



# Breast Ultrasound Microvascular Imaging and Radiogenomics

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Microvascular ultrasound (US) techniques are advanced Doppler techniques that provide high sensitivity and spatial resolution for detailed visualization of low-flow vessels. Microvascular US imaging can be applied to breast lesion evaluation with or without US contrast agents. Microvascular US imaging without a contrast agent uses a sophisticated wall filtering system to selectively obtain low-flow Doppler signals from overlapped artifacts. Microvascular US imaging with second-generation contrast agents amplifies flow signals and makes them last longer, which facilitates hemodynamic evaluation of breast lesions. In this review article, we will introduce various microvascular US techniques, explain their clinical applications in breast cancer diagnosis and radiologic-histopathologic correlation, and provide a summary of a recent radiogenomic study using microvascular US.

**Keywords:** Breast; Ultrasound; Ultrasonography; Microvascular imaging; Contrast agent; Radiogenomics; Radiomics

## INTRODUCTION

The development and progression of cancer are attributed to the non-physiological proliferation of immature tumor vessels in response to tumor hypoxia, which is called tumor angiogenesis [1, 2]. Doppler ultrasound (US) can roughly demonstrate these histologic changes, which present as increased vascularity and irregular or penetrating vessels within the breast cancer. However, the practical role of Doppler US is debatable when distinguishing small or hypovascular cancers from hypervascular benign tumors

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because of its low sensitivity for detecting microvessels (< 0.1 mm in diameter) within cancer [3].

Microvascular US imaging refers to an advanced Doppler technique that provides improved sensitivity to low-flow vessel signals. Microvascular US imaging can be divided into two types depending on whether or not contrast agents are used. Microvascular US imaging without contrast agents applies an intelligent wall filtering system to preserve low-flow vessel signals and allows the visualization of more microvessels with high resolution [4-6]. Contrast-enhanced US (CEUS) with second-generation contrast agents detects amplified and continuous vessel signals and facilitates real-time scanning of tumor perfusion [4, 7]. Recent investigations have reported the clinical utility of microvascular US imaging in differentiating benign from malignant breast tumors [8-23] and correlated the microvascular US features of breast cancer with corresponding histopathologic vascular changes [12, 24-26].

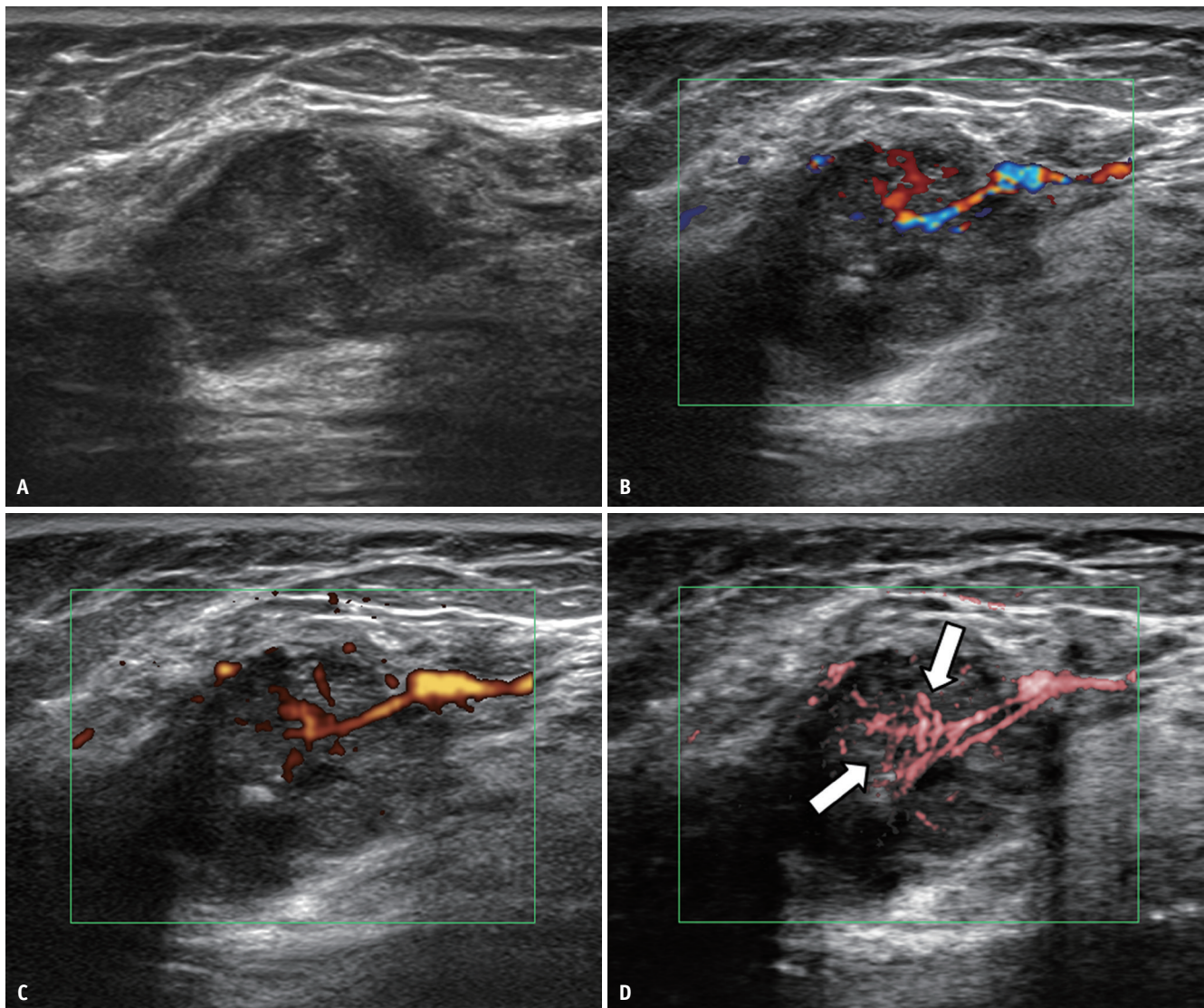
Therefore, the purpose of this review article is to introduce various microvascular US techniques, explain their clinical applications in breast cancer diagnosis and radiologic-histopathologic correlation, and provide a summary of a recent radiogenomic study using microvascular US.

## Microvascular US Imaging without Contrast Agents

### US Techniques

Recently developed microvascular US imaging techniques apply smart wall filtering systems to improve their sensitivity for detecting low-flow vessels without the injection of contrast agents. Superb microvascular imaging (SMI, Canon Medical Systems) is one of the earliest developed and best-known microvascular US imaging modalities. During the Doppler US examination, low-frequency clutter signals, which are derived from patients' motion, pulsation, and respiration, overlap with low-

velocity flow signals [5]. Conventional Doppler techniques apply a single-dimension wall filter to remove clutter artifacts, and overlapping low-velocity flow signals are removed together. However, SMI uses a multi-dimensional wall filter to separate low-velocity flow signals from overlapping clutter artifacts; this allows the visualization of more low-flow vessels that are not visualized within breast lesions on conventional Doppler US (Fig. 1) [4-6]. Other high-end US equipment also provide various microvascular imaging techniques, such as AngioPLUS (SuperSonic Imagine), MicroFlow imaging (Philips Healthcare), and MV-Flow (Samsung Medison), by applying custom smart wall filtering systems and high-frequency sampling techniques



**Fig. 1. Color Doppler US, power Doppler US, and SMI findings of breast cancer.**

**A.** A B-mode US image shows an irregular indistinct hypoechoic mass in the breast that is a surgically-proven invasive ductal carcinoma. Color Doppler US (**B**) and power Doppler US (**C**) images show a penetrating vessel in the mass. **D.** A SMI image shows a penetrating vessel with more branching and irregular vessels (arrows) in the mass. SMI = superb microvascular imaging, US = ultrasound

[5, 27, 28].

Several researchers have reported the superiority of SMI for evaluating breast tumor vascularity when compared with performance of conventional Doppler techniques [8-11, 29]. Park et al. [8] compared the characterizations of lesion vascularity of 191 breast masses by SMI, color Doppler, and power Doppler imaging and reported that SMI detected twice as many tumor vessels as color or power Doppler imaging and identified more detailed vessel morphology and distribution. Ma et al. [9] compared SMI with color Doppler imaging by grading the vascularity of 123 breast lesions on a scale of 0 to 3, and SMI had higher grades for detecting tumor vessels than color Doppler imaging. Zhan et al. [10] evaluated 82 breast lesions that were assessed as category 3 or 4 and avascular on color Doppler imaging and reported that SMI detected higher numbers of penetrating vessels in breast lesions. These results imply that SMI is more sensitive for detecting tumor vessels than conventional color or power Doppler imaging, and it provides more detailed information on microvessels in breast lesions.

### Clinical Applications for Differentiating Benign from Malignant Breast Lesions

Recent investigations have revealed that SMI can be useful for differentiating malignant from benign breast masses [8-11, 30]. Several studies have suggested that SMI depicts malignant vascular features, such as hypervascularity, the presence of penetrating vessels, or irregular vessel morphology better, and it can improve the diagnostic performance of B-mode US or conventional color or power Doppler imaging when used as their adjunct (Fig. 2) [8, 9, 11]. In a recent meta-analysis based on 15 preceding SMI studies, the pooled sensitivity, specificity, and area under the curve (AUC) of the summary receiver operating characteristic curve for SMI for distinguishing breast cancer from benign tumors were 81%, 71%, and 0.87, respectively, and there was no publication bias according to the diagnostic criteria and US equipment [30]. Some researchers evaluated the utility of AngioPLUS imaging in breast cancer diagnosis and found that more vessels and the combination of internal and peripheral vessels suggest breast malignancy [15, 27].

Other studies have investigated whether SMI is useful for differentiating between ductal lesions [16, 17, 31]. Bakdik et al. [17] reported that all malignant ductal lesions showed 3 or more vessels on SMI. Kim et al. [16] reported that malignant ductal lesions showed more vessels, periductal

and intraductal vessel distributions, and penetrating or branching vessel morphology on SMI. The diagnostic performance of SMI was superior to that of power Doppler imaging, and SMI improved the diagnostic performance of B-mode US as an adjunct for differentiating ductal malignancy from benign ductal lesions [16, 17].

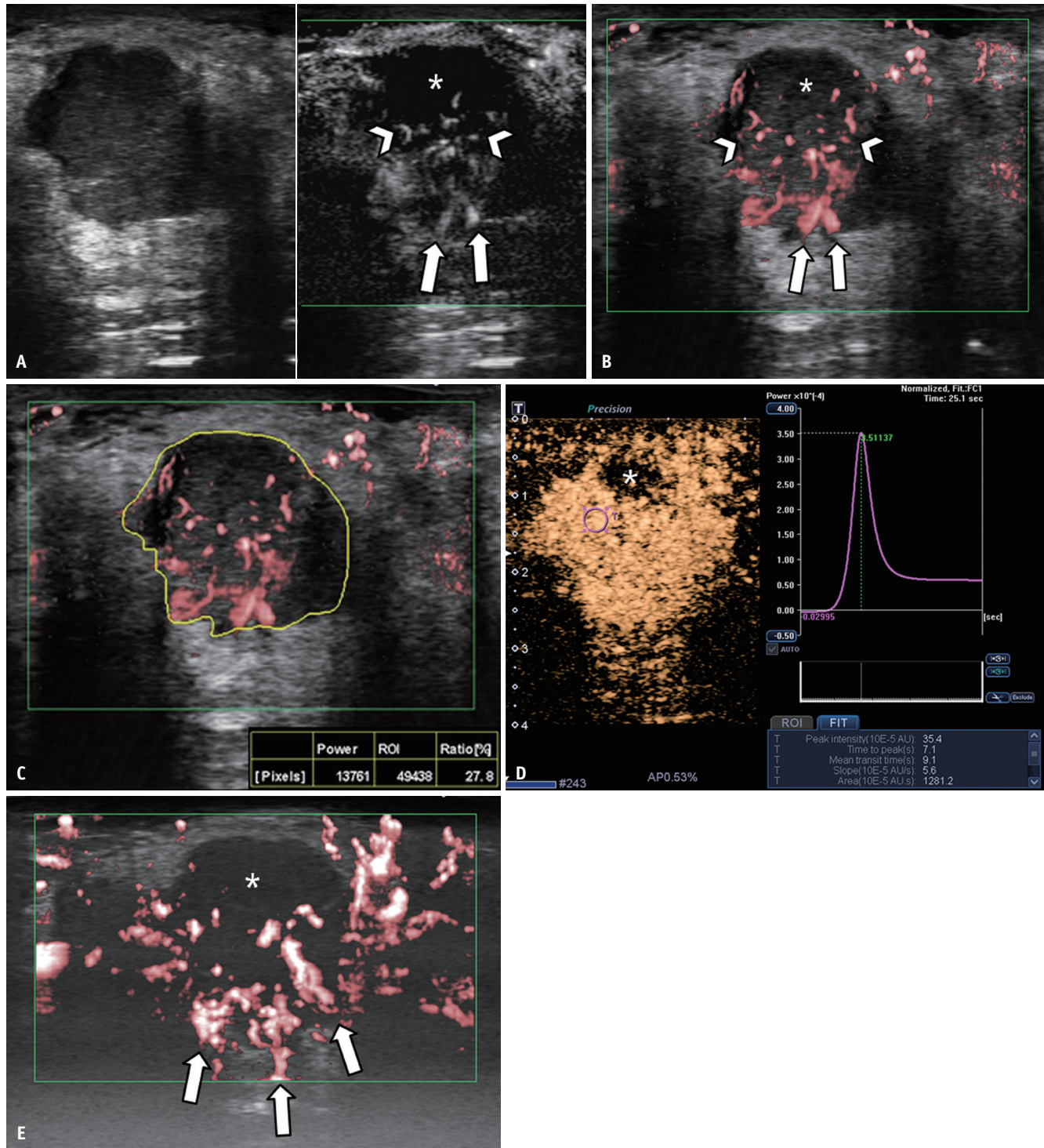
More recent investigations of SMI use an objective parameter, called the vascular index, to estimate the degree of vascularity. The vascular index (%) indicates the ratio of the number of pixels used to depict the Doppler signal to that used for the total lesion, and it can be automatically measured using built-in packages for analysis or offline analysis software (Fig. 2) [4]. Recent studies have reported that malignant breast tumors have a higher vascular index than benign tumors [12-14]. Park et al. [12] reported that the vascular index, with a cut-off value of 8.9%, yielded the highest accuracy for differentiating malignant from benign masses among various 2-5 vascular parameters of SMI and CEUS (76.5%), and this could reduce 26 unnecessary biopsies out of 38 needed for category 4A masses. Zhang et al. [13] measured the vascular index of breast tumors using a 3-dimensional SMI image, and a cut-off value of 4.0% showed a sensitivity of 76%, a specificity of 66%, and an accuracy of 71%. Chae et al. [14] reported that the use of the vascular index with a cut-off value of 3.0% improved the diagnostic performance of B-mode US alone (AUC, 0.853-0.912 vs. 0.795-0.824).

However, investigations on microvascular US imaging without contrast agents are limited to a few US equipment, and the diagnostic criteria or the cut-off values of the vascular index for discriminating breast cancer from benign tumors vary among studies. Further large-scale studies with various microvascular US techniques are expected to establish clinically useful vascular criteria for breast cancer diagnosis.

### Correlation between US Features and Histologic Features

Microvessel density (MVD) assessment is the gold standard for quantifying intratumoral angiogenesis using immunohistochemical staining of blood vessels [2]. High MVD in breast cancer suggests poor relapse-free and overall survival [2]. Several researchers have attempted to verify the predictive value of microvascular imaging features of breast tumors for the degree of histologic MVD (Figs. 3, 4)[12, 15, 24]. In SMI studies, the semi-quantitative grade of the vascular amount or vascular index (%) was significantly correlated with MVD (Spearman's





**Fig. 2. Microvascular US imaging of a breast cancer.**

**A.** B-mode US and monochrome SMI images show a non-parallel irregular indistinct hypoechoic mass with penetrating vessels (arrows), central irregular vessels (arrowheads), and a perfusion defect (asterisk) in the breast that is a surgically-proven invasive ductal carcinoma. **B.** A color SMI image shows penetrating vessels (arrows), central irregular vessels (arrowheads), and a perfusion defect (asterisk) in the mass. **C.** A color SMI image with vascular index measurement shows increased vascular index (27.8%). The vascular index (%) is the ratio between pixels for the Doppler signal and those for the total lesion. **D.** A CEUS image with time-intensity curve analysis shows hyperenhancement of the mass with a perfusion defect (asterisk) and a strong and rapid enhancement (high peak intensity, slope, and area under the curve) when the ROI (pink circle) is set in the area with the strongest enhancement. **E.** An SMI image after CEUS examination shows more vessels, more penetrating vessels (arrows), and a constant perfusion defect (asterisk) than an SMI image before CEUS examination (**B**). CEUS = contrast-enhanced ultrasound, ROI = region of interest, SMI = superb microvascular imaging, US = ultrasound

rank correlation coefficient [ $r$ ] = 0.63–0.82) [12, 24]. In a similar study using AngioPLUS, the combination of the internal and peripheral vascular distributions was significantly associated with a higher MVD than no vessel or internal vascular distribution [15].

In addition to MVD evaluation, microvascular US features of breast cancer were associated with the histologic findings predictive of tumor aggressiveness. Son et al. [32] found that malignant vascular signs on AngioPLUS, such as hypervascularity with radial orientation and a central or penetrating distribution, were significantly associated with histologic tumor size and human epidermal growth factor receptor 2 (HER2) positivity.

## Microvascular US Imaging with Contrast Agents

### US Techniques

CEUS is another microvascular US technique that applies microbubble US contrast agents. US contrast agents are injected intravenously and reach breast tumors after cardiovascular circulation. US contrast agents in the tumor vessels enhance the backscatter of US waves, amplify blood flow signals, and provide microvascular information on breast tumors [4]. Early US contrast agents were easily destroyed in the vessels within 2 minutes, and their contrast effects were not prolonged enough during continuous US scanning. However, the second-generation US contrast agents introduced in 2001 contain an internal slowly-diffusing gas, and their contrast effects last for up to 10–15 minutes, which allows continuous hemodynamic evaluation of breast lesions [4, 7, 33]. Various US contrast agents are commercially available, and Sonovue (Bracco SpA) or Sonazoid (Daiichi-Sankyo) are commonly used in breast tumor evaluation.

In addition, the low mechanical index (< 0.3) technique, with the contrast-specific mode, is essential for CEUS examination with second-generation US contrast agents [4, 33]. The mechanical index is the maximum amplitude of the pressure pulse in the tissue. A lower mechanical index minimizes the destruction of microbubbles and prolongs the contrast effects [33]. The contrast-specific mode is a special technology in US machines that can discriminate the nonlinear signals generated by the US contrast agents from the linear signals generated by the tissue and the contrast agents. At a low mechanical index, linear signals from tissues and US contrast agents are difficult to differentiate with routine US techniques. However, by using the contrast-

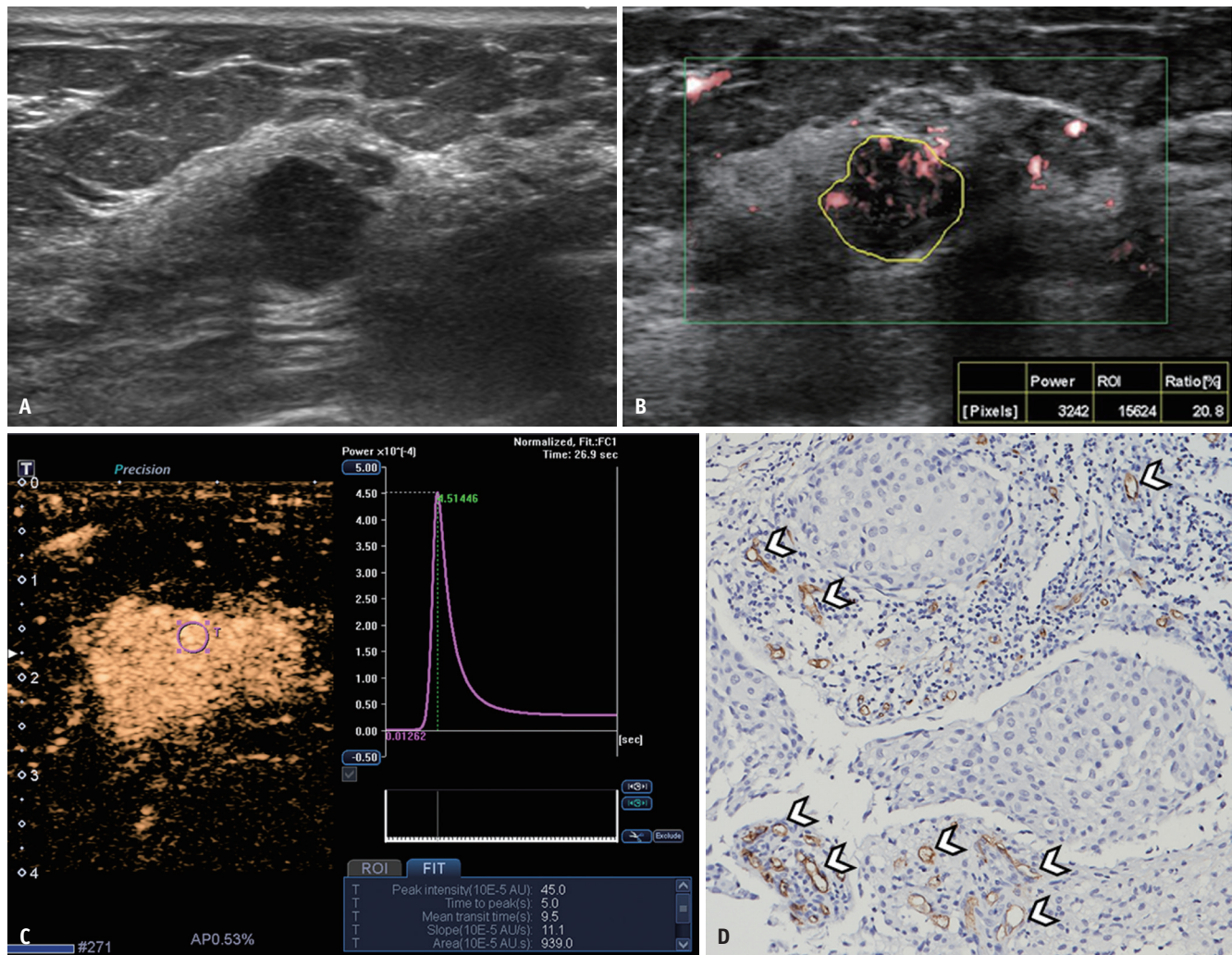
specific mode, it is possible to detect and differentiate the nonlinear signal from the US contrast agents and obtain CEUS images [33].

We can evaluate tumor vascularity qualitatively and quantitatively using CEUS examinations with second-generation contrast agents. Qualitative evaluation involves the subjective evaluation of the enhancement pattern of lesions, such as the degree of enhancement relative to adjacent normal tissue, enhancement order, margin, internal homogeneity, penetrating vessels, and perfusion defects. Quantitative evaluation involves the time-intensity curve analysis using built-in analysis packages or offline analysis software. With the placement of a region of interest in the area of the strongest enhancement, the time-intensity curve is drawn and various quantitative parameters, such as peak intensity, time-to-peak, mean transit time, wash-in velocity, and area under the time-intensity curve, are automatically analyzed (Fig. 2) [4, 7].

### Clinical Applications for Differentiating Benign from Malignant Breast Lesions

Several studies have investigated whether CEUS with a second-generation contrast agent can help distinguish malignant from benign breast tumors. Common qualitative features suggestive of malignancy on CEUS include heterogeneous hyperenhancement of lesions in comparison with adjacent normal tissue, centripetal enhancement order, and the presence of intralesional perfusion defects or penetrating vessels. This implies the extensive angiogenesis of breast cancer, the complex histologic composition including tumor cell clusters and desmoplastic stroma, and the central necrosis or fibrosis [12, 18–21, 34]. In addition, common quantitative malignant vascular features on the time-intensity curve of CEUS include a high peak intensity and slope, short time-to-peak, and a high area under the time-intensity curve, which implies the strong and rapid contrast uptake by malignant breast tumors (Fig. 2) [12, 18–21]. With the use of these malignant vascular features, most CEUS studies for breast cancer diagnosis have stated that the addition of CEUS could improve the diagnostic performance of B-mode US alone [7, 22, 23]. The most recent meta-analysis by Li et al. [23] reported that the diagnostic performances of CEUS and B-mode US in 9 studies were 93% and 87% for pooled sensitivity and 86% and 72% for pooled specificity, respectively. In 5 studies comparing B-mode US plus CEUS with B-mode US alone, the diagnostic performances were 94% and 87% for





**Fig. 3. Microvascular US imaging of a breast cancer with histologic MVD assessment.**

**A.** A B-mode US image shows a non-parallel irregular indistinct hypoechoic mass that is surgically-proven as an invasive ductal carcinoma. **B.** A color SMI image with vascular index measurement shows multiple irregular vessels in the mass and an increased vascular index (20.8%). **C.** A CEUS image with time-intensity curve analysis shows hyperenhancement of the mass and a rapid and strong enhancement (high peak intensity, slope, and area under the curve), when the ROI (pink circle) is set within the area with the strongest enhancement. **D.** Histologic MVD assessment using immunohistochemical staining of microvessels (arrowheads) with a CD34 antibody shows a high MVD, 45 (x 200 magnification). CEUS = contrast-enhanced ultrasound, MVD = microvessel density, ROI = region of interest, SMI = superb microvascular imaging, US = ultrasound

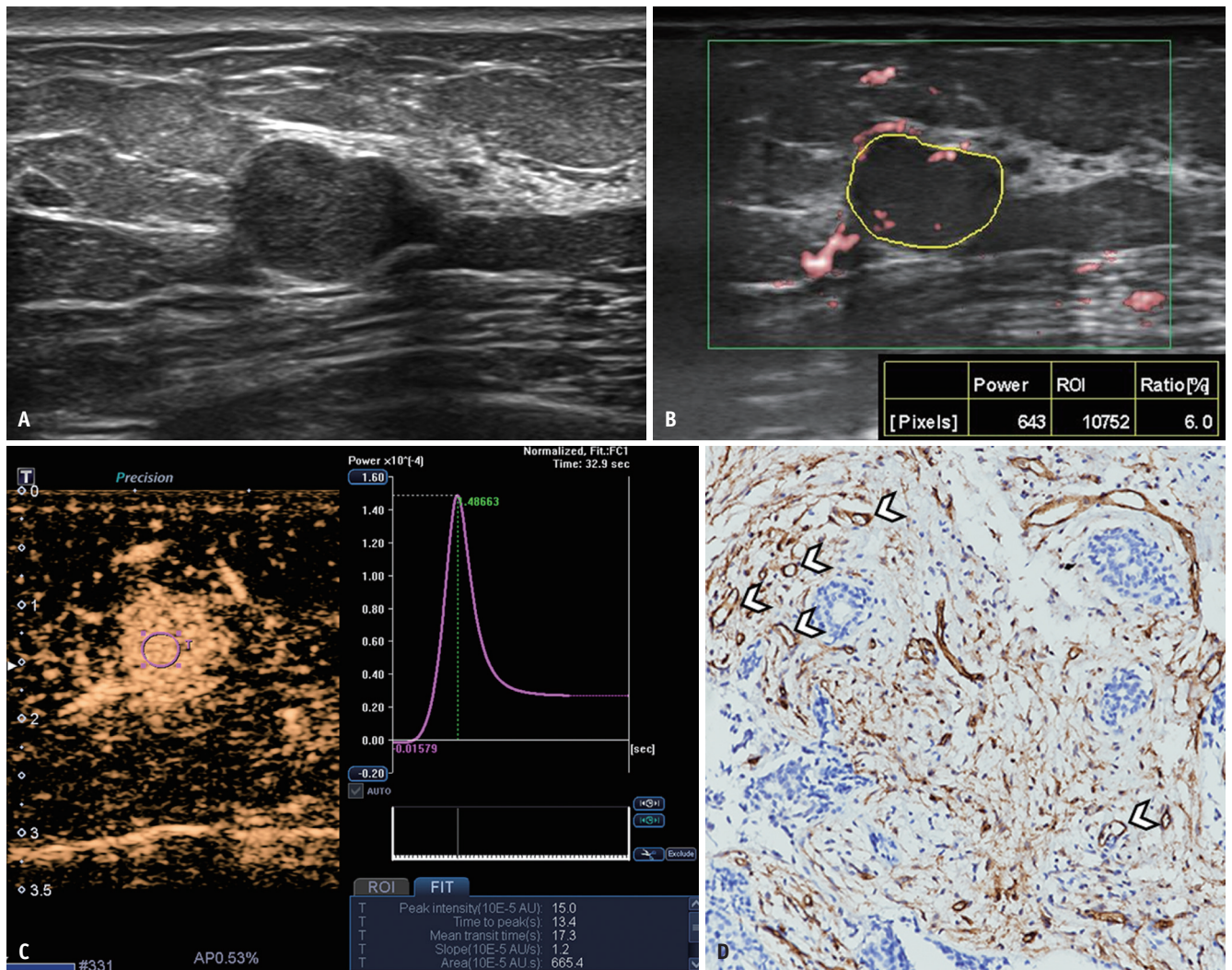
pooled sensitivity and 86% and 80% for pooled specificity, respectively [23]. B-mode US plus CEUS also had a better area under the summary receiver operating characteristic curve for diagnosing breast malignancy than B-mode US alone (AUC, 0.965 vs. 0.911;  $p < 0.001$ ) [23].

Two recent studies compared the diagnostic performances of SMI and CEUS for differentiating breast cancer from benign tumors [12, 35]. Xiao et al. [35] reported that the diagnostic performance of SMI was equivalent to that of CEUS, using root hair-like and crab claw-like vascular patterns as diagnostic criteria (AUC, 0.865 vs. 0.891). Park et al. [12] evaluated various quantitative and qualitative parameters of SMI and CEUS, and reported that their

diagnostic performances were not different (AUC, 0.863 vs. 0.841). Therefore, microvascular US imaging without contrast agents is clinically useful for evaluating tumor vascularity, considering the limitations of CEUS, such as the need for intravenous contrast injection and the time-consuming processes of imaging acquisition and post-imaging analysis.

#### Correlation between US Features and Histologic Features

Several quantitative and qualitative CEUS parameters are correlated with histologic MVD. Qualitative parameters, including hyperenhancement, centripetal enhancement order, and perfusion defects, and qualitative parameters, including



**Fig. 4. Microvascular US imaging of a benign tumor with histologic MVD assessment.**

**A.** A B-mode US image shows an oval circumscribed hypoechoic mass that is biopsy-proven as a fibroadenoma. **B.** A color SMI image with vascular index assessment shows a few peripheral dot-like vessels in the mass and a low vascular index (6.0%). **C.** A CEUS image with time-intensity curve analysis shows hyperenhancement of the mass, but a slow and weak enhancement (low peak intensity, slope, and area under the curve) when the ROI (pink circle) is set within the area with the strongest enhancement. **D.** Histologic MVD assessment using immunohistochemical staining of microvessels (arrowheads) with a CD34 antibody shows a low MVD, 22 (x 200 magnification). CEUS = contrast-enhanced ultrasound, MVD = microvessel density, ROI = region of interest, SMI = superb microvascular imaging, US = ultrasound

high peak intensity, stiff wash-in slope, and high area under the time-intensity curve, were associated with increased MVD in recent studies ( $r = 0.43-0.57$ ) [12, 25, 26].

CEUS features were associated with other histologic features predictive of tumor aggressiveness. Two recent studies reported that perfusion defects on CEUS were associated with estrogen receptor negativity and HER2 positivity [21, 25]. In these studies, there were also significant associations between penetrating vessels and poor histologic grade, as well as between stiff wash-in slope and Ki-67 positivity. Therefore, microvascular US features with or without contrast agents can reflect histologic

vascular changes in breast cancer and predict prognosis in breast cancer.

### Radiogenomic Study Using Microvascular US

Radiogenomics is an emerging research field that correlates imaging phenotypes with underlying genes, mutations, and expression patterns [36]. Radiogenomic studies are attracting attention because they can predict clinical outcomes and identify new imaging biomarkers, which can facilitate better treatment. Most radiogenomic studies on breast cancer have focused on the correlation



between radiomic features on breast MRI and genomic information, such as genetic mutations, molecular subtype, and recurrence scores [36].

Recently, however, a new radiogenomic study correlated morphologic and vascular US features of 31 breast cancers and their RNA sequencing results [37]. In this study, 13 US phenotypes on B-mode US, SMI, and CEUS were associated with 340 differentially expressed genes that were upregulated or downregulated by more than 4-fold. Among them, 21 genes associated with eight microvascular US features were relevant to tumor growth, metastasis, hormone receptor status, and drug resistance (Table 1)

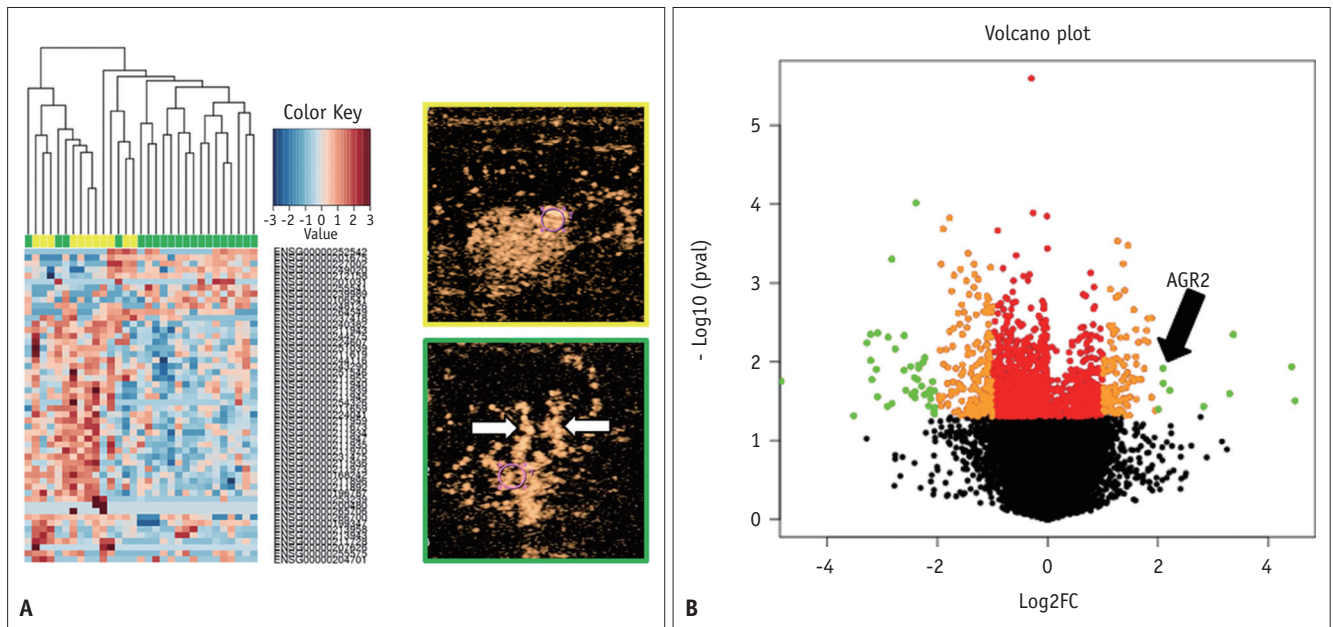
[37]. Breast cancer with complex vessel morphology on SMI showed a downregulation of the *CRIPAK* gene (5.4-fold) and upregulation of the *FZD8* gene (4.1-fold) when compared with the expression of these genes in breast cancers with simple or no vessels [37]. *CRIPAK* gene confers anti-angiogenic effects [38] and, thus, the downregulation of *CRIPAK* can activate tumor angiogenesis, which can be manifested as irregular tumor vessels on SMI. *FZD8* is known to be a key gene for drug resistance in triple-negative breast cancer [39]. Therefore, *FZD8*-targeted agents may be therapeutic for triple-negative breast cancer with complex vessel morphology. In addition, penetrating vessels on SMI

**Table 1. Genes Relevant with Breast Cancer according to the Microvascular US Phenotypes**

US Phenotypes	Genes	Related Functions	Fold Change*	P
Super microvascular imaging				
Vascular index	<i>MIR1307</i>	Cisplatin resistance in breast cancer	11.6	0.002
	<i>HIST2H2BE</i>	Endocrine resistance in ER-positive cancer	4.1	< 0.001
	<i>MIR597</i>	Tumor suppressor	0.2	0.048
Vessel morphology	<i>FZD8</i>	Drug resistance in triple-negative breast cancers, metastasis	4.1	0.01
	<i>NMI</i>	Tumor suppressor	0.2	< 0.001
	<i>IGF1R</i>	High expression in ER-positive cancers, low-grade tumor	0.2	0.006
	<i>UBB</i>	Cell proliferation	0.2	0.006
	<i>CRIPAK</i>	Anti-angiogenic effect	0.2	0.01
	<i>SNHG20</i>	Cell proliferation, invasion, migration	0.2	0.04
Penetrating vessel	<i>SNHG12</i>	Cell proliferation, apoptosis and migration in triple-negative breast cancer	0.1	0.01
	<i>CST1</i>	Cell proliferation, migration, and invasion	6.3	0.003
	<i>CRIPAK</i>	Anti-angiogenic effect	0.2	0.009
	<i>AREG</i>	Cell proliferation and migration of HER2-positive cancer, development of ER-positive breast cancer	0.1	0.007
Contrast-enhanced US				
Enhancement order	<i>SNHG12</i>	Cell proliferation, apoptosis and migration in triple-negative breast cancer	0.2	0.002
	<i>MIR562</i>	Angiogenesis	0.1	0.003
	<i>VTRNA2-1</i>	Tumor suppressor	0.02	0.007
Enhancement margin	<i>TFF1</i>	High expression in ER-positive cancer, metastasis	7.7	0.02
	<i>STC2</i>	Cell migration and invasion	6.3	0.002
	<i>HOXB5</i>	Cell proliferation and invasion	4.5	< 0.001
	<i>PHLDA2</i>	Tumor suppressor	0.3	0.003
Internal homogeneity	<i>CXCL10</i>	Cell proliferation and invasion	0.2	0.007
	<i>HLA-DQA1</i>	Development of breast cancer	9.8	< 0.001
Penetrating vessel	<i>AGR2</i>	Metastasis, high expression in ER-negative cancer	4.3	0.01
	<i>MIR562</i>	Angiogenesis	0.1	0.01
Perfusion defect	<i>HLA-DQA1</i>	Development of breast cancer	7.0	0.002
	<i>AREG</i>	Cell proliferation and migration of HER2-positive cancer, development of ER-positive breast cancer	0.2	0.02

\*Fold change means the gene expression potential in breast cancers with each malignant microvascular feature (vascular index  $\geq 16.1\%$ , complex vessel morphology, the presence of penetrating vessel, centripetal enhancement order, uncircumscribed enhancement margin, heterogeneous internal enhancement, and the presence of perfusion defect) compared to those without each malignant microvascular feature. ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, US = ultrasound





**Fig. 5. Radiogenomic analysis using CEUS and RNA sequencing.**

**A.** A heat map shows 52 differentially expressed genes stratified by the presence of penetrating vessels on CEUS. The columns represent 31 individual breast cancers with (yellow) or without (green) penetrating vessels, and the rows represent 52 individual differentially expressed genes (Ensemble Gene ID reference [45]). The color key indicates the degree of differential gene expression during upregulation (red) or downregulation (blue). The CEUS image with the yellow border shows a breast cancer without penetrating vessels in a 50-year-old woman, and the CEUS image with a green border shows a breast cancer with penetrating vessels (white arrows) in a 74-year-old woman. **B.** A volcano plot demonstrates the differentially expressed genes in breast cancers with penetrating vessels compared with those without penetrating vessels ( $p < 0.05$ ). The x-axis represents the degree of the differential gene expression (log two-fold change [ $\log_2FC$ ]) of individual genes, and the y-axis represents the negative logarithm of their  $p$  value to base 10. Positive  $\log_2FC$  values represent upregulation in cancers with penetrating vessels, and negative values represent downregulation. Green circles represent differentially expressed genes in cancers with penetrating vessels and those without penetrating vessels with  $p$  values of less than 0.05 and  $\log_2FC$  greater than 2.0 or less than -2.0 ( $> 4$ -fold of upregulation or downregulation). The *AGR2* gene (the green circle indicated by a black arrow) is 4.3-fold upregulated in cancers with penetrating vessels compared with those without penetrating vessels ( $\log_2FC = 2.09$ ,  $p = 0.01$ ). Reprinted with permission from RSNA [37]. CEUS = contrast-enhanced ultrasound

and CEUS were associated with the upregulation of *CST1* (6.3-fold) and *AGR2* (4.3-fold), respectively, which are associated with breast cancer cell proliferation, invasion, and metastasis (Fig. 5) [40, 41]. Therefore, the presence of penetrating vessels suggests breast cancer aggressiveness. An elevated vascular index on SMI was also associated with the upregulation of *MIR1307* (11.6-fold) and *HIST2H2BE* (4.1-fold), which can develop drug resistance in breast cancer [42, 43], and the downregulation of *MIR597* (6.2-fold), which can act as a tumor suppressor in breast cancer [44]. This implies that increased vascularity on SMI can predict poor drug response and disease progression in breast cancer. This study suggests that microvascular US features that are easily assessed in routine practice can reflect important genetic alterations related to breast cancer and angiogenesis, and they can provide information for better predictions of prognosis and potential therapeutic targets.

However, radiogenomic exploration that compares

microvascular US features with genetic alterations is now developing. Further investigations involving a larger study population are needed to verify the preceding results, identify novel genes associated with breast cancer, and correlate the findings with actual clinical outcomes in the future.

## CONCLUSION

Microvascular US techniques with or without contrast agents provide more useful and detailed vascular information on breast lesions than conventional color or power Doppler techniques. The supplementary use of microvascular US imaging could improve the diagnostic performance of B-mode US in differentiating breast malignancy from benign tumors. Furthermore, macroscopic vascular features presented on microvascular US can reflect histologic microvascular features and biomarkers of breast tumors and, by extension, predict their genomic

features. Further large-scale investigations are needed to establish standardized criteria for cancer diagnosis for various microvascular US imaging techniques. In addition, an increase in radiogenomic analyses using microvascular US techniques is anticipated for the identification of novel genetic mutations that may be suitable potential therapeutic targets or prognostic markers.

### Conflicts of Interest

Author Bo Kyoung Seo has received research grants from Korea government and Korea University. The remaining authors declare that they have no conflict of interest.

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### Author Contributions

Conceptualization: Bo Kyoung Seo. Funding acquisition: Bo Kyoung Seo. Investigation: Ah Young Park, Mi-Ryung Han. Methodology: all authors. Resources: Bo Kyoung Seo. Supervision: Bo Kyoung Seo. Visualization: Mi-Ryung Han. Writing—original draft: all authors. Writing—review & editing: all authors.

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