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# Regional Contrast-Enhanced Ultrasonography (CEUS) Characteristics of Breast Cancer and Correlation with Microvessel Density (MVD)

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
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**Background:** The aim of this study was to investigate the perfusion characteristics of different breast lesion regions in contrast-enhanced ultrasonography (CEUS).


**Material/Methods:** A total of 161 malignant and benign breast lesion cases were subjected to CEUS. Perfusion parameters were analyzed and compared between the central and peripheral lesion regions, and surrounding tissue. Mass section was marked with methylene blue. Samples were subjected to immunohistochemistry, and microvessel density (MVD) was calculated.

**Results:** There were significant differences in perfusion performance between the central and peripheral lesion regions, and surrounding tissue. In the malignant tumors, the fast-in and fast-out pattern was the most common type in the peripheral region (57.98%), while the slow-in and slow-out patterns were the major types in the central region and surrounding tissue (49.58% and 57.98%, respectively). Compared with the surrounding tissue, the peripheral region in the cancers exhibited hyperechoic enhancement and fast-in and slow-out pattern, with large area under the curve (AUC), while the central region showed isoechoic enhancement and equally-in and slow-out pattern, with large AUC. In the benign lesions, the peripheral region exhibited hyperechoic enhancement and fast-in and fast-out pattern, with small AUC, while the central region showed isoechoic enhancement and equally-in and -out pattern, with the same AUC value. Moreover, the perfusion parameters in the central and peripheral regions were significantly associated with MVD.

**Conclusions:** It is more objective to evaluate the perfusion performance of breast lesions with the reference of surrounding tissue. Compared with the central region, the peripheral region could better reflect the perfusion characteristics of malignant lesions.

**MeSH Keywords:** **Contrast Media • Microvessels • Nuclear Receptor Coactivator 3**

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## Background

In the past few decades, the annual incidence of breast cancer has been rising in China. Routine diagnostic screening and imaging of breast cancer mainly depends on mammography, radiology and conventional ultrasound, which have limited capacities in distinguishing breast cancers from benign tumors and characterizing tumor blood supply [1]. Tumor angiogenesis is an important indicator of tumor growth and metastasis, which has become an ideal target for gene therapy [2]. Microvessel density (MVD) is currently the criterion standard for characterization of tumor angiogenesis. However, measurement of MVD by biopsy is an invasive procedure. Due to tumor heterogeneity, the biopsy site might not fully reflect the entire tumor microvasculature, and it is not possible to dynamically monitor the microvascular circulation and function. It is, therefore, critical to achieve a safe and non-invasive evaluation of tumor microvasculature.

Contrast-enhanced ultrasound (CEUS) imaging with high spatial and temporal resolution has provided a great opportunity for visualization of microcirculation. Intravascular contrast agents enable conventional imaging, with fewer influencing factors than CT or MR perfusion imaging [4]. The disadvantage of CT and MR enhanced angiography is that the enhancement depends on the tissue blood supply and the extravascular volume. Further, CEUS facilitates the morphological analysis of cancers as well as the functional evaluation of microcirculation and hemodynamic characteristics [5,6]. However, in enhanced CT and MR examinations, the early changes in the time-intensity curve (TIC) of region of interest (ROI) are associated with the blood supply per unit tissue, and the late enhancement is related to the extravascular space [3]. The CEUS quantitative parameters can be obtained from the TIC, which provides objective and reproducible estimation of cancer vascularization [7]. Previous imaging studies of breast cancer mainly focused on the whole cancer, ignoring the imbalance of tumor microcirculation.

In this study, for the CEUS, the breast cancer lesion had been divided into different regions. Perfusion characteristics of the peripheral region of breast cancer were summarized with the quantitative analysis of different cancer regions, and its clinical significance is discussed. Perfusion parameters were also optimized according to the relevance between the regional perfusion parameters and MVD.

## Material and Methods

### Study population

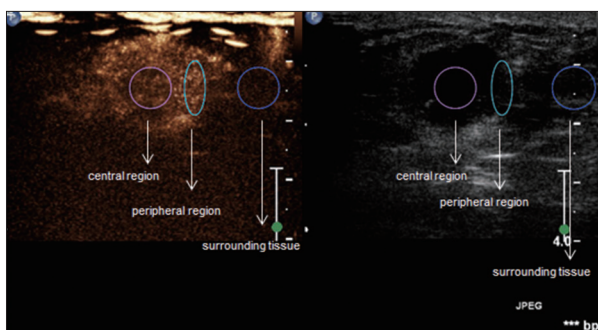
Clinically confirmed breast cancer patients consenting to undergo surgical resection were eligible for the study. The

following subjects were excluded: breast cancer patients who had received fine-needle puncture in the breast, without chemotherapy, radiotherapy, or endocrine therapy; patients who were allergic to contrast agent; patients with failure of heart, liver, kidney, or other major organs; and patients who were contraindicated for surgery due to physical and/or economic conditions. A total of 158 female subjects, aged 26 to 85 years (with an average age of 41.89 years), were included in this study, from Aug 2013 to Aug 2014. Of these 158 patients (with 161 tumors), 116 had breast cancer (with 119 tumors) and 42 had benign solid tumors (with 42 tumors). Cancers included intraductal carcinoma (n=6), infiltrating ductal carcinoma (n=80), invasive lobular carcinoma (n=20), and mixed type (n=13). Axillary lymph node metastases were observed in 53 cases. Benign lesions included intraductal papilloma (n=6) (with 2 cases of mild to moderate dysplasia) and fibroadenoma (n=12). The maximum diameter of these malignant and benign tumors ranged from 0.6 cm to 8 cm, with an average of 2.8 cm. Prior written and informed consent was obtained from every patient and the study was approved by the Ethics Review Board of the First Affiliated Hospital of Xinjiang Medical University.

### Ultrasonographic examination

Gray-scale ultrasonography was performed using the Philips iU22 scanner (Philips Healthcare Solutions, Bothell, WA, USA), with a 5- to 12-MHz probe. Ultrasonography was conducted 1 week before surgery on patients, in supine position, exposing the chest. The probe was used to scan the bilateral breasts, starting from the outer upper quadrant, in a spoke-like fashion, from the periphery to the nipple. The tumors were first carefully observed with two-dimensional ultrasound, and the blood flow distribution was determined with color Doppler imaging, based on which the tumor areas were selected. In patients with multiple tumors, the tumor with more compact appearance and less interference with respiratory motion was selected.

SonoVue (Bracco, Milan, Italy) was used as the ultrasound contrast agent. The CEUS was performed with 5 mL SonoVue bolus injected via the cubital vein, followed by injection of 5 mL saline. A 3-min continuous recording was stored in the instrument. The imaging data were analyzed using the QLAB software. Regions of interest (ROI) were selected and defined as follows: the central region covered the cancer center, with the diameter of about 1 cm (the area would be reduced for relatively smaller tumor); the peripheral region was defined as an ellipse whose boundary coincided with the enhancement boundary of CEUS, with the area and depth close to the central region (tumor feeding vessels should be necessarily avoided); and the surrounding region, which covered the mammary gland tissue, with the same area and depth as the central



**Figure 1.** Regions of interest (ROI) in CEUS. The central region covered the cancer center, with the diameter of about 1 cm (purple circle); the peripheral region was defined as an ellipse whose boundary coincided with the enhancement boundary of CEUS, with the area and depth close to the central region (sky-blue ellipse); and the surrounding tissue, which covered the mammary gland tissue, with the same area and depth as the central region (dark blue circle).

region (Figure 1). The mass section studied was marked with methylene blue (MB), which was not contained by the peripheral tissue sampling, and a line was drawn across the skin layer. The following perfusion parameters were analyzed: the median intensity (MI), peak intensity (PI), rise time (RT), rising slope (RS), initial time of perfusion (ITP), average transit time (TT), area under the curve (AUC), time from peak to one-half (DT/2), descending slope (DS), and time to peak (TTP). MI and PI represented the degree of enhancement, AUC interpreted the total perfusion volume, and RT, RS, ITP, TT, DT/2, DS, and TTP described the perfusion pattern. The morphological analysis of the time-intensity curve (TIC) involved the absolute and relative shape. The absolute TIC shapes were classified into 4 types, according to the RS and the DS: type 1, a fast-in and fast-out pattern (RS >1.5 and DS >1.5); type 2, a fast-in and slow-out pattern (RS >1.5 and DS <1.5); type 3, a slow-in and fast-out pattern (RS <1.5 and DS >1.5); and type 4, a slow-in and slow-out pattern (RS <1.5 and DS <1.5). The relative TIC shapes were also divided into the 4 types mentioned above, based on the comparison with the adjacent breast tissue as reference.

### Microvessel density (MVD) measurement

After surgery, based on the MB marking, the tissue samples from the 3 regions were obtained, embedded with paraffin, and cut into sections. These sections were subjected to CD34 immunohistochemical staining. MVD measurement was performed according to a previously reported method from Weidner et al. [8]. The entire section was first observed at low magnification (100×), and 3 regions with the highest vascular densities were selected, i.e., the hot spot. Then, the vessels stained brown with CD34 were counted at high magnification (200×), and the mean MVD was calculated accordingly.

Microvessel numbers were counted, in which the standard criteria for microvessel determination was not a complete lumen. Whether with or without lumen, 1 microvessel was defined as the close arrangement of single or multiple endothelial cells.

### Statistical analysis

Data normality was tested with the  $\chi^2$  goodness-of-fit test. Normally distributed data were expressed as mean  $\pm$ SD, or expressed as median with interquartile range, as appropriate. SPSS 20.0 software was used for statistical analysis. The Wilcoxon rank-sum test was used to analyze the TIC types and the differences in perfusion parameters for the central and peripheral regions, and the surrounding tissue, of the malignant and benign breast lesions. Multivariate analysis of variance was performed to compare the general differences in TIC index for the central and peripheral regions, and the surrounding tissue, of the malignant and benign breast lesions. Pearson correlation coefficient analysis was conducted to analyze the reference between the regional perfusion parameters and MVD.  $P < 0.05$  was considered as statistically significant.

## Results

### Comparison of absolute TIC shapes between different regions in malignant and benign breast lesions

The absolute TIC shapes between the central and peripheral regions of the breast cancers, and the surrounding tissue, were first investigated. As shown in Table 1, our results revealed significant different patterns for the central and peripheral regions of breast cancers, and the surrounding tissue ( $P < 0.001$ ). There were also differences between the 3 different regions in benign and malignant lesions for each imaging modality. In the malignant tumors, the fast-in and fast-out pattern was the most common type in the peripheral region (57.98%), while the slow-in and slow-out patterns were the major types in the central region (49.58%) and in the surrounding tissue (57.98%). On the other hand, no significant differences were observed between the different regions in the benign cases ( $P > 0.05$ ).

### Comparison of relative TIC shapes between different regions in malignant and benign breast lesions

The relative TIC shapes were next analyzed and compared across different regions of the breast cancers, and the surrounding tissue. As shown in Table 2, for the peripheral region of the cancers, the MI, PI, RT, TT, and AUC were all significantly higher than in the central region and the surrounding tissue ( $P < 0.05$ ). The initial ITP was significantly lower than in the central region and surrounding tissue ( $P < 0.05$ ). The RS was

**Table 1.** Comparison of absolute TIC shapes between different regions in malignant and benign breast lesions.

Perfusion pattern	Peripheral region (malignant/benign)		Central region (malignant/benign)		Surrounding tissue (malignant/benign)		P
	N	%	N	%	N	%	
Fast-in and fast-out	69/14	57.98/33.33	33/14	27.73/33.33	9/13	7.56/30.95	<0.001/>0.05
Fast-in and slow-out	26/16	21.85/38.09	26/14	21.85/33.33	39/15	32.77/35.71	<0.001/>0.05
Slow-in and fast-out	1/0	0.84/0.00	1/0	0.84/0.00	2/0	1.69/0.00	<0.001/>0.05
Slow-in and slow-out	23/12	19.33/28.57	59/14	49.58/33.33	69/14	57.98/33.33	<0.001/>0.05

**Table 2.** Comparison of perfusion parameters in malignant breast lesions.

Perfusion parameters	Peripheral region		Central region		Surrounding tissue		$\chi^2$	P
MI	13.93	[10.1, 18.55]	7.99	[5.95, 10.47]**	7.02	[5.24, 9.27]**	69.29	<0.001
PI	14.79±5.72		2.82	[1.39, 4.59]**	2.15	[1, 4.99]**###	107.78	<0.001
RT	2.45	[1.58, 3.49]	1.31	[0.78, 2.44]**	1.06	[0.68, 1.95]**	33.00	<0.001
RS	2.83	[1.7, 5.21]	2.66±1.34**		1.75	[0.92, 3.27]**	47.97	<0.001
ITP	30.18	[18.79, 43.27]	39.32	[28.33, 56.65]**	45.45	[28.29, 66.88]**###	149.75	<0.001
Average TT	362.72	[223.66, 645.1]	147.55	[55.06, 325.71]**	87.48	[30.07, 210.24]**	36.74	<0.001
AUC	33.85	[23.91, 44.85]	30.9	[21.07, 45.44]**	30.14	[15.1, 54.2]**###	109.14	<0.001
DT/2	1.67	[1.02, 2.3]	1	[0.46, 1.5]	0.9	[0.27, 1]	4.02	0.134
DS	18.81	[15.3, 26.61]	19.67	[16.14, 24.61]**	21.92	[16.93, 34.45]**###	79.02	<0.001
TTP	9.58	[5.81, 12.72]	5.82	[3.34, 7.76]	5.19	[2.59, 6.92]*	6.18	0.046

MI – median intensity; PI – peak intensity; RT – rise time; RS – rising slope; ITP – initial time of perfusion; TT – transit time; AUC – area under the curve; DT/2 – time from peak to one-half; DS – descending slope; TTP – time to peak. Normally distributed data were expressed as mean ±SD, or otherwise, expressed as median with interquartile range. Compared with the peripheral region, \*  $P<0.05$ ; \*\*  $P<0.01$ ; compared with the central region, #  $P<0.05$ ; ###  $P<0.01$ .

significantly lower than that of the central region ( $P<0.01$ ), but was significantly higher than that of the surrounding tissue ( $P<0.01$ ). The DS was significantly lower than that of the central region and the surrounding tissue ( $P<0.05$ ). The TTP in the peripheral region was significantly longer than in the surrounding tissue ( $P<0.05$ ), with no significant difference in TTP between the central and peripheral regions ( $P>0.05$ ). The DT/2 did not significantly differ between these different regions (Table 2). As shown in Table 3, for the benign lesions, the MI in the peripheral region were significantly higher ( $P<0.05$ ), while the ITP was significantly lower ( $P<0.05$ ) than in the central region and surrounding tissue. There was no significant difference in the AUC between the peripheral and central regions in the benign lesions, while the AUC values in both the 2 regions were lower than the surrounding tissues. The PI in the peripheral region was significantly lower than in the central region, but higher than in the surrounding tissue ( $P<0.05$ ).

The TTP was significantly longer than in the surrounding tissue, and the DS was higher than in the central region, but lower than in the surrounding tissue. Taken together, these results suggest that the relative TIC shapes differ between the different regions for the malignant and benign breast lesions. Therefore, compared with the surrounding tissues, the peripheral region of the breast cancers exhibited the fast-in and slow-out pattern, with hyperechoic enhancement, while the central region displayed the equally-in and slow-out pattern, with isoechoic enhancement. On the other hand, the peripheral region of the benign breast lesions showed fast-in and fast-out pattern, together with hyperechoic enhancement, while the central region exhibited equally-in and -out pattern, with isoechoic enhancement. Compared with the central region of breast cancer, the peripheral region of breast cancer was associated with the fast-in and slow-out pattern, with hyperechoic enhancement.

**Table 3.** Comparison of perfusion parameters in benign breast lesions.

Perfusion parameters	Peripheral region	Central region	Surrounding tissue	$\chi^2$	P
MI	8.24±3.81	5.78±3.24**	5.38 ± 2.92**	15.526	0.001
PI	4.38 [2.48, 6.22]	9.01±4.20**	2.34 [1.72, 4.73]**	23.677	<0.001
RT	2.14 [1.36, 2.67]	1.09 [0.83, 1.42]	0.95 [0.7, 1.65]**	3.969	0.137
RS	5.67 [3.91, 8.84]	3.11 [1.52, 6.4]**	2.83 [1.49, 5.89]**	19.277	<0.001
ITP	6.73±3.83	38.27 [24.65, 51.45]**	41.7 [27.79, 53.43]**	19.100	<0.001
Average TT	288.5 [206.94, 529.77]	37.50 ± 18.57	134.08 [56.96, 330.36]	2.739	0.254
AUC	33.56 [23.85, 38.63]	33.01 [24.68, 47.5]	37.42 [27.75, 54.61]**##	11.901	0.003
DT/2	1.47 [0.77, 1.98]	0.76 [0.46, 1]	38.13±21.30	1.059	0.589
DS	23.24 [18.24, 27.3]	21.12 [15.61, 28.25]**	24.35 [18.67, 46.63]**	21.540	<0.001
TTP	7.8[5.62, 11.42]	21.80 ± 11.22	5.23 [3.73, 7]**	7.819	0.020

MI – median intensity; PI – peak intensity; RT – rise time; RS – rising slope; ITP – initial time of perfusion; TT – transit time; AUC – area under the curve; DT/2 – time from peak to one-half; DS – descending slope; TTP – time to peak. Normally distributed data were expressed as mean ±SD, or otherwise, expressed as median with interquartile range. Compared with the peripheral region, \* P<0.05; \*\* P<0.01; compared with the central region, # P<0.05; ## P<0.01.

**Table 4.** MVD assessment for different regions in malignant and benign breast lesions.

	Peripheral region M (P <sub>25</sub> , P <sub>75</sub> )	Central region M (P <sub>25</sub> , P <sub>75</sub> )	Surrounding tissue M (P <sub>25</sub> , P <sub>75</sub> )	$\chi^2$	P
Malignant lesions	49.51 (18.00, 73.00)	30.27 (14.00, 48.50)	19.33 (9.50, 29.00)	59.47	<0.001
Benign lesions	10.58 (5.81, 12.73)	6.82 (4.34, 7.86)	5.69 (3.59, 7.92)	6.18	0.046

**Comparison of overall perfusion performance between different regions in malignant and benign breast lesions**

The overall CEUS perfusion performance was next analyzed and compared, between different regions in malignant and benign breast lesions, with the multivariate analysis of variance. Our results showed significant differences in the CEUS perfusion performance between the central and peripheral regions, and the surrounding tissue, for both the malignant (F=7.783, P<0.001) and benign (F=3.101, P<0.001) breast lesions.

**Relationship between the perfusion parameters and MVD in malignant and benign breast lesions**

The MVD values for different regions in the lesions were calculated based on the detection of the microvascular-rich regions, i.e., the hot spots. The relationship between the perfusion parameters and MVD in the malignant and benign lesions was next investigated. Our results showed that, in the 119 cases of breast cancer, significant differences in MVD were observed between different regions. The MVD in the peripheral region was significantly higher than in the central region, and the MVD

in the central region was higher than in the surrounding tissue (P<0.05). In the other 42 benign breast lesions, these were also significant differences in MVD between different regions (P<0.05) (Table 4). Results for the relevance analysis between perfusion parameters and MVD are shown in Table 5. For the cancers, the MI, RS, ITP, DS, TTP, PI, and AUC in the central and peripheral regions were significantly related to MVD (P<0.05 for RS and TTP; and P<0.001 for MI, ITP, DS, PI, and AUC) (Figure 2). However, in the surrounding tissue, only the RS and DS were significantly associated with MVD (P<0.05). On the other hand, for both the malignant and benign breast lesions, the RT, TT, or DT/2 was not significantly associated with MVD, for either the central region, peripheral region, or surrounding tissue (P>0.05).

**Discussion**

CEUS technology enables determination of the diffusion pattern and real-time quantification of the contrast agent within the organs, as well as non-invasive evaluation of tumor angiogenesis and hemodynamic alteration. CEUS enhancement mainly depends on the tissue blood supply and available fluid volume.

**Table 5.** Relevance between perfusion parameters and MVD in malignant and benign breast lesions.

Perfusion parameters	Malignant lesions			Benign lesions		
	Peripheral region <i>r, P</i>	Central region <i>r, P</i>	Surrounding tissue <i>r, P</i>	Peripheral region <i>r, P</i>	Central region <i>r, P</i>	Surrounding tissue <i>r, P</i>
MI	0.524	0.411	0.134	0.227	0.261	0.088
	<0.001	<0.001	0.146	0.004	0.001	0.268
PI	0.471	0.533	0.037	0.512	0.373	0.07
	<0.001	<0.001	0.693	<0.001	<0.001	0.376
RT	-0.008	-0.053	-0.066	-0.057	-0.063	-0.018
	0.927	0.567	0.476	0.527	0.455	0.629
RS	0.227	0.182	0.214	0.239	0.216	0.168
	0.013	0.047	0.024	0.002	0.006	0.033
ITP	0.407	0.427	0.072	0.373	0.32	0.115
	<0.001	<0.001	0.437	<0.001	<0.001	0.145
TT	-0.054	-0.125	-0.05	-0.123	-0.184	-0.06
	0.556	0.177	0.586	0.785	0.629	0.455
AUC	0.432	0.252	0.03	0.311	0.381	0.051
	<0.001	0.006	0.745	<0.001	<0.001	0.524
DT/2	0.16	0.06	-0.066	0.129	0.056	0.037
	0.081	0.514	0.479	0.104	0.478	0.642
DS	0.291	0.305	0.182	0.264	0.259	0.16
	0.001	0.001	0.047	0.001	0.001	0.043
TTP	-0.16	-0.196	-0.082	-0.156	-0.355	-0.129
	0.043	0.033	0.373	0.048	0.008	0.104

MI – median intensity; PI – peak intensity; RT – rise time; RS – rising slope; ITP – initial time of perfusion; TT – transit time; AUC – area under the curve; DT/2 – time from peak to one-half; DS – descending slope; TTP – time to peak.

Therefore, early alteration in the TIC is associated with blood flow within the tissues, while subsequent enhancement mainly depends on extravascular space [9]. The ultrasound contrast agent is intravascular and does not diffuse into the interstitial space. Therefore, compared with CT and MRI, CEUS offers additional functional data. In the present study, the quantitative CEUS perfusion parameters for different regions of malignant and benign breast lesions were analyzed and compared to improve the accuracy of breast cancer diagnosis.

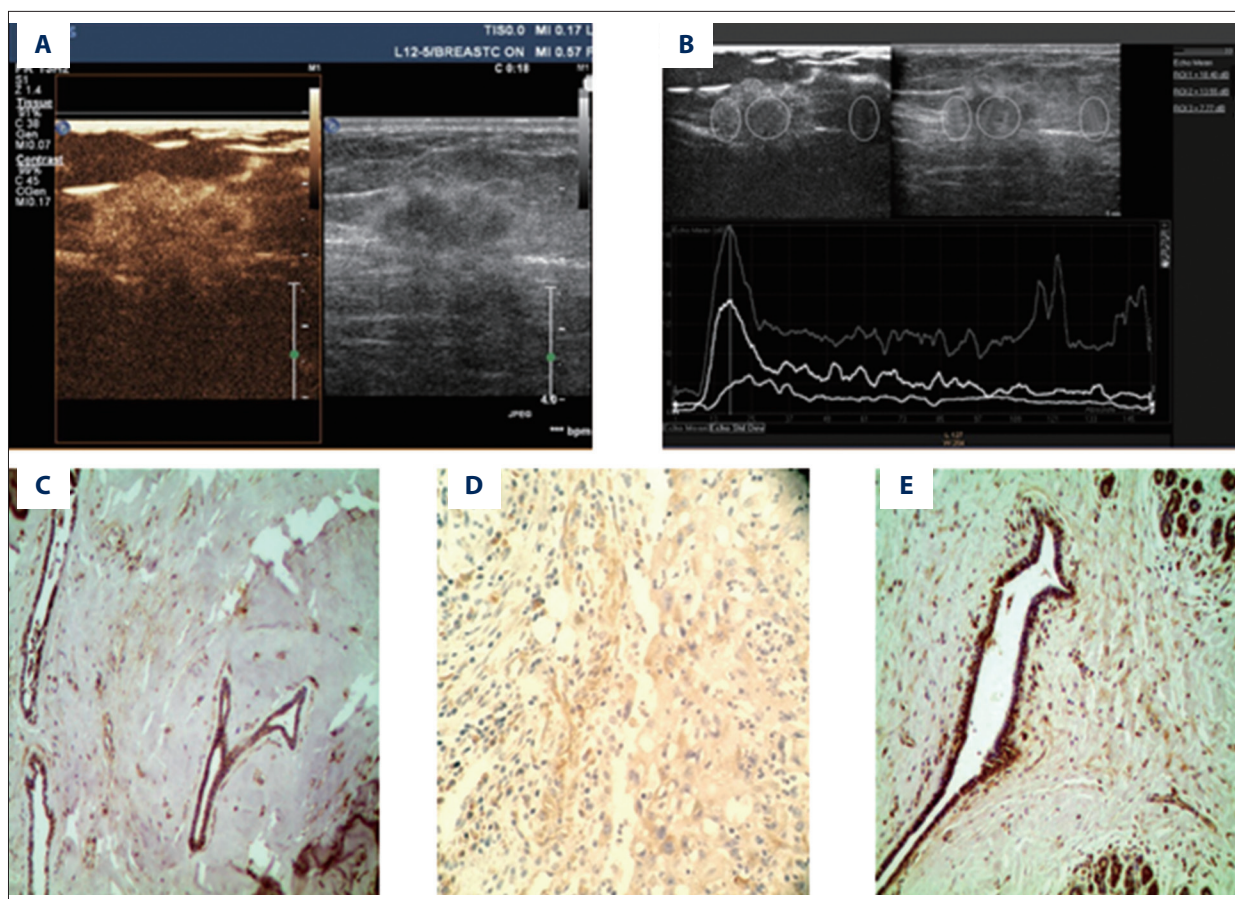
#### Absolute perfusion pattern for peripheral regions of malignant breast cancers

For the analysis of absolute shape of TIC, our results showed that the fast-in and fast-out pattern was the dominant CEUS

perfusion pattern in the peripheral region of cancers. It differed from that of the outflow pattern for enhanced CT TIC of breast cancers, in which the enhancement was prolonged and the DS was lower due to diffusion of the contrast agent into the interstitial space [9]. Further, the central region of the cancers and the surrounding tissue were mainly characterized by slow-in and slow-out pattern. In contrast, in the benign lesions, the proportion of perfusion patterns was similar across different regions.

#### Perfusion characteristics of peripheral region of malignant breast cancers in comparison with other regions

To eliminate the impact of variation between individuals in quantitative analysis, the surrounding tissue was used as a



**Figure 2.** CEUS perfusion characteristics and MVD assessment for a 59-year-old patient with invasive ductal carcinoma (lesion size: 2.9×1.5 cm). **(A)** Uneven hyperechoic enhancement in CEUS, with larger enhancement area compared with the 2-dimensional ultrasound. **(B)** TIC analysis of the central and peripheral regions, and the surrounding tissue. Compared with the central region, the peripheral region was characterized by hyperechoic enhancement and fast-in and fast-out pattern. Compared with the surrounding tissue, the central region showed fast-in and slow-out pattern, as well as higher perfusion level (lower perfusion level during the initial enhancement). **(C–E)** MVD assessment with CD34 immunohistochemical staining (100×). MVD in the peripheral region **(C)** was successively higher than the central region **(D)** and the surrounding tissue **(E)**.

reference to investigate the perfusion pattern of different sites of breast cancers. Our results showed that, compared with the surrounding tissue, the peripheral region of the cancers was characterized by hyperechoic enhancement, fast-in and slow-out pattern, and large AUC, while the central region was characterized by isoechoic enhancement, equally-in and slow-out pattern, and large AUC. Further, the degree of enhancement, perfusion time of contrast agent, and the total perfusion volume in the peripheral region of the cancers were all higher than in the central region, with earlier enhancement compared with the central region, which mainly showed hyperechoic enhancement, fast-in and slow-out pattern, and large AUC. This result was probably due to the differences in the microcirculation between the central and peripheral regions of cancers. Increased neoangiogenesis in the peripheral region of cancers, with thin vascular walls, lacking muscular layer, easily results in arteriovenous fistula [10,11]. Therefore, the contrast

agents are enhanced from the periphery towards the inside, that why the ITP for the peripheral region was earlier than in the central region.

Retention of high concentrations of contrast agent in the tumor vascular bed would result in greater perfusion. However, the formation of tumor thrombus within the veins and peripheral lymphatic vessels aggravated interstitial edema, leading to increased resistance. Therefore, the contrast agent extinction was slower in the peripheral region of cancers than that of the central region. Ultrasound contrast agent could only be visualized in the blood pool. Angiogenesis was more active in the peripheral region of the cancers than in the central region. The increased permeability of the blood vessels due to lack of wall structure resulted in slightly faster wash-out of the contrast agent in the peripheral region compared with the central region. Contrast agent accumulated in the peripheral region of

the cancers, resulting in a larger AUC than in the central region and surrounding tissue. The central region of the cancers is full of connective and fibrous tissues, with hard texture, which is not conducive to angiogenesis, resulting in poor vascularization and degeneration, necrosis, and calcification of cancers [12]. Therefore, perfusion was lower in the central region of the cancers than in the peripheral region. Based on previous findings, with no division in the regions of breast cancers, compared with the surrounding tissue, the overall breast lesion showed hyperechoic enhancement and fast-in and slow-out pattern [13,14], suggesting that the perfusion characteristics of malignant breast cancer would be mainly attributed to the peripheral region. In addition, our results also suggested a significantly different perfusion performance in the peripheral region of the cancers compared with the central region and the surrounding tissue.

### **Perfusion characteristics of peripheral region of benign breast lesions in comparison with other regions**

Our results showed that, in the benign lesions, there were no significant differences in the absolute shape of the perfusion curves between different regions. However, when the surrounding tissues were used as reference, the investigation of the relative shapes of the perfusion curves for different regions was of great importance. The peripheral region showed hyperechoic enhancement and fast-in and fast-out pattern, while the central region displayed isoechoic enhancement, equally-in and equally-out pattern. The analysis of the TIC parameters showed that the degree of enhancement and the total perfusion volume in the peripheral region of the benign lesions were higher than in the central region, showing hyperechoic enhancement and fast-in and fast-out pattern. The analysis revealed that the perfusion pattern in the peripheral region of the benign lesions was different from the central region and the surrounding tissue. Therefore, compared with the surrounding tissue, the benign lesions were characterized by fast-in and fast-out pattern, in contrast to the peripheral region of the cancers (fast-in and slow-out). According to Tables 2 and 3, the relative morphological analysis of TIC with the surrounding tissue as reference was superior to the observation of absolute curve type alone (Table 1) in diagnosing malignant and benign breast lesions.

### **Relationship between the perfusion parameters for malignant and benign breast lesions and MVD**

Our results showed that significant differences are observed in the MVD between the central and peripheral regions, and the surrounding tissue, for the malignant breast cancers, and the microvascular-rich region was located in the surrounding tissue. Moreover, the CEUS analysis also showed that the differences in the PI, ITP, AUC, and DS between these different

regions were in line with the differences in MVD, which suggests the differential perfusion performance in these different regions. In comparison with the central region, the peripheral region was associated with relatively high MVD, hyperechoic enhancement, slow-out pattern, and low level of perfusion in the initial enhancement.

Due to the vascular heterogeneity in the peripheral region of breast cancers, obvious structural defects are observed in the tumor blood vessel wall, which might increase vascular permeability, and induce interstitial edema and interstitial reflux obstruction, further leading to excessive accumulation of contrast agent microbubbles. Therefore, compared with the central region, slower RS in the TIC curve would be observed in the peripheral region, suggesting higher perfusion strength. The lower perfusion strength in the initial perfusion period in the peripheral region might be associated with resistance caused by microbubbles. Excessive endothelial cells and abnormal pericytes might induce the vessel retortion and dilatation, and even the formation of cystic blood pool and blood vessel lake [15,16], resulting in increased initial perfusion resistance.

Our results showed that, compared with the central region, the peripheral region exhibited higher level of perfusion, which is in line with the MVD measurement (the vascularization degree in the peripheral region was higher than in the central region). These results suggest the heterogeneity of neonatal microvascular distribution within the tumors, which is mainly distributed in the active peripheral region of the tumor. The correlation analysis further confirmed that, in the breast lesions, the perfusion parameters of MI, ITP, DS, PI, and AUC were significantly associated with MVD, for both the central and peripheral regions. These results suggest the consistency of the vascular heterogeneity of breast cancer in the pathological characteristics and hemodynamics.

## **Conclusions**

In conclusion, compared with the central and surrounding tissue, the perfusion pattern of the peripheral regions of cancers was characterized by hyperechoic enhancement and fast-in and fast-out pattern. In contrast, the perfusion pattern of the peripheral region in the benign lesions was characterized by isoechoic enhancement and slow-in and slow-out pattern. Compared with the surrounding tissue, the peripheral region of the cancers exhibited hyperechoic enhancement and fast-in and slow-out pattern, while the peripheral region of the benign lesions displayed hyperechoic enhancement and fast-in and fast-out pattern, with significant differences. Therefore, it is more objective to evaluate the lesion perfusion strength and pattern with the reference of the surrounding tissue, while it is biased to perform the absolute curve shape-based



assessment of benign and malignant lesions, regardless of the gland background differences. As an independent diagnostic factor, the perfusion performance of the peripheral region of breast cancer might contribute to the assessment of malignant and benign breast lesions based on the perfusion characteristics. Moreover, the perfusion parameters of MI, ITP, DS, PI, and AUC in the peripheral region were significantly associated

with MVD, which might serve as the preferred parameters for disease diagnosis in clinical practice.

## Disclosures

All authors declare no financial or non-financial competing interests.

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