Diagnostic and prognostic values of contrast-enhanced ultrasound combined with diffusion-weighted magnetic resonance imaging in different subtypes of breast cancer

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Abstract. The present study aimed to investigate the diagnostic and prognostic values of contrast-enhanced ultrasound (CEUS) combined with diffusion-weighted magnetic resonance imaging (DW-MRI) in different subtypes of breast cancer (BC). CEUS and DW-MRI were conducted in 232 patients with BC prior to surgical treatment. Patients were categorized as having the luminal A subtype, the luminal B subtype, triple-negative subtype or the human epidermal growth factor receptor 2 (Her-2)-positive subtype according to their expression of the estrogen receptor (ER), progesterone receptor (PR) and Her-2, as detected by immunohistochemistry. The CEUS and DW-MRI parameters of patients with different subtypes of BC were obtained and analyzed. The risk factors for the prognosis of patients with different subtypes of BC were analyzed using Kaplan-Meier and COX regression analyses. The diagnostic accuracy rate of CEUS combined with DW-MRI (93.10%) was higher than that of CEUS (88.79%) or DW-MRI (82.33%) alone. The local recurrence rate and distant metastasis rate of the Her-2-positive subtype were the highest among all the subtypes. Furthermore, patients with Her-2-positive BC exhibited a higher proportion of lesions with indistinct margins and histological grade III. Lymph node metastasis and BC subtype were independent risk factors for the prognosis of BC. The overall survival and disease-free survival of patients with the luminal A subtype were higher than those of patients with the Her-2-positive subtype. The results of the current study therefore indicate that CEUS combined with DW-MRI is more effective at diagnosing the different subtypes of BC than either CEUS or DW-MRI alone.

Introduction

Breast cancer (BC), the second most common cause of cancer-associated mortality in women worldwide, is a heterogeneous disorder accompanied by systemic symptoms, including hot flushes, insomnia and mood changes, as well as local symptoms, including urogenital atrophy and vaginal dryness (1,2). According to a World Health Organization report published in 2015, the incidence of BC is 19.4 per 100,000 women in East Africa and 89.7 per 100,000 women in Western Europe (3). In China, >1.6 million people are diagnosed with BC each year, and the mortality rate of BC is 1.2 million (4). The mortality rate of BC differs among Asian countries; the mortality rate of BC is decreasing in Hong Kong and Singapore, but is increasing in Taiwan, Japan and Korea (5).

The heterogeneity of cancer is regarded as a major obstacle inhibiting the development of effective treatment strategies, as unique diagnostic, prognostic and therapeutic techniques are required to treat the same type of cancer in different patients (6). Human breast tumors are diverse and may respond to the same treatment in different ways (7). Based on the cluster analysis of 465 genes, Zavyalova et al (8) classified BC into the following subtypes: Luminal A, luminal B, human epidermal growth factor receptor 2 (Her-2)/neu positive and triple negative. As each subtype of BC has its own specific clinical features in terms of recurrence pattern and prognosis, different treatments are required for each subtype (9).

Contrast-enhanced ultrasound (CEUS) is considered to be an advancement of traditional grayscale ultrasound imaging, which is one of the most frequently used modalities in clinical imaging (10). Due to the rapid development of medical imaging, CEUS has been used widely in China since 2004 and is currently used to diagnose patients with BC (11,12). CEUS is able to characterize mass lesions, stage invasive cancer, evaluate tumor perfusion in real time with minimal invasiveness, detect tumor recurrence and predict the tumor response to neoadjuvant chemotherapies in BC (13-15). Diffusion-weighted imaging (DWI) is a sensitive but nonspecific modality...
able to detect locoregional or metastatic BC disease (16). Diffusion-weighted magnetic resonance imaging (DW-MRI) is able to differentiate between benign and malignant focal hepatic lesions (17). Additionally, it has been determined that DW-MRI is a cancer imaging biomarker (18). Therefore, the present study evaluated the effectiveness of CEUS combined with DW-MRI in the diagnosis and treatment of BC.

Patients and methods

Patients. A total of 232 patients pathologically diagnosed with invasive BC and treated at the China-Japan Union Hospital of Jilin University (Jilin, China) between January 2011 and January 2013, were recruited in the present study. All patients were female with a mean age of 44.3±7.2 years and underwent CEUS and DW-MRI prior to surgery. The present study was performed with the approval of the Clinical Ethics Committee of China-Japan Union Hospital of Jilin University. Written informed consent was provided by all patients included in the current study.

The inclusion criteria of patients was as follows: i) Pathological diagnosis with BC at China-Japan Union Hospital of Jilin University, with complete clinical information; ii) no history of breast implants, mastitis, chemoradiotherapy or any breast-associated surgery and no identification of pregnancy or lactation; iii) standard treatment, apart from endocrine therapy, received within 6 months according to the standards of National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology (19); and iv) willingness to participate in the current study and the signing of written informed consent. The exclusion criteria were as follows: i) No pathological diagnosis of BC; ii) an interval of >1 month between CEUS or DW-MRI and surgery; iii) recurrence or distant metastasis following treatment; iv) diagnosis of mental illness; and v) non-completion of the questionnaire regarding patient quality of life. Included patients consisted of those with different subtypes of BC (7): Luminal A (n=59), luminal B (n=79), Her-2 positive (n=48) and triple-negative (n=38).

Immunohistochemistry (IHC). IHC was performed to detect the expression of estrogen receptor (ER) and progesterone receptor (PR) in BC tissues taken from patients included in the current study. The expression of Her-2 was measured based on the expression of estrogen receptor (ER) and progesterone receptor (PR). Tissues were sent to the pathology department ≤30 min following collection. Subsequently, tissues were scored as ER- and PR-positive if >10% of tumor cells exhibited intensive ER or PR staining in the nucleus (6).

Histological grading. According to the graded standards of diagnosis and treatment of common malignant tumors in China, BC tissues were histologically graded based on the sum scores of gland duct formation, polymorphism of the nucleus, irregular chromatin and the number of nuclear fissions as follows: Grade I (3-5 points), grade II (6-7 points) and grade III (8-9 points) (21). The detailed code used to assign points is listed below: i) 1 point for clear duct formation and well-differentiated ducts, 2 points for moderately differentiated ducts and 3 points for solid or cord-like ducts in some regions; ii) 1 point for a regular nucleus, 2 points for a moderately irregular nucleus and 3 points for a polygonal nucleus; and iii) Based on the amount of increased chromatin and nuclear fission that can be observed in a high-power field: 1 point for 1 chromat or nuclear fission, 2 points for 2-3 chromatins or nuclear fission and 3 points ≥3 chromatins or nuclear fission.

CEUS examination. Prior to surgery, all included patients were examined using CEUS performed using the Philips IU22 color Doppler ultrasound machine (Philips Medical Systems B.V., Amsterdam, Netherlands) using a frequency of 5-12 MHz. Patients were lying in a supine, dorsal or side-lying position with bilateral upper limbs pointing up to fully expose their breasts and bilateral axillae. The nipple was considered to be the center and the entire breast was scanned in a radial pattern. Multi-slice scanning was performed to ensure that no area was missed. The amount, shape, size, margin, internal and posterior echo, calcification, aspect ratio and axillary lymph node of lumps were observed and recorded. CEUS was performed on suspected lesions using an L9-3 linear array probe (Philips Medical Systems B.V.). In addition, pulse-inversion harmonic imaging with a mechanical index of 0.07 was used. The Sonovue contrast agent (Bracco Spa, Milan, Italy) was diluted with 5 ml normal saline and continuously shaken; 3 ml diluted contrast agent was injected into the median cubital vein of each patient. Subsequently, CEUS imaging was performed for 3 min. QLAB software 8.1 (Philips Medical Systems B.V.) was used for dynamic image analysis. To evaluate the effectiveness of CEUS at diagnosing the different subtypes of BC, the radial enhancement around lesions, enhancement pattern, characteristics of perfusion and lesion enhancement, internal characteristics at peak time and characteristics of lesion margin were observed and analyzed. The diagnostic accuracy
rate of CEUS in the detection of BC subtypes was determined by comparison with the IHC results.

**DW-MRI.** All patients were preoperatively scanned using a 1.5 T MRI scanner (Siemens AG, Munich, Germany) and a phased array coil with eight receiver channels. Patients were in the prone position with the breast hanging on the slot of the coil and the chest clinging to the coil. Scans were obtained from as much of the breasts, axilla and thorax included in the field of view. MRI parameters were as follows: T1-weighted image (WI)-FLASH set as the repetition time (TR)=8.6 ms; echo time (TE)=4.7 msec; matrix=336x448; slice thickness=1 mm; space=0 cm; and T2 WI-T2 magnetic resonance set as TR=5,600 msec; TE=56 msec; T1=170 msec; field of view (FOV)=34x34 cm; matrix=314x384; slice thickness=4 cm; space=0.8 cm. DWI images were acquired in the axial plane with an echo planar sequence using the echo-planar imaging technique and 2 diffusion-sensitive factors (b=50, 800 sec/mm²) were selected, with imaging parameters set as TR=5,800 msec; TE=83 msec; FOV=34x34 cm; matrix=306x384; slice thickness=5 mm; space=1 mm. Additionally, the dynamic scanning images were managed using Syngo Numaris software version 4 (Siemens AG), according to which apparent diffusion coefficient (ADC) images were automatically drawn using a computer. The formula for ADC was as follows: ADC=ln (Sb0/Sb1)/(b1-b0), in which Sb0 and Sb1 referred to the signal intensity of the region of interest (ROI), b1=800 sec/mm² and b0=50 sec/mm². The image of the layers similar to the largest cross section of focus was used as the standard image, and the cystic degeneration, necrosis and bloody areas were avoided. The maximum ADC (ADCmax), minimum ADC (ADCmin) and mean ADC (ADCmean) were calculated based on the ROI for the elevation of the availability of DW-MRI in the diagnosis of different subtypes of BC and the accuracy rate was compared with the results of IHC.

**Image analysis.** The results of IHC, CEUS and DW-MRI were compared to analyze the accuracy of CEUS alone, DW-MRI alone and CEUS combined with DW-MRI in detecting the subtypes of BC, respectively.

**Follow-up.** Patients were followed up until July 30, 2016. Out of the 232 included patients, 210 cases completed follow-up, indicating that the follow-up completion rate was 90.52%. Telephone and outpatient reviews were conducted for all patients every 15 days for 6-42 months following surgery. Recorded indices were: Local recurrence, including lymph node recurrence in the chest wall, subcutaneous tissue or axilla and around the sternum, and cancerous nodules in the surgical skin, axilla or chest wall, together with supraclavicular lymph node metastasis and distant metastasis. The recurrence time was analyzed according to the clinical features and results of DW-MRI and CEUS.

**Statistical analysis.** All the data were analyzed using SPSS 19.0 software (IBM Corp, Armonk, NY, USA). The enumeration data were presented as case numbers and percentages and comparisons between two groups were performed using a $\chi^2$ test. Measurement data were expressed as the mean ± standard deviation. Comparisons among multiple groups were performed using one-way analysis of variance and pairwise comparisons were conducted using the least significant difference test. The prognosis of patients with different subtypes was analyzed using the Kaplan-Meier method and multivariate analysis.
Cox regression analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinicopathological characteristics and IHC analysis of patients with different subtypes of BC. No significant differences were noted in age, tumor size and lymph node metastasis among patients with the four different subtypes of BC (Table I). However the histological grade of BC tissue differed significantly between patients with different subtypes of BC. The number of patients with grade I was significantly higher in those with luminal A and B subtypes of BC than in those with the Her-2-positive and triple negative subtypes (P<0.05), while the number of patients with grade III was significantly lower (P<0.05; Table I). According to the expression of ER, PR and Her-2 as determined by IHC, ER and PR were positive but HER2 was negative in patients with the luminal A subtype; ER, PR and HER2 were all positive in the patients with the luminal B subtype; ER and PR were negative but HER2 was positive in patients that were Her-2-positive; and the expression of ER, PR and HER2 were all negative in patients with triple negative BC (Fig. 1).

CEUS parameters of patients with different subtypes of BC. The tumor margin of different subtypes was as follows (Table II): Luminal B: Smooth edge (17.24%), lobulated (6.90%), with burr (10.34%) and indistinct (65.52%); luminal A: Smooth edge (5.08%), lobulated (11.86%), with burr (15.25%) and indistinct (67.80%); Her-2 positive: Smooth edge (14.58%) and lobulated (85.42%); triple negative: Smooth edge (13.16%); lobulated (7.89%); with burr (15.79%) and indistinct (63.16%).
Compared with the Her-2-positive group, there were significantly more patients with lobulated and with burr subtypes in the luminal B, luminal A and triple negative groups (P<0.05). Furthermore, compared with the triple negative group, there were significantly more patients with the indistinct subtype in the luminal B, luminal A and Her-2-positive groups (P<0.05). There were no significant differences in tumor morphology, internal calcification, histological grade or lymph node metastasis among the four subtypes of BC (Table II).

**Table II. CEUS parameters of patients with different subtypes of breast cancer.**

| Parameter                  | Luminal B (n=87) | Luminal A (n=59) | Her-2 positive (n=48) | TNBC (n=38) | χ² | P-values |
|----------------------------|------------------|------------------|-----------------------|-------------|----|---------|
| Tumor morphology (%)      |                  |                  |                       |             |    |         |
| Regular                   | 5 (5.75)         | 3 (5.08)         | 2 (4.17)              | 4 (10.53)   | 1.754 | 0.625  |
| Irregular                 | 82 (94.25)       | 56 (94.92)       | 46 (95.83)            | 34 (89.47)  |    |         |
| Tumor diameter (%)        |                  |                  |                       |             |    |         |
| ≤2 cm                     | 6 (2.08)         | 6 (10.17)        | 1 (2.08)              | 4 (10.53)   | 3.242 | 0.356  |
| >2 cm                     | 81 (97.92)       | 53 (89.83)       | 47 (97.92)            | 34 (89.47)  |    |         |
| Margin (%)                |                  |                  |                       |             |    |         |
| Smooth                    | 15 (17.24)       | 3 (5.08)         | 7 (14.58)             | 5 (13.16)   | 19.250 | 0.023a |
| Lobulated                 | 6 (6.90)         | 7 (11.86)        | 0                     | 3 (7.89)    |    |         |
| With burr                 | 9 (10.34)        | 9 (15.25)        | 0                     | 6 (15.79)   |    |         |
| Indistinct                | 57 (65.52)       | 40 (67.80)       | 41 (85.42)            | 24 (63.16)  |    |         |
| Calcification (%)         |                  |                  |                       |             |    |         |
| Yes                       | 64 (73.56)       | 46 (77.97)       | 32 (66.67)            | 27 (71.05)  | 1.793 | 0.617  |
| No                        | 23 (26.44)       | 13 (22.03)       | 16 (33.33)            | 11 (28.95)  |    |         |
| Histological grade (%)    |                  |                  |                       |             |    |         |
| I                         | 8 (9.20)         | 5 (8.47)         | 5 (10.42)             | 2 (5.26)    | 5.317 | 0.504  |
| II                        | 35 (40.23)       | 16 (27.12)       | 17 (35.42)            | 10 (26.32)  |    |         |
| III                       | 44 (50.57)       | 38 (64.41)       | 26 (54.17)            | 26 (68.42)  |    |         |
| Lymph node metastasis (%) |                  |                  |                       |             |    |         |
| Yes                       | 49 (56.32)       | 31 (52.54)       | 27 (56.25)            | 21 (55.26)  | 0.234 | 0.972  |
| No                        | 38 (43.68)       | 28 (47.46)       | 21 (43.75)            | 17 (44.74)  |    |         |

Associations were evaluated using the χ² test. *P<0.05. Her-2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

**Table III. ADC (x10⁻⁶ mm³/s) value of patients with different subtypes of breast cancer.**

| Group                   | ADC<sub>max</sub> | ADC<sub>mean</sub> | ADC<sub>min</sub> |
|-------------------------|-------------------|--------------------|-------------------|
| Luminal A (n=59)        | 1.34±0.29<sup>a</sup> | 1.12±0.23<sup>a</sup> | 0.74±0.16<sup>ab</sup> |
| Luminal B (n=87)        | 1.34±0.31<sup>a</sup> | 1.31±0.26<sup>a</sup> | 0.79±0.17<sup>ab</sup> |
| Her-2 positive (n=48)   | 1.31±0.28<sup>a</sup> | 1.15±0.25<sup>a</sup> | 0.65±0.13<sup>b</sup> |
| TNBC (n=38)             | 1.08±0.31          | 1.00±0.17          | 0.66±0.18          |
| Total (n=232)           | 1.29±0.30          | 1.18±0.48          | 0.73±0.16          |

Differences between groups were assessed using one-way analysis of variance followed by the least significance difference test. *P<0.05 vs. the TNBC group; *P<0.05 vs. the Her-2 positive group. BC, breast cancer; Her-2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer; ADC<sub>max</sub>, maximum ADC; ADC<sub>mean</sub>, minimum ADC; ADC<sub>mean</sub>, mean ADC; ADC, apparent diffusion coefficient.

**Table III. Cell parameters of patients with different subtypes of breast cancer.**

| Group | ADC<sub>max</sub> | ADC<sub>mean</sub> | ADC<sub>min</sub> |
|-------|-------------------|--------------------|-------------------|
| Luminal A (n=59) | 1.34±0.29<sup>a</sup> | 1.12±0.23<sup>a</sup> | 0.74±0.16<sup>ab</sup> |
| Luminal B (n=87) | 1.34±0.31<sup>a</sup> | 1.31±0.26<sup>a</sup> | 0.79±0.17<sup>ab</sup> |
| Her-2 positive (n=48) | 1.31±0.28<sup>a</sup> | 1.15±0.25<sup>a</sup> | 0.65±0.13<sup>b</sup> |
| TNBC (n=38) | 1.08±0.31          | 1.00±0.17          | 0.66±0.18          |
| Total (n=232) | 1.29±0.30          | 1.18±0.48          | 0.73±0.16          |

Differences between groups were assessed using one-way analysis of variance followed by the least significance difference test. *P<0.05 vs. the TNBC group; *P<0.05 vs. the Her-2 positive group. BC, breast cancer; Her-2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer; ADC<sub>max</sub>, maximum ADC; ADC<sub>mean</sub>, minimum ADC; ADC<sub>mean</sub>, mean ADC; ADC, apparent diffusion coefficient.

**Accuracy of CEUS, DW-MRI and CEUS combined with DW-MRI in the diagnosis of BC subtypes.** The diagnostic accuracy rates of the luminal A, luminal B, Her-2-positive and triple negative subtypes by CEUS in the preoperative diagnosis of BC were 88.14, 87.36, 77.08 and 71.05%, respectively.
The total diagnostic accuracy rate was 82.76%. The diagnostic accuracy rates of the luminal A, luminal B, Her-2-positive and triple negative subtypes with DW-MRI were 81.36, 88.51, 79.17 and 71.05%, respectively. The total diagnostic accuracy rate was 81.90%. The diagnostic accuracy rates of luminal A, luminal B, Her-2-positive and triple negative subtypes using CEUS combined with DW-MRI were 91.53, 94.25, 87.50 and 81.58%, respectively. The total diagnostic accuracy rate was 90.09% (Table IV). The diagnostic accuracy rate following the combined use of CEUS and DW-MRI was significantly higher compared with CEUS alone or DW-MRI alone in the diagnosis of BC (each, P<0.05).

Kaplan-Meier analysis of the diagnostic accuracy rate of patients with different subtypes of BC. Among the 210 patients that successfully completed follow-up, 12 patients (5.71%, 1 with luminal A BC, 4 with luminal B BC, 0 with triple negative and 7 with Her-2-positive BC) experienced local recurrence and 20 patients (4 with luminal A BC, 2 with luminal B BC, 11 with Her-2-positive BC, 3 with triple negative BC) had distant metastasis. There was a significant difference in the rate of local recurrence and distant metastasis among patients with different types of BC (all P<0.05). The rates of local recurrence and distant metastasis in patients with the Her-2-positive subtype (28.21%) were significantly higher.
than in all other subtypes (P<0.05; Table V). Additionally, patients with the Her-2-positive subtype exhibited the lowest disease-free survival (DFS) rate (P<0.05) and overall survival (OS) rate (P<0.05) compared with patients with the other three subtypes, indicating that patients with the Her-2 positive subtype of BC have the poorest prognosis (Fig. 3).

**Table IV. Diagnostic accuracy rate of CEUS, DW-MRI and CEUS combined with DW-MRI in the diagnosis of breast cancer.**

|                      | Luminal A | Luminal B | Her-2 positive | TNBC | Total |
|----------------------|-----------|-----------|----------------|------|-------|
| CEUS                 |           |           |                |      |       |
| Negative (0-3 stage) | 7         | 11        | 11             | 11   | 40    |
| Positive (4-5 stage) | 52        | 76        | 37             | 27   | 192   |
| Total                | 59        | 87        | 48             | 38   | 232   |
| Diagnostic accuracy rate, % | 88.14 | 87.36 | 77.08 | 71.05 | 82.76* |
| MR-DWI               |           |           |                |      |       |
| Negative (0-3 stage) | 11        | 10        | 10             | 11   | 42    |
| Positive (4-5 stage) | 48        | 77        | 38             | 27   | 190   |
| Total                | 59        | 87        | 48             | 38   | 232   |
| Diagnostic accuracy rate, % | 81.36 | 88.51 | 79.17 | 71.05 | 81.90* |
| Combination of CEUS with DW-MRI | | | | | |
| Negative (0-3 stage) | 5         | 5         | 6              | 7    | 23    |
| Positive (4-5 stage) | 54        | 82        | 42             | 31   | 209   |
| Total                | 59        | 87        | 48             | 38   | 232   |
| Diagnostic accuracy rate, % | 91.53 | 94.25 | 87.50 | 81.58 | 90.09 |

Associations were evaluated using the χ² test. *P<0.05 vs. CEUS combined with DW-MRI. CEUS, contrast-enhanced ultrasound; DW-MRI, diffusion-weighted magnetic resonance imaging; Her-2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

Figure 3. Kaplan-Meier curves of OS and DFS in patients with different subtypes of breast cancer. (A) The ROC for DFS in all subtypes of BC; (B) the ROC for OS in all subtypes of BC; ROC, receiver operating characteristic; CEUS, contrast-enhanced ultrasound; DW-MRI, diffusion-weighted magnetic resonance imaging; OS, overall survival; DFS, disease-free survival.

COX regression analysis of risk factors affecting the prognosis of patients with different subtypes of BC. Risk factors for the prognosis of BC were analyzed using COX regression analysis, with the survival status of patients as the dependent variable. Lymph node metastasis and the different BC subtypes were included as independent variables in the Cox model. The
results suggested that lymph node metastasis and subtypes were independent factors in the prognosis of BC. Compared with patients with the Her-2-positive subtype, patients with the luminal A and B subtypes exhibited significantly higher DFS and OS, indicating that they had better prognoses (all P<0.05; Tables VI and VII).

### Discussion

Previous studies have indicated that CEUS and DW-MRI are successful at identifying BC; however, few studies have investigated the combined use of the two methods to diagnose BC (10-14). To identify a more effective approach for the diagnosis of BC, combined CEUS and DW-MRI were used in the present study.

In the diagnosis of BC with DW-MRI alone, it has been demonstrated that the perfusion-related diffusion coefficient and ADC values of luminal B BC are significantly lower than those of luminal A BC; furthermore, the signal enhancement ratio of luminal B BC is significantly higher than that of luminal A BC (22). In the diagnosis of BC with CEUS, the diagnostic accuracy rate of the Her-2-positive subtype is significantly higher than that of the Her-2-negative subtype (23). Compared with the other three subtypes of BC, a larger size, a round/oval mass shape, a smooth mass margin and rim enhancement on DW-MRI are significantly associated with a diagnosis triple negative BC (24). The current study compared the accuracy rate of the two methods and their combination in the diagnosis of BC and it was determined that CEUS and DW-MRI combined exhibited higher accuracy.
and specificity in diagnosing BC subtypes compared with either CEUS or DW-MRI alone. The diagnostic accuracy rates of luminal A, luminal B, Her-2-positive and triple negative subtypes following the use of CEUS combined with DW-MRI were 91.53, 94.25, 87.50 and 81.58%, respectively. Similarly, Wang et al (25) revealed that contrast-enhanced harmonic ultrasonography plus DW-MRI was more effective at diagnosing prostate transition-zone cancer than either method alone, as indicated by its higher sensitivity and accuracy rate. It has been demonstrated that, although CEUS is more effective at diagnosing tumors than traditional ultrasound, sometimes difficulties arise when distinguishing between different enhancement patterns (12). Additionally, DW-MRI is able to differentiate between benign and malignant focal hepatic lesions using the ADC value, as normal tissues and benign lesions generally have higher ADC values than malignant lesions (18).

The results of the current study also demonstrated that lymph node status and BC subtype are independent prognostic factors of patients with BC. Following the prognostic analysis of different BC molecular subtypes, Yang et al (26) indicated that lymph node status is an independent prognostic factor affecting the OS and DFS rates of patients. The results of a study of Liu et al (27) indicated that the OS and DFS of patients were affected by lymph node metastasis and that the recurrence and metastasis of patients were closely associated with the number of positive lymph nodes. It has been demonstrated that patients with metastases of 2 mm or smaller axillary lymph nodes exhibit lower OS and DFS rates (28). Additionally, it has been reported that 20-30% of patients with BC will still develop distant metastases, although progress has been made in its treatment (29). Furthermore, patients with the Her-2-positive subtype exhibit the highest rate of distant metastasis compared with patients with other subtypes. Similarly, it has been proven that patients with luminal/HER2 and HER2-enriched tumors exhibit a significantly higher rate of distant metastases than those with luminal A tumors (30).

Although survival in early BC is predominantly estimated by primary tumor size, histological grade and nodal status, these factors cannot explain the heterogeneity of the disease (31). Intrinsic gene sets have been developed to classify BC into four molecular subtypes with distinct prognoses and responses to treatment (32). Importantly, the results of the current study indicated that BC subtype is an independent prognostic factor in patients. It has been proven that patients with different types of BC exhibit different epidemiology, responses to therapy and prognoses (33). Finetti et al (34) demonstrated that BC is a heterogeneous disease consisting of various subtypes with different prognoses. In addition, Kim et al (35) reported that the prognosis of patients with BC differs according to genotype.

The results of the current study indicate that patients with the luminal A and B subtypes exhibit higher OS and DFS than patients with the Her-2-positive subtype. By connecting the results of IHC and expression profile assignments, Cheang et al (36) indicated that the luminal A and B subtypes are hormone receptor-positive and Her2-negative tumors, respectively. Additionally, Puig-Vives et al (37) determined that, compared with Her-2-positive and triple-negative subtypes, patients with hormone receptor-positive tumors exhibit a better prognosis. Additionally, Kim et al (35) indicated that patients with luminal-type BC, including those with luminal A and B subtypes, exhibited good prognosis compared with the other types, including the Her-2 and triple-negative subtypes. Wang et al (38) also indicated that patients with the Her-2 positive subtype exhibited the poorest DFS and OS prognosis among the four subtypes of BC. In accordance with the results of the current study, Yang et al (26) indicated that patients with the Her-2-positive subtype had the lowest OS and DFS rates compared with the luminal A, luminal B and basal-like subtypes.

In conclusion, the results of the current study indicated that the combination of CEUS and DW-MRI is more accurate at distinguishing between the different subtypes of BC. The results also indicated that determining the molecular subtype of BC is important when predicting the prognosis of patients with BC. However, the number of patients included in the current study was limited; therefore, further studies involving larger samples are required to confirm the results of the current study.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
GFL and ZQW designed the study. SHZ and XFL collected the data. LL, YYM and SNY drafted and revised the manuscript. GFL and ZQW designed the study. All authors approved the final version of the manuscript.

Ethics approval and consent to participate
The present study was approved by the Clinical Ethics Committee of China-Japan Union Hospital of Jilin University (Changchun, China). All patients provided written informed consent for their inclusion in the current study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.
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