, which represents the stiff tumor, indicates no strain in the entire hypoechoic lesion (i.e., the entire lesion is blue not including its surrounding area); an elasticity score of 5, which represents the very stiff tumor, indicates no strain in the

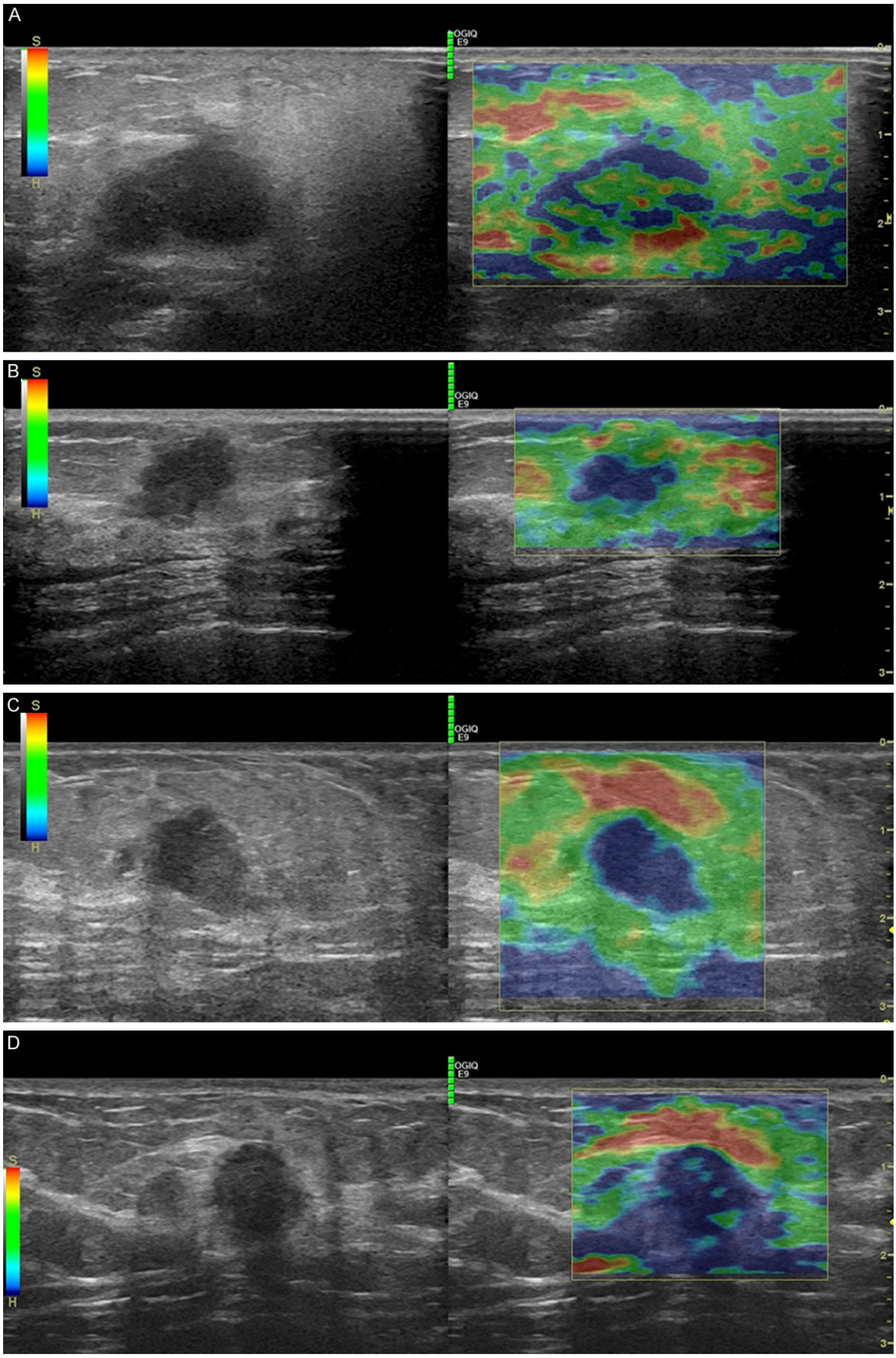
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Figure 1. A: Ultrasound elastography of a 17.6-mm-sized, grade 3 primary breast IDC in a 40-year-old woman without axillary nodal metastasis showed the hypoechoic lesions with a mosaic pattern of green and blue. The elasticity score was 2. B: Ultrasound elastography of an 11.1-mm-sized, grade 2 primary breast IDC in a 61-year-old woman without axillary nodal metastasis showed the green periphery and blue center. The elasticity score was 3. C: Ultrasound elastography of a 12.5-mm-sized, grade 2 primary breast IDC in a 41-year-old woman without axillary nodal metastasis showed the entire lesion was blue (non-including surrounding area). The elasticity score was 4. D: Ultrasound elastography of a 12.5-mm-sized, grade 2 primary breasts IDC in a 41-year-old woman with axillary nodal metastasis showed both the entire hypoechoic lesion and its surrounding area were blue. The elasticity score was 5.

entire hypoechoic lesion or the surrounding area (i.e., both entire hypoechoic lesion and its surrounding area are blue).

Histopathological evaluation

Breast-conserving surgery or mastectomy was performed for all the breast cancers. ALND was done for patients who refused to receive SLNB and those who underwent SLNB and had positive results. The histological type and grade of breast tumors, lymphovascular invasion, immunohistochemical indicators (ER, PR, HER2, P53, P16, Ki-67), and axillary nodal status were derived from the histopathological report. Histological type was determined according to the World Health Organization (WHO) histological classification of breast tumors [15]. Tumor histological grade was determined based on the modified Scarff-Bloom-Richardson (SBR) system [16]. Tumors with HER2 3+ and those with HER2 2+ and presence of gene amplification in fluorescence *in situ* hybridization were considered HER2 positive. The cutoff value of 10% was used to define ER, PR, P53 and P16 positive, and 14% to define Ki-67 positive [17]. According to the immunohistochemical findings, the breast tumors were also divided into four molecular subtypes: luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+, HER2+), HER2/neu (ER-, P-, HER2+) and Triple-negative (ER-, PR-, HER2-).

In addition, 102 pathological specimens of breast malignant tumors were processed for Masson's trichrome staining for the detection of collagen content. Seven tumor samples were excluded due to unsatisfactory staining. Finally, 95 tumors undergoing Masson's trichrome staining were used for analysis, including 20 with lymph node metastasis and 75 without lymph node metastasis. Above samples were obtained from the Department of Pathology of the First Affiliated Hospital of Guangxi Medical University. The collagens in tumors were quantified with the image processing software of Plus 6.0 (IPP 6.0) and the percentage of areas with collagen fibers was recorded.

Statistical analysis

All statistical analyses were performed using SPSS software version 17.0 (SPSS, Chicago, IL). Two-sample *t*-test, chi-squared test and Mann-Whitney rank test were used to investigate the relationships between imaging, clinical, pathological variables and axillary nodal involvement. Variables with significant correlation in univariate analysis were further included into a multivariate logistic regression model, and binary regression analysis was performed. The independent factors of axillary nodal metastasis were identified according to the odds ratio and 95% confidence interval (CI). A value of *P* less than 0.05 was considered statistically significant.

Results

The small breast invasive ductal carcinoma of 102 patients was clinically diagnosed at T1N0M0. Among them, 56.9% (58/102) were luminal A type, and the percentage of luminal B, Her2/neu and triple negative breast carcinoma (TNBC) was 12.7% (13/102), 9.8% (10/102) and 20.6% (21/102), respectively. A total of 20.6% (21/102) patients had axillary lymph node metastasis. The number of involved lymph nodes ranged from 1 to 11, and only five patients had ≥4 involved lymph nodes. The median size of tumor as measured by US was 15.5 mm (range, 6.1-20 mm). A total of 6, 28, 38 and 30 tumors had an elasticity score of 2, 3, 4 and 5, respectively (**Figure 1**). The median elasticity score was 3.9 (range, 2-5).

Results of univariate analysis of US image variables, clinical, pathological and axillary nodal statuses are shown in **Tables 1** and **2**. Tumor elasticity score (P=0.005), lymphovascular status (P<0.001), and histologic grade (P=0.028) were significantly correlated with the axillary

Table 1. Lymph node involvement in relation to clinical and ultrasonic variables

Variables	Number of patients	Node positive (%)	*P*-value
Age (mean ± SD; year)	50.6 ± 11.3	49.0 ± 14.1	0.487
<40	15	7 (46.7)	0.441
40-50	36	4 (11.1)	
50-60	32	5 (15.6)	
>60	19	5 (26.3)	
Menopausal status			
Pre	45	9 (20)	0.896
Post or peri	57	12 (20.1)	
Location			
Outer	66	14 (21.2)	0.855
Areola	7	2 (28.6)	
Inner	29	5 (17.2)	
Size (mm)	15.5 ± 3.5	16.4 ± 2.8	0.212
<10	10	1 (10)	0.236
10-15	30	5 (16.7)	
>15	62	15 (24.2)	
BI-RADS			
3	2	0 (0)	0.028
4a or 4b	21	2 (9.5)	
4c	13	1 (7.7)	
5	66	18 (27.3)	
Elasticity score (mean ± SD)	3.9 ± 0.9	4.4 ± 0.7	0.005
2	6	0 (0)	
3	28	2 (7.1)	
4	38	9 (23.7)	
5	30	10 (33.3)	
Elasticity score group			
<4	34	2 (5.9)	0.009
≥4	68	19 (27.9)	

nodal metastasis. A higher incidence of nodal metastasis was noted in patients with a higher elasticity score group/a lymphovascular invasion/a histologic grade of 3. However, the statuses of ER, PR, HER2, P53 and P16 had no significant correlation with axillary nodal status. In multivariate analysis, lymphovascular invasion was the most powerful predictor of nodal metastasis ($P<0.001$), elasticity score and histologic grade were independent predictors of nodal metastasis in small IDC ($P=0.026$) (**Table 3**). The small IDC with elasticity score of 4 or 5 had nearly 5.7 times (OR, 5.68) increment in the risk for lymphatic metastasis as compared to those with elasticity score of 3. The negative predictive value (NPV) of low tumor elasticity score (<4) was 94.1% for lymphatic metastasis.

Of 95 tumor samples undergoing Masson's trichrome staining, the mean collagen proportion in tumors with high elasticity score and low elasticity score was 30.3% (range, 11.4%-68.2%) (**Figure 2A** and **2B**) and 17.3% (range, 4.0%-40.3%) (**Figure 2C** and **2D**), respectively, showing a significant difference between them ($P<0.001$). Furthermore, the mean collagen proportion of patients with nodal metastasis was significantly higher than that of patients without lymph nodal involvement [(30.5.4 ± 12.7)% vs. (24.6 ± 13.2)%; $P=0.047$] (**Table 4**).

Discussion

Due to its convenience, non-invasiveness, good repeatability and no extra cost, RTE is widely applied in clinical settings. According to the 5-point score system, RTE is routinely used to distinguish malignant breast lesions from benign ones [14, 18, 19], although it is insensitive to the tumor depth and tumor volume. In the present study, all the elasticity images of breast tumors were scored by an experienced radiologist and the results are in high reliability.

Our results showed that the elasticity score as determined by RTE was an independent variable associated with axillary nodal metastasis in T1 stage, clinically lymph node-negative IDC. Malignant tumors are stiffer than normal tissues on a macroscopic scale [20, 21] mainly due to the desmoplastic reaction including increased collagen matrix deposition, stroma cell recruitment and activation, as well as the highly linearized and cross-linked tumor-associated collagens [22, 23]. This stiffer environment enhances the cell invasion and migration [24, 25]. Given this, to investigate the relationship between elasticity score and axillary nodal metastasis, Masson's trichrome staining was performed, and the collagen content was deter-

Table 2. Lymph node involvement in relation to histological variables

Variables	Number of patients	Node positive (%)	*P*-value
Histological grade			
1 or 2	77	12 (15.6)	0.028
3	25	9 (36)	
Lymphovascular invasion			
Presence	21	12 (57.1)	<0.001
Absence	81	9 (11.1)	
ER status			
Positive	64	10 (15.6)	0.108
Negative	38	11 (28.9)	
PR status			
Positive	60	11 (18.3)	0.501
Negative	42	10 (23.8)	
Her2 status			
Positive	23	6 (26.1)	0.468
Negative	79	15 (19.0)	
Ki-67 status			
Positive	67	17 (25.4)	0.098
Negative	35	4 (11.4)	
P53 status			
Positive	48	8 (16.7)	0.356
Negative	54	13 (24.1)	
P16 status			
Positive	52	7 (13.5)	0.069
Negative	50	14 (28)	
Subtype			
Luminal A	58	9 (15.5)	0.139
Luminal B	13	3 (23.1)	
Her2/neu	10	3 (30)	
Triple-negative	21	6 (28.6)	

Footnotes: ER: oestrogen receptor; PR: progesterone receptor.

Table 3. Multivariate logistic regression analysis

	Odds ratio	95% confidence interval	*P*-value
Lymphovascular invasion			
Yes	9.74	2.96-32.05	<0.001
No	1.00		
Elasticity score	2.56	1.153-5.663	0.021
≥4	5.68	1.04-31.12	0.045
<4	1.00		
Histological grade			
1 or 2	1.00	1.12-10.24	0.031
3	3.38		

mined in the present study. Results showed that breast tumors with a high elasticity score had significantly more collagen than those with a low elasticity score, and the elasticity score of breast cancer was closely related to the collagen content. Some studies on SWE and piston compression device in mouse breast cancer models showed a significant correlation between tumor internal stiffness or bulk modulus value and collagen content [26, 27]. However, no study has been conducted to investigate this relationship in human breast cancer.

The relationship between collagen content of breast cancers and lymph node metastasis is still unclear and few studies have been performed before. Epithelial tumor cells progress within collagen-dense microenvironments, and collagen density may promote the mammary tumor initiation, progression, and metastasis [28]. One study using a xenograft model of human breast tumor showed Col1 fiber density was significantly higher in the primary human breast cancer with nodal metastasis, in which Col1 fiber was detected by second harmonic generation (SHG) microscopy and Col1 fiber density was quantified using a SHG image analysis software [29]. Our findings from women with T1 stage IDC were in agreement with the findings of Kakkad et al [29].

Collagen content may influence the stiffness of breast cancer and regional lymph node metastasis, which partly explains why the stiffness of tumor measured by sonoelastography was independently associated with axillary nodal involvement in T1 stage IDC in our study.

This study had several limitations. First, all the breast cancers were IDC, without other histopathologic types of breast cancer included. Thus, the relationship between breast cancer of other pathological types and lymph node metastasis was not studied. Second, the number of tumors smaller than 10 mm in size was small, and thus the conclusion of other studies, in which tumor size was independently associated with

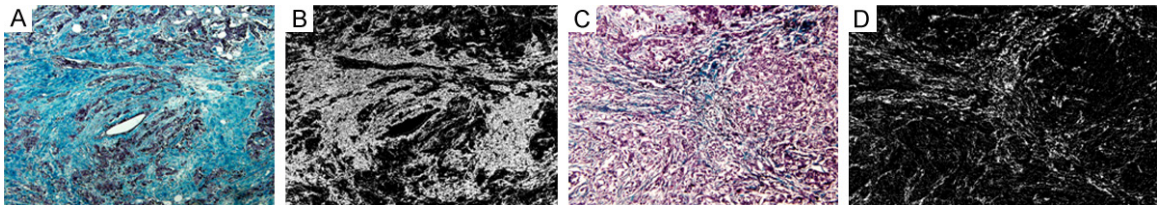

Figure 2. Masson's trichrome staining of breast tumors with elasticity scores of 3 (A) and 5 (C) showed collagens were blue-green. Values are the percentages of area occupied by collagens in each field. The color photos were converted to black-white ones using a image processing software, and the proportions of bright areas were measured as collagen content (B, D).

Table 4. Tumor collagen content in relation to lymph node status and elasticity score

Variables	Collagen content (mean ± SD)	*P*-value
Lymph node status		
Positive (n=17)	27.7 ± 11.9	0.015
Negative (n=17)	18.6 ± 8.4	
Elasticity score		
<4	21.1 ± 12.4	0.018
≥4	33.4 ± 14.9	

axillary nodal metastasis [7, 8, 30, 31], was not obtained in the present study Third, this study was to use elasticity scores of breast cancer to predict the axillary nodal metastasis, which may help avoid excessive surgical treatment in a part of small breast cancers. Hence, patients with positive nodal involvement as determined by clinical or imaging examinations were excluded before study, and only T1 stage, clinically lymph node-negative IDC was included for analysis. Fourth, although the association of RTE with axillary nodal involvement in T1 stage ductal invasive is statistically significant, the specificity is low and there was only 33.3% even for the score of 5. At last, due to the complex structure of breast cancer and the disorganized arrangement of collagen fibers, the findings from this ingle-centered study with a small sample size are still needed to be confirmed by future studies. In further work, studies with large sample size are required to validate the relationship between breast cancer stiffness and collagen content, as well as between collagen content and regional lymph node metastasis. At the same time, the ARFI or SWE may be added to further quantify the stiffness of breast cancer.

In conclusion, our results show an independent association between stiffness of T1 stage ductal invasive breast cancer and axillary nodal metastasis. The elasticity evaluation of breast cancer, which is easily obtained in clinical practice, is useful in the assessment and staging of small breast cancer, and may also join the predictive model of lymph node involvement in small breast cancers.

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Disclosure of conflict of interest

None.

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