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Original Article

Tumor size of breast invasive ductal cancer measured with contrast-enhanced ultrasound predicts regional lymph node metastasis and N stage

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Abstract: Purpose: This study aimed to determine the role of breast invasive ductal cancer (BIDC) size measured with Contrast-enhanced Ultrasound (CEUS) in the prediction of regional lymph node metastasis (LNM) and N stage. Methods: One hundred and six consecutive patients with breast lesions underwent ultrasound imaging within 2 weeks before mastectomy and axillary lymph node dissection. The largest transverse (width) and anteroposterior (depth) diameter were measured under CEUS by using calipers. The correlation between tumor size and regional LNM metastasis and N stage was evaluated. Results: Univariate analysis showed the diameters measured with CEUS were associated with lymph node metastasis ($P < 0.05$). The tumor size could distinguish grouped N stage (all $P < 0.05$). Tumor area (TA) might be an indicator that can differentiate No BIDC from N1-3 BIDC (cutoff = 5.37 cm^2), N0-1 BIDC from N2-3 BIDC (cutoff = 6.48 cm^2), and N0-2 BIDC from N3 BIDC (cutoff = 8.23 cm^2) with the sensitivity of 71%, 72% and 83%, respectively, and the specificity of 79%, 68% and 84%, respectively. Conclusions: The TA of BIDC measured with CEUS may be a predictor of regional LNM and N stage.

Keywords: Breast invasive ductal cancer, N stage, lymph node metastasis, contrast-enhanced ultrasound

Introduction

Breast invasive cancer is the most common malignancy in women, and long-term therapeutic efficacy of surgical intervention depends on accurate staging of breast cancer [1]. The prognosis of breast cancer women depends on tumor size and regional lymph node status. For patients who need surgical treatment, lymph node involvement can contribute to the clinical classification and determine the following treatment, and thus to predict the regional lymph node involvement is very important. Physical examination of the subaxillary lymph nodes is notoriously inaccurate. One study demonstrates that the false-positive rate is 53% in breast cancer women [2]. Breast ultrasound and mammography are the most commonly used diagnostic imaging modalities in the estimation of primary tumor size at diagnosis [3, 4].

Some studies have shown that sonography is a non-invasive tool for the detection of actual size of breast cancer with axillary LNM metastasis, and the size of primary tumor is an independent factor and has a significant relationship with lymph node metastasis [5-7]. However, there are no reports regarding the correlation of pri-

mary tumor size measuring by CEUS with the regional LNM and N stage. Other studies reveal that the breast tumor size is associated with the clinical outcome. Thus, we speculate that an increase in tumor size might correlate with the occurrence of regional LNM and worsen the N stage. This study was to prospectively assess the size of BICD measured with non-contrast US and CEUS, and to determine the role of tumor size in the prediction of regional LNM and N stage.

Materials and methods

Patients

Written informed consent was obtained from all the patients before study and this study was approved by the Ethics Committee of our institute.

From September 2011 to March 2014, a total of 190 consecutive patients with biopsy-confirmed breast cancer were recruited. Of them, 52 were treated with pre-operative neoadjuvant chemotherapy, 3 did not undergo breast resection due to contraindications, 27 were diagnosed with invasive lobular carcinoma and others, and 2 had images of poor quality. These patients were excluded from the study. In addition, 3 patients with contraindications to surgery had malignant hypertension and hyperthyroidism, and also excluded. Finally, 106 patients were included for analysis.

Of 106 patients, all were female and the mean age was 43.69±8.77 years (range: 21-62 years). The tumors located in the upper-outer quadrant of the breast in 58 patients, the upper-inner quadrant in 17, the lower-inner quadrant in 13 and the lower-outer quadrant in 15 and the center in 3 patients. All patients underwent pre-operative conventional ultrasound and contrast-enhanced ultrasound examinations.

Of 106 patients, 90 had solitary lesion and 16 had multiple lesions. When a patient had multiple lesions, only the largest or the most suspicious lesion was evaluated.

After conventional US examination of the breast, the enrolled patients underwent preoperative contrast-enhanced ultrasound examinations. Subsequently, they were scheduled for modified radical breast resection or mastectomy with complete axillary lymph node dissection at the ipsilateral axillary. The interval between contrast-enhanced ultrasound and surgery ranged from 2 to 10 days (mean: 4 days). According to the postoperative histopathology and American Joint Committee on Cancer (AJCC) criteria [8], well, moderately and poorly differentiated cancer was confirmed in 33, 60 and 13 patients, respectively; and breast cancer at T1, T2 and T3 stage was found in 18, 69 and 19 patients, respectively. The number of resected LNs per patient ranged from 6 to 25 with a mean of 15. According to the number of resected metastatic LNs, the N stage was clinically determined on the basis of AJCC criteria [8]. When the number of postoperative metastatic LNs is 0, 1 to 3, 4 to 9 and ≥ 10, the N stage is confirmed as N0, N1, N2 and N3, respectively.

Conventional and contrast-enhanced ultrasonography

All the ultrasound examinations were performed by one experienced sonographer who has 9-year experience in ultrasound examination. The breast was initially examined with conventional B-mode ultrasonography to identify the lesion. Ultrasound imaging was obtained by using a Siemens-Acuson S2000 scanner (Siemens Medical Solutions, CA, USA) with a 9L4 7-14MHz linear array transducer. The probe offers a lateral resolution of 0.35 mm and an axial resolution of 10.25 mm.

On non-contrast ultrasonography, the maximum imaging plane of the mass, which included the mass and its surrounding normal tissues if possible, was selected for CEUS. Contrast pulse sequence (CPS) imaging mode was used to evaluate the tumor perfusion and detection was done as follows: the mechanical index was between 0.06 and 0.08, the dynamic range was 78 dB, the depth of imaging was 3 cm or 4 cm, a single focus was placed at the bottom of the image, the probe was stabilized manually, and no pressure was exerted to avoid weakening the contrast-enhanced signals. These procedures were adjusted at the beginning and maintained constantly during the experiments. The contrast agent was SonoVue (Bracco SpA, Milan, Italy), which is a lyophilized powder of phospholipid-stabilized micro-bubbles contain-

Table 1. Univariate analysis of clinical characteristics, histopathological findings and tumor size for predicting regional lymph node metastasis

	Positive LNs (n = 61)	Negative LNs (n = 45)
Age (mean, years)	36.42±6.57	49.64±6.45
Anatomical distribution		
Upper-outer quadrant	38	20
Lower-outer quadrant	9	7
Upper-inner quadrant	6	13
Lower-inner quadrant	8	5
Differentiation		
Well	14	19
Moderate	35	25
Poor	12	1
T-stage		
T1	6	12
T2	36	33
T3	19	0
Tumor width (CEUS)		
< 2.7	18	36
≥ 2.7	43	9
Tumor depth (CEUS)		
< 1.95	21	34
≥ 1.95	40	11
Tumor area		
< 5.37	18	27
≥ 5.37	43	18

ing sulfur hexafluoride gas with a mean diameter of 2.5 μm; the solution was reconstituted by addition of 5 mL of sterilized saline. After a bolus injection of 4.8 mL of SonoVue manually via a 20-gauge cannula placed in the antecubital vein, administration of contrast medium was performed, followed by flushing with 5 ml of saline. The selected plane remained unchanged during the examination, and the real-time imaging was recorded for up to 90 s. All the static and dynamic images were digitally stored on the drive of US systems in digital imaging and communication in medicine (DICOM) format.

Tumor size measurement and analysis

The sonographer measured the lesions in centimeters, including the echogenic rim around the lesion, when present. The probe was vertical to the skin, multi-faceted multi-angle scanning was done, and weak echo was obtained to determine the maximum tumor diameter section for measurement of diameter. For measurements, the tumor edge was defined as the end of the hypoechoic mass before the wide border denoting the transition between the tumor and the surrounding normal tissues [9]. The greatest transverse (width) and anteroposterior (depth) dimensions of the tumor were measured by CEUS using calipers, the tumor size was measured from the edge of the area in the enhanced range [10]. The tumor area (TA) was determined using the greatest dimensions in two orthogonal planes [11]. In order to reduce measurement bias, the tumor size was measured two times in every patient to test the intra-observer variability of tumor length, width and depth. All the images were preoperatively analyzed and assessed by two sonographers.

Statistical analysis

Univariate analysis was performed to determine whether tumor size and clinicopathological factors could predict LNM. Tumor size was compared among patients with breast cancer of N stages using non-parametric Mann-Whitney test and Bonferroni correction for multicomparisons, and 95% confidence interval was calculated. If significant difference was observed in Mann-Whitney tests, the cut-off values of tumor dimensions were determined with receiver-operating characteristic (ROC) analysis for predicting N stages. Statistical analysis was carried out with SPSS version 19.0 (SPSS, Chicago IL, USA). A value of *P* less than 0.05 was considered statistically significant.

Results

Intra-observer variability of tumor size measurement

The intraobserver variability of tumor size measurement was small, and thus the mean tumor size from two measurements was used as the final tumor size.

Table 2. Tumor size of BIDC in patients stratified by N stages

	AREA (cm^2)	Tumor width (cm)	Tumor depth (cm)
N0 (45)	3.93±1.77	22.07±5.71	16.96±4.19
N1 (43)	6.66±2.28	28.95±4.73	22.59±5.65
N2 (6)	5.86±2.05	28.83±8.38	20.17±3.56
N3 (12)	13.18±5.07	44.20±7.41	29.58±9.38

Table 3. The *P* values for statistical comparisons of size of BIDC among N stages

N stages	AREA	Tumor Width	Tumor Depth
N0 vs. N1-3	< 0.0001[a,b]	< 0.0001[a,b]	< 0.0001[a,b]
N1 vs. N2	0.511	0.760	0.258
N1 vs. N3	< 0.0001[a,b]	< 0.017[a,b]	< 0.0001[a,b]
N2 vs. N3	0.001[a,b]	0.041	0.005
N0-1 vs. N2-3	< 0.0001[a,b]	0.007	< 0.0001[a,b]
N0-2 vs. N3	< 0.0001[a,b]	0.001	< 0.0001[a,b]

Note: Statistical analysis was done with Mann-Whitney test. [a]$P < 0.05$. [b]$P < 0.05$ after Bonferroni correction.

Univariate analysis of primary tumor size in CEUS and possible clinicopathological factors for predicting LNM

The median of the area, width and depth in CEUS were 5.37 cm^2, 2.35 cm and 1.95 cm, respectively. In addition, 57.5% of patients (61/106) had positive LNs, whereas 42.5% (45/106) patients had not. Univariate analysis showed the potential factors able to predict LNM included the age, anatomical distribution, differentiation, T stage and primary tumor size and are shown in **Table 1**.

According to the univariate analysis, the age, anatomical distribution, differentiation, T stage, tumor width, tumor depth and AREA of primary tumor could predict LNM (all $P < 0.01$). LNM was more likely to be present in patients with tumor AREA of ≥ 5.37 cm^2 (vs. < 5.37 cm^2), tumor width of ≥ 2.35 cm (vs. < 2.35 cm) and tumor depth of ≥ 1.95 cm (< 1.95 cm).

Multivariate analysis of primary tumor size on non-contrast US and clinicopathological factors for predicting regional LNM

Although tumor width, depth and width could reflect the primary tumor size, the area might be a better parameter because area was calculated based on the greatest dimensions of two sections.

Tumor size corresponding to N stage

In this cohort, LNM was found in 57.5% (61/106) of patients, and 42.5% (45/106) had no LNM. According to the AJCC criteria, N0 stage was found in 45, N1 stage in 43, N2 stage in 6 and N3 stage in 12. The tumor size of BIDC at different N stages is shown in **Table 2**. Tumor size could distinguish N0 BIDC from N1-3, N0-1 and N2-3 BIDC and N0-2 BIDC from N3 BIDC on the basis of tumor area, depth and width. In addition, there were also significant differences in the tumor area, depth and width between N1 BIDC and N3 BIDC as well as between N2 BIDC and N3 BIDC, whereas there was no difference between N1 BIDC and N2 BIDC (**Table 3**).

ROC analysis of tumor size of BIDC for detection of grouped N stages

There was overlap in the tumor width between N0 BIDC and N1-3 BIDC, and between N0-2 BIDC and N3 BIDC compared with tumor area and depth. ROC analysis (**Table 4**) showed AREA has the highest performance when compared with tumor width and depth, having a relatively larger area under the curve (AUC), and higher sensitivity and specificity for the differentiation of N0 BIDC from N1-3 BIDC. For the differentiation of N0 BIDC from N1-3 BIDC with AREA, an AUC of 0.84 had a sensitivity of 71% and specificity of 79% when the AREA was 5.37 cm^2 or larger.

Discussion

LNM is one of the most common patterns of BIDC spread. Accurate clinical staging is significant for the scientific and rational treatment, prognosis and comparisons of therapeutic efficacy among different treatment groups. Currently, the staging of breast cancer is based primarily on the AJCC TNM staging system [8]. As demonstrated in AJCC, the N stage of the tumor is clinically determined according to the number of metastatic LNs. When the number is 0, 1-3, 4-9 and ≥ 10, the cancer may be classified as N0, N1, N2 and N3, respectively [8]. Axillary lymph node status is an important independent factor affecting the prognosis of breast cancer patients, and may guide the adjuvant therapy of breast cancer [12]. However, the axillary lymph node may not be identified by

Table 4. Receiver-operating characteristic analysis (ROC) of BIDC tumor size associated with N stages

Cut-off value	Different N stages	AUC	Sensitivity (%)	Specificity (%)
AREA (cm^2)				
5.37	N0 vs. N1-3	0.840	71	79
6.48	N0-1 vs. N2-3	0.811	72	68
8.23	N0-2 vs. N3	0.823	83	84
Tumor width (cm)				
2.47	N0 vs. N1-3	0.836	82	72
3.12	N0-1 vs. N2-3	0.850	78	73
3.40	N0-2 vs. N3	0.956	92	90
Tumor depth (cm)				
1.92	N0 vs. N1-3	0.77	69	73
2.18	N0-1 vs. N2-3	0.702	67	63
2.42	N0-2 vs. N3	0.792	75	76

ultrasound before surgery if they are small. Thus, we speculate if the size of primary BIDC may predict the LNM and N stage, aiming to improve the diagnostic accuracy. Previous studies have confirmed that the T stage and degree of differentiation correlated with LNM [5, 12]. However, there are no reports regarding the role of tumor size of BIDC measured by CEUS in the prediction of regional LNM and N stage. In this study, our results showed that the tumor area, width and depth of the primary tumor measured by CEUS could predict regional LNM as well as N stage of BIDC.

According to the "Size-Note" theory, both the tumor size and the number of positive lymph nodes independently contribute to the lethality of invasive breast cancer [13]. There is lower risk for axillary lymph node metastasis when the breast tumor is small (diameter < 2 cm), and the tumor size is an independent predictor of nodal positivity [14]. Thus, we postulate that the tumor size seems to predict the survival by influencing regional LNM because the larger the tumor size, the larger the area of adjacent tissues invaded by the cancer is, and the higher risk for LNM is. CEUS plays an important role in the evaluation of vascularity of breast cancer, and its sensitivity and specificity are higher than those of conventional ultrasound [15]. Some studies have indicated that the pathologic findings corresponding to the region of size increased at CEUS are malignant in most malignant lesions [14, 16]. For the breast cancer with poorly defined margins (i.e., ill-defined, spiculated, hyperechoic halo, microlobulated or angulated), it is necessary to perform CEUS examination to assess the extent of cancer invasion.

Previous studies have shown that the tumor length is one of risk factors for LNM in patients with esophageal cancer, and the presence of LNM is an important indicator for the staging and determination of appropriate therapeutic strategies [17, 18]. There was study using the tumor volume to predict the LNM for the volume is more comprehensive [19]. However, we can only measure the greatest longitudinal depth and transverse width without moving in CEUS [10]. Thus, the area was employed as a factor to predict LNM.

Because significant differences were observed in the tumor width, depth and TA in BIDC at different N stages, tumor size measurements could be used as factors to differentiate BIDC at N stages. The sensitivity and specificity of higher than 70% were achieved when the TA cut-off value was 5.37 cm^2 for differentiating N0 BIDC from N1-3 BIDC, 6.48 cm^2 for differentiating N0-1 BIDC from N2-3 BIDC and 8.23 cm^2 for differentiating N0-2 BIDC from N3 BIDC based on the ROC analysis. However, TA had a higher AUC and specificity when compared with the tumor width and depth in the differentiation of BIDC at different N stages. This may be explained that TA takes both the tumor width and depth into consideration.

The TA of BIDC was able to predict the N stage before therapy, which could affect the clinical decision making and the prognosis. We speculate that the N stages predicted by TA may also affect the clinical decision making and the prognosis in some ways similar to the stages determined by AJCC criteria. We will perform further studies to determine whether the N stages predicted by TA are reliable for clinical decision making.

There were several limitations in this study. Firstly, the accurate largest section was not obtained from all the patients, and we could not measure all bigger lesions one-time with

CEUS examination. Secondly, the small size of our study was still small. However, our findings still revealed that the tumor size measurements determined by CEUS were helpful to predict the regional LNM and N stages. Further studies are required to detect the three-dimensional size [20] to confirm our findings.

In summary, TA of BIDC measured by CEUS may be an important risk factor of LNM. The cut-off values of TA at 5.37 cm^2, 6.48 cm^2 and 8.23 cm^2 may be used to discriminate N0 BIDC from N1-3 BIDC, N0-1 BIDC from N2-3 BIDC, and N0-2 BIDC from N3 BIDC, respectively. This study may provide evidence for the prediction of regional LNM, which is helpful for the rational selection of therapeutic strategy.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[2] Lanng C, Hoffmann J, Galatius H and Engel U. Assessment of clinical palpation of the axilla as a criterion for performing the sentinel node procedure in breast cancer. Eur J Surg Oncol 2007; 33: 281-284.

[3] Bosch AM, Kessels AG, Beets GL, Rupa JD, Koster D, van Engelshoven JM and von Meyenfeldt MF. Preoperative estimation of the pathological breast tumour size by physical examination, mammography and ultrasound: a prospective study on 105 invasive tumours. Eur J Radiol 2003; 48: 285-292.

[4] Kald BA, Boiesen P, Ronnow K, Jonsson PE and Bisgaard T. Preoperative assessment of small tumours in women with breast cancer. Scand J Surg 2005; 94: 15-20.

[5] Gajdos C, Tartter PI and Bleiweiss IJ. Lymphatic invasion, tumor size, and age are independent predictors of axillary lymph node metastases in women with T1 breast cancers. Ann Surg 1999; 230: 692-696.

[6] Westenend PJ, Meurs CJ and Damhuis RA. Tumour size and vascular invasion predict distant metastasis in stage I breast cancer. Grade distinguishes early and late metastasis. J Clin Pathol 2005; 58: 196-201.

[7] Herrada J, Iyer RB, Atkinson EN, Sneige N, Buzdar AU and Hortobagyi GN. Relative value of physical examination, mammography, and breast sonography in evaluating the size of the primary tumor and regional lymph node metastases in women receiving neoadjuvant chemotherapy for locally advanced breast carcinoma. Clin Cancer Res 1997; 3: 1565-1569.

[8] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL and Trotti A. AJCC cancer staging manual. New York: Springer; 2010.

[9] Shoma A, Moutamed A, Ameen M and Abdelwahab A. Ultrasound for accurate measurement of invasive breast cancer tumor size. Breast J 2006; 12: 252-256.

[10] Wang JW, Zheng W, Liu JB, Chen Y, Cao LH, Luo RZ, Li AH and Zhou JH. Assessment of early tumor response to cytotoxic chemotherapy with dynamic contrast-enhanced ultrasound in human breast cancer xenografts. PLoS One 2013; 8: e58274.

[11] Ryu EB, Chang JM, Seo M, Kim SA, Lim JH and Moon WK. Tumour volume doubling time of molecular breast cancer subtypes assessed by serial breast ultrasound. Eur Radiol 2014; 24: 2227-2235.

[12] Martinez-Ramos D, Escrig-Sos J, Alcalde-Sanchez M, Torrella-Ramos A and Salvador-Sanchis JL. Disease-free survival and prognostic significance of metastatic lymph node ratio in T1-T2 N positive breast cancer patients. A population registry-based study in a European country. World J Surg 2009; 33: 1659-1664.

[13] Garami Z, Benko K, Kosa C, Fulop B and Lukacs G. [The effect of mammographic screening on tumor size, axillary node status and the degree of histologic anaplasia]. Magy Seb 2006; 59: 383-387.

[14] Aitken E and Osman M. Factors affecting nodal status in invasive breast cancer: a retrospective analysis of 623 patients. Breast J 2010; 16: 271-278.

[15] Ricci P, Cantisani V, Ballesio L, Pagliara E, Sallusti E, Drudi FM, Trippa F, Calascibetta F, Erturk SM, Modesti M and Passariello R. Benign and malignant breast lesions: efficacy of real time contrast-enhanced ultrasound vs. mag-

netic resonance imaging. Ultraschall Med 2007; 28: 57-62.
[16] Jiang YX, Liu H, Liu JB, Zhu QL, Sun Q and Chang XY. Breast tumor size assessment: comparison of conventional ultrasound and contrast-enhanced ultrasound. Ultrasound Med Biol 2007; 33: 1873-1881.
[17] Eloubeidi MA, Desmond R, Arguedas MR, Reed CE and Wilcox CM. Prognostic factors for the survival of patients with esophageal carcinoma in the U.S.: the importance of tumor length and lymph node status. Cancer 2002; 95: 1434-1443.
[18] Griffiths EA, Brummell Z, Gorthi G, Pritchard SA and Welch IM. Tumor length as a prognostic factor in esophageal malignancy: univariate and multivariate survival analyses. J Surg Oncol 2006; 93: 258-267.
[19] Kimura Y, Sumi M, Ichikawa Y, Kawai Y and Nakamura T. Volumetric MR imaging of oral, maxillary sinus, oropharyngeal, and hypopharyngeal cancers: correlation between tumor volume and lymph node metastasis. AJNR Am J Neuroradiol 2005; 26: 2384-2389.
[20] Kuriakose MA, Loree TR, Hicks WL, Welch JJ, Wang H and DeLacure MD. Tumour volume estimated by computed tomography as a predictive factor in carcinoma of the tongue. Br J Oral Maxillofac Surg 2000; 38: 460-465.