Title

Reproducibility and diagnostic performance of a quantitative parameter of superb microvascular imaging in real-time breast ultrasound for evaluating breast masses

Abbreviated title

Vascular index of SMI in real-time breast US

Eun Ji Lee, MD, Yun-Woo Chang, MD, PhD, Eunsun Oh, MD, Jiyoung Hwang, MD, PhD, Hyun-joo Kim MD, PhD, Seong Sook Hong, MD, PhD

1Department of Radiology, Soonchunhyang University Seoul Hospital, Seoul, Korea

Corresponding author: Yun-Woo Chang
Soonchunhyang University Seoul Hospital, 59 Daesagwan-ro, Yongsan-gu, Seoul, Korea
Tel: +82-2-709-9397
Fax: +82-2-709-9397
E-mail: ywchang@schmc.ac.kr

Type of manuscript

Original research

ORCID

Eun Ji Lee : 0000-0002-4860-2495
Yun-Woo Chang : 0000-0001-9704-8112
Eunsun Oh : 0000-0001-5160-390X
Jiyoung Hwang : 0000-0002-3070-4880
Hyun-joo Kim : 0000-0001-5386-1881
Seong Sook Hong : 0000-0003-2893-6583

Acknowledgments

This research was supported by the Soonchunhyang University research fund.

Disclosures

The authors declare that they have no relevant financial disclosures.
Reproducibility and diagnostic performance of a quantitative parameter of superb microvascular imaging in real-time breast ultrasound for evaluating breast masses
Abstract

Purpose: To evaluate the reproducibility and diagnostic performance of a quantitative parameter of superb microvascular imaging (SMI) in real-time breast ultrasound (US) for differentiating benign from malignant breast masses.

Materials and Methods: 87 breast masses in 75 patients who underwent both B-mode US and SMI before US-guided core needle biopsy were included in this study. Two radiologists performed B-mode US and measured the vascular index (VI) of SMI respectively for each lesion in real-time. Intraobserver and interobserver agreements were analyzed for the VI of SMI. Diagnostic performances of B-mode US using a BI-RADS lexicon and combined use with VI of SMI were evaluated compared to pathology.

Results: The median value of VI of malignant masses (n = 32) was significantly higher than that of benign masses (n = 55) (7.6% and 2.6%, respectively, p < 0.001). Intraobserver agreements of VI were excellent regardless of the pathology, size, or depth of the lesion. Interobserver agreement of VI was excellent regardless of the measurement interval. Interobserver agreement for final diagnostic decision was improved with combined B-mode US and VI (κ = 0.883) in comparison with B-mode US only (κ = 0.617). There were significant improvements of specificity, accuracy, and positive predictive value of B-mode US by adding VI in both observers in compared with B-mode US alone (all p < 0.001).

Conclusion: VI of SMI obtained in real-time breast US is highly reproducible with improved diagnostic performance for differentiating between benign and malignant breast lesions when it
is combined with B-mode US.

**Keywords:** Breast, Ultrasound, Doppler, Superb microvascular imaging, Neoplasm
Introduction

Assessment of vascularity of the breast mass is important for differentiating benign from malignancy as malignant masses show more profound tumor vascularity than benign masses due to neoangiogenesis induced by cancerous cells [1]. Although Breast Imaging Reporting and Database System (BI-RADS) categorization based on morphology in B-mode ultrasound (US) takes precedence over other US features in the evaluation of breast masses, tumor vascularity can be noninvasively assessed by Doppler US evaluation of vascular number and morphology of breast lesions [2-4]. A recent multicenter prospective study has shown that the use of color Doppler US in additional to B-mode US can significantly increase specificity in evaluating breast lesions when age is taken into consideration [5]. However, the conventional color Doppler US removes clutter caused by tissue motion together with a low speed of blood flow signals, thus limiting detailed evaluation of tumor vascularity with only qualitative evaluation [6,7].

Superb microvascular imaging (SMI) is an emerging Doppler US technique that can depict slow blood flow apart from clutter using a multi-dimensional filter. By using SMI, it is possible to evaluate more detailed vascularity of a breast mass. It has been reported that qualitative or semi-quantitative evaluation of the tumor vascularity using SMI is superior to that of color or power Doppler US, showing better diagnostic performance in discriminating between malignant and benign breast lesions [8-12]. Larger numbers of tumor vessels, central distribution, and branching and penetrating vessels are known as vascular features associated with malignant breast tumors [8-12]. Microvessels assessed with microvascular Doppler US are also correlated
with microvascular density (MVD) on pathologic specimen, showing stronger correlations than those assessed with color Doppler US [13,14]. These results indicate that SMI has advantages in non-invasive evaluation of vascularity of breast tumors. Introduction of vascular index (VI) has enabled quantitative evaluation of tumor vascularity in SMI [15]. A few studies have reported the diagnostic value of VI in addition to B-mode US for differentiating benign from malignant breast masses [16-18]. However, these studies were focused on the diagnostic performance of the use of VI in additional to B-mode US without assessing inter-observer or intra-observer agreement.

Although it has been reported that the combination of quantitative value of SMI with conventional B-mode US can enhance the diagnostic performance in differentiating benign from malignant breast mass, validating the reproducibility of quantitative values is essential for clinical application. In a few previous studies on the diagnostic performance of SMI using VI, VI of each lesion was retrospectively measured by manually drawing a ROI in a representative image selected using a post-processing program by the operator [16,17,19]. There have been no studies reporting the reproducibility or diagnostic performance of VI measured in real-time US performed by different operators.

Thus, the purpose of this study was to evaluate the reproducibility and diagnostic performance of a quantitative parameter of SMI in real-time breast US and effects of measurement interval, histopathologic type, and intrinsic factors of lesions on the reproducibility.

**Materials and Methods**
**Patients**

This study involved retrospective analysis of prospectively acquired data. It was approved by our Institutional Review Board and the need to obtain informed consent was waived. From November 2019 to June 2020, both B-mode US and SMI were performed by two radiologists for 128 patients who underwent US-guided core needle biopsy for breast lesions on the basis of imaging findings or physician’s decision. Among them, 53 patients were excluded due to the following reasons: prior history of breast cancer with surgery, chemotherapy or radiotherapy (n = 9), prior vacuum-assisted biopsy in the biopsy site (n = 2), lactating women (n = 1), and lesion which the VI cannot be obtained due to the inability to clearly distinguish the region of interest (ROI) such as non-mass lesion (n = 44). Breast US exams were performed including B-mode images and SMI at the time of biopsy or within one month before the biopsy. Finally, 75 patients who had both B-mode US and SMI were included. Twelve patients had two breast masses. A total of 87 breast masses in 75 patients (mean age, 46 years; range, 21-83 years) were included in this study.

**Real-time B-mode ultrasound examination**

All US examinations were performed using one of two identical types of US equipment, Aplio 800 system (Cannon Medical Systems Corporation, Tokyo, Japan) using 7- to 18- MHz multi-frequency ultra-wide band linear array transducer. Examinations were performed by two board-certified radiologists who had 18 and 3 years of experience in breast imaging and had approximately one year of experience in SMI, respectively. US images of B-mode and SMI were
obtained for each breast lesion. Operators were aware of clinical information and mammographic findings before US exams. Two orthogonal B-mode images were obtained for each breast mass and the longest diameter of the lesion was recorded. Lesion depth was recorded as the vertical diameter from the skin to the central portion of the mass and breast thickness was recorded as the vertical diameter from the skin to pectoralis muscle on B-mode image. The ratio of the lesion depth and the breast thickness was divided into three equal parts in the total distribution and classified into superficial, medium, and deep location according to the location where the mass was found. Each lesion was categorized based on features on B-mode according to the American College of Radiology’s BI-RADS, 5th edition[20].

Real-time SMI ultrasound examination

SMI was obtained using the same depth, focus, and time gain of B-mode images. For quantitative evaluation of breast masses, VI of each lesion was obtained from SMI. In each lesion, VI was obtained by manually drawing an ROI along the margin of the mass in a still image with the maximum Doppler signals during a real-time US exam. VI is the percentage ratio between the pixels for Doppler signal within the ROI and those for the total lesion. It is automatically calculated with a dedicated software provided with the US equipment (Figure 1). SMI and VI measurements were done more than twice for the same lesion to evaluate intra-observer agreement. If the patient performed breast US and core needle biopsy at the same day, both observers performed VI measurements on the same day. One observer performed real-time US including B-mode and measurement of the VI of SMI, followed by the other observer.
obtaining additional VI measurements for the target lesion in real-time. If the patient performed scheduled core needle biopsy after breast US, the measurement of VI was performed on a different day by two observers. The VI of SMI was measured by each observer at different days within one month of US exams. If the VI measurement was performed on the same day, it was recorded that the measurement time interval was absent, and if it was performed on the different day, it was recorded as having a measurement time interval. Each observer performed US exams without knowledge of images obtained by another operator. Image parameters for SMI were as follows: velocity scale, 2.5 cm/sec; dynamic range, 21-40 dB; and frame rate, 13-52 frames/sec. Data acquisition for US exams including B-mode and SMI took about 2-3 minutes per lesion.

Data and statistical analysis

To compare the size and VI of SMI between benign and malignant masses, Chi-square test or Mann-Whitney U test was used. BI-RADS assessment categories of lesions based on B-mode US were divided into two groups for statistical analysis. Positive results consisted of categories 4A, 4B, 4C, and 5 while negative results consisted of category 3. Histopathologic results from core needle biopsy or surgical excision were used as reference standards. If surgical excision was subsequently performed followed by core needle biopsy, histopathologic results from the surgical specimen were used.

Intra-observer and inter-observer agreements were assessed by calculating interclass correlation coefficient (ICC) [21] for VI of SMI. Weighted κ values were used to evaluate inter-observer agreements of BI-RADS category assessment and final category assessment in
combination of B-mode and VI of SMI [22]. \( \kappa \) values were interpreted as poor (\( \kappa = 0.0 \)), small/slight (\( \kappa = 0.0–0.20 \)), fair (\( \kappa = 0.21–0.40 \)), moderate (\( \kappa = 0.41–0.60 \)), substantial (\( \kappa = 0.61–0.80 \)) and almost perfect (\( \kappa = 0.81–1.00 \)) agreement between examiners [22]. Measurement reliability was classified as excellent (ICC > 0.75), fair to good (ICC = 0.40–0.75), and poor (ICC ≤ 0.40) [23]. Both ICC scores and \( \kappa \) values were reported with 95% confidence intervals (CIs). Inter-observer agreements of VI according to histopathologic type and intrinsic factors including lesion size and lesion depth were also analyzed through calculation of ICC. Inter-observer agreement of the final assessment based on B-mode US only and combination of B-mode US with VI of SMI of breast masses was assessed using weighted \( \kappa \) values.

The optimal cutoff value was obtained as the average value of the lesion VI measured by two observers. The maximum of Youden index in receiving operating characteristics (ROC) curve analysis was used to obtain the optimal cutoff value of VI. To evaluate additional diagnostic value of VI of SMI to B-mode US, diagnostic performances based on binary results of positive or negative of B-mode US alone and combined B-mode US and VI of SMI were compared. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were included as diagnostic performance indices. For the evaluation of the additional value of VI of SMI, we re-assessed BI-RADS category 4A masses by combining VI of SMI with B-mode US. If VI of SMI was less than the cutoff value, downgrade was performed to make the final result negative. Overall diagnostic indices between B-mode and a combination of B-mode and VI of SMI were compared for both observers using the McNemar’s test. All statistical analyses were performed using Rex 3.1.2 version (rexsoft.org). \( P \)-values less than 0.05 were
considered statistically significant.

**Results**

*Lesion characteristics and histopathologic diagnoses*

Among a total of 87 lesions, 55 (63.2%) were diagnosed as benign and 32 (36.8%) were diagnosed as malignant on US-guided core needle biopsy (n=64), vacuum-assisted biopsy (n=6) or surgical excision (n=17). BI-RADS assessment category of the lesions based on B-mode US was category 3 (n=30), category 4A (n=27), category 4B (n=9), 4C (n=10) and 5 (n=11) in observer 1 and category 3 (n=29), category 4A (n=26), category 4B (n=17), 4C (n=6) and 5 (n=9) in observer 2, Histopathologic diagnoses of breast masses are summarized in Table 1. The median size of malignant masses was significantly ($p = 0.022$) larger than that of benign masses. Malignant masses showed a higher VI than benign masses: 7.3% (interquartile range [IQR]: 3.21-10.28) and 0.9% (IQR: 0 - 3.08) for malignant versus benign lesions, respectively ($p < 0.001$) (Table 1).

*Intra-observer and inter-observer agreement of vascular index of SMI in real-time US*

Intra-observer agreements of VI of SMI were excellent regardless of pathologic type, size, or depth of the lesion (Table 2). Regarding the pathologic type, the intra-observer agreement was excellent for both benign and malignant lesions (ICC: 0.945 and 0.972, respectively). When lesions were divided into three groups according to their sizes (<10mm, 10-20mm and >20mm),
all three groups showed excellent intra-observer agreement (ICC: 0.945, 0.971, and 0.985, respectively). The intra-observer agreement was also excellent regardless whether the lesion depth was superficial, medium, or deep (ICC: 0.935, 0.973, and 0.977, respectively). The inter-observer agreement was excellent regardless of the presence of time interval between measurements of VI (absent of interval ICC, 0.948; presence of interval ICC, 0.925) (Table 3) (Figure 1, figure 2).

There was a substantial agreement in BI-RADS assessment category based on B-mode (κ = 0.722). The agreement was moderate (κ = 0.617) when BI-RADS assessment category was dichotomously divided. When a new dichotomous diagnostic decision was made by applying VI of SMI to downgrade of BI-RADS category 4A lesions, the inter-observer agreement was improved to have a substantial agreement with an increase of kappa value (κ = 0.883) in comparison with a dichotomous diagnostic decision based on B-mode US alone (Table 3) (Figure 2).

*Diagnostic performances of B-mode US alone and combined B-mode with VI of SMI*

The optimal cutoff value of the VI of SMI was 3.37% with a sensitivity of 72.7%, a specificity of 78.1%, and an area under the ROC curve (AUC) of 0.793. When the optimal cutoff value of VI was applied to downgrade BI-RADS category 4A lesions, there was an improvement of diagnostic performance for both observers. In observer 1, 21 out of 27 category 4A lesions, and in observer 2, 18 out of 26 category 4A lesions were downgrade after applying the optimal cutoff value of VI. In both observers, VIs of all category 3 lesions were below the cutoff value. In ROC
curve analyses, AUC values were significantly higher in combined use of B-mode US and VI of SMI compared to those with B-mode US alone for both observers (observer 1 AUC: 0.748 vs. 0.906, \( p < 0.001 \); observer 2 AUC: 0.715 vs. 0.842, \( p < 0.001 \)) (Figure 3). Diagnostic indices for B-mode US and a combination of B-mode US and VI of SMI are summarized in Table 4. For both observers, specificity, PPV, and accuracy were significantly improved in the combined VI of SMI and B-mode US (specificity from 52.7\% to 87.2\% \((p < 0.000)\), PPV from 55.1\% to 81.5\% \((p = 0.000)\), and accuracy from 69.3\% to 89.7\% \((p = 0.000)\) in observer 1; specificity from 49.0\% to 83.6\% \((p < 0.000)\), PPV from 52.6\% to 75.6\% \((p = 0.001)\), and accuracy from 65.9\% to 84.0\% \((p = 0.005)\) in observer 2). For both observers, there were no statistically significant differences in sensitivity or NPV with either imaging method (B-mode US alone or a combination of B-mode US with VI of SMI).

**Discussion**

Our results showed that VI was a highly reproducible and objective quantitative parameter of SMI. Intra-observer reproducibility of VI in real-time US was excellent regardless of pathology, size or depth of the lesion.

In our study, the inter-observer agreement of VI in real-time US was excellent for cases with or without a measurement interval between the observers. In the case of VI measurements performed on different days, there might be differences in ROI placement or SMI settings of the US equipment including dynamic range or frame rate, which might affect the degree of
agreement between examiners. Although there was a mild decrease in the interobserver agreement when there were intervals between VI measurements, the inter-observer agreement was still excellent, suggesting a high reproducibility of VI of SMI.

When VI was used combined with B-mode US, the diagnostic performance for distinguishing benign and malignant breast masses was improved compared to that with B-mode alone. The inter-observer variability in diagnostic decision was also improved. In a previous study, we have investigated the value of combining quantitative parameters of SWE and SMI with B-mode US and found that adding VI alone to B-mode US could increase the diagnostic performance compared to the use of B-mode alone [18]. In that previous study, VI values measured by different observers were used without analyzing intra-observer or inter-observer reproducibility. Chae et al.[19] evaluated interobserver agreement of VI of SMI and added value of the VI with B-mode US. They reported that combination of B-mode US and SMI with VI improved the characterization of breast masses. However, the reader performance was significantly improved only one reader and they used manually drawn region of interest using the previous obtained image video clip[19]. To the best of our knowledge, our study is the first study to evaluation of intra- and inter-observer reproducibility by measuring the real-time VI of SMI.

The optimal cutoff value obtained in the present study was 3.37%, similar to 3.35% in our previous study [18]. However, different of values were reported in previous studies (4% [16] or 8.9% [14], 2.95%[19]). Such differences in cutoff values of VI might be due to different measurement methods and different learning curve of the performers. These studies obtained the
VI by post-processing software that previously obtained image, and the imaging parameters of SMI were some different [14,18,19]. One of the reasons for the difference in VI cut off values of studies may be explained differences in the study population. Our study included BI-RADS category 3, 4 and 5 lesions, but reported the highest cutoff value of VI study were enrolled patients who underwent US-guided biopsy for breast lesions classified as BI-RADS category 4 and 5 lesions [14].

The AUC of VI in this study was 0.793, consistent with results of previous studies (0.776 [16], 0.844 [14], and 0.778 in our prior study[18]). To evaluate the value of adding VI to B-mode US, we applied VI for downgrading BI-RADS category 4A lesions. With a combination of VI with B-mode US, the diagnostic performance was improved, showing statistically significant improvements of specificity, PPV, accuracy, and AUC without significant loss of sensitivity or NPV for both observers. These results are consistent with previous studies using VI of SMI [14,18,19], suggesting that applying VI for downgrading category 4A lesions can reduce unnecessary biopsy.

We also evaluated the effect of combined use of VI with B-mode US on interobserver variability in the final diagnostic decision. The interobserver agreement of dichotomous division of final diagnostic decision based on B-mode alone was improved after adding VI of SMI to B-mode US. Considering that observers in this study had different experiences in breast US, these results meant that the addition of SMI could decrease the variability of diagnostic decision between observers with different expertise in B-mode US.
The present study has a few limitations. First, this study included a limited number of patients with a retrospective design. Thus, it might have a selection bias. In addition, all included patients were scheduled for biopsies. Assessments of their lesions might have been overrated, which might have lowered the specificity. Second, VI value did not reflect the overall vascularity of the lesion. Since measurements of VI were obtained by selecting a 2-D till image of SMI in real-time and drawing ROI manually, it was impossible to quantify volumetric total vascularity of the lesion. This will be possible with the development of technology that enables quantification of 3-D volume data of SMI. Third, the two observers had about one year of accumulated experience of VI measurements with a similar learning curve which might have resulted in a high inter-observer agreement. Lastly, the time interval of VI measurement was variously different within one month, which could lead to bias between two observers. For a generalized use of the quantitative parameter of microvascular Doppler US, further studies using various techniques of microvascular Doppler US including a larger number of patients are warranted.

In conclusion, VI of SMI obtained in real-time breast US showed substantial intra-observer and inter-observer agreements regardless of intrinsic factors of the lesion or the presence of measurement intervals. Adding VI of SMI to conventional B-mode US improved diagnostic performance for differentiating benign from malignant breast lesions. It also improved the inter-observer agreement of the final diagnostic decision. Thus, VI, a highly reproducible quantitative parameter of SMI, might provide additional objective information useful for distinguishing benign from malignant breast masses.
References


12. Zhan J, Diao XH, Jin JM, Chen L, Chen Y. Superb Microvascular Imaging-A new vascular
detecting ultrasonographic technique for avascular breast masses: A preliminary study. Eur J
Radiol 2016;85:915-921.

13. Ma Y, Li J, Ren W, Deng L. Correlation between superb microvascular imaging and

of Ultrasound Microflow Assessment to Distinguish Malignant from Benign Solid Breast
Masses: Association between Ultrasound Parameters and Histologic Microvessel Densities.

15. Park AY, Seo BK. Up-to-date Doppler techniques for breast tumor vascularity: superb

superb microvascular imaging can help to differentiate malignant and benign breast lesion.
Cancer management and research 2019;11:5481-5487.

of Ultrasound Microflow Assessment to Distinguish Malignant from Benign Solid Breast
Masses: Association between Ultrasound Parameters and Histologic Microvessel Densities.

18. Lee EJ, Chang Y-W. Combination of Quantitative Parameters of Shear Wave Elastography and

Superb Microvascular Imaging for the Evaluation of Breast Masses: Comparison With

Imaging Reporting and Data System. 5th ed. Reston, VA: American College of Radiology,
2013:1-173.

21. Stanish WM, Taylor N. Estimation of the Intraclass Correlation Coefficient for the Analysis of

22. Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or
partial credit. Psychol Bull 1968;70:213-220.

23. Hudson JM, Milot L, Parry C, Williams R, Burns PN. Inter- and intra-operator reliability and
repeatability of shear wave elastography in the liver: a study in healthy volunteers. Ultrasound
Figures Legends

Fig. 1
A 49-year-old women with invasive ductal carcinoma. (A) B-mode ultrasound (US) showing 13 mm sized microlobulated irregular hypoechoic mass in a medium depth of the left breast. (B) Each observer independently measured vascular index (VI) of a superb microvascular imaging in real-time by manually drawing a line along the margin of the mass in a still image. Measurement of VI was obtained by each observer on the same day. The vascular index value obtained by each observer was 11.1% (B, observer 1) or 12.2% (C, observer 2). Both observers assessed the mass as BI-RADS category 4B based on B-mode US. The VI of SMI obtained by each observer was over the cutoff value of 3.37%.

Fig. 2
A 37-year-old women with a left breast mass. B-mode US (A) showing an oval, angular, hypoechoic mass in the deep glandular tissue of the left breast. Both observers independently performed B-mode US and SMI on different days. The mass was assessed as BI-RADS category 4A based on real-time B-mode US by both observers (A). Superb microvascular imaging of observer 1 (B) and observer 2 (C) shows minimal Doppler signals within the mass. The VI is 1.0%
by observer 1 and 0.5% by observer 2. As each VI of SMI measured by each observer is less than the cutoff value (3.37%), the final assessment of combined B-mode US and VI of SMI is negative for both observers. The mass is pathologically diagnosed as fibroadenoma on core needle biopsy.

Fig. 3
In ROC curve analyses, AUC values are significantly higher in combined use of B-mode US and VI of SMI that those with B-mode US alone for both observers.
Table 1. Comparison of size and vascular index between benign and malignant

<table>
<thead>
<tr>
<th>N</th>
<th>Benign 55</th>
<th>Malignant 32</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign proliferative breast lesion 25, Fibroadenoma 21, Intraductal papilloma 3, Duct ectasia 2, Sclerosing adenosis 2, Abscess 1, Intramammary lymph node 1</td>
<td>IDC 25, DCIS 6, ILC with DCIS 1</td>
<td></td>
</tr>
<tr>
<td>Size, mm*</td>
<td>9.0 (7.0, 15.5)</td>
<td>13.5 (9.0, 21.0)</td>
<td>0.022</td>
</tr>
<tr>
<td>VI, %*</td>
<td>0.9 (0, 3.08)</td>
<td>7.3 (3.21, 10.28)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

N: number, Data are expressed as median (interquartile range), VI: vascular index, IDC: invasive ductal carcinoma, DCIS: ductal carcinoma in situ, ILC: invasive lobular carcinoma
Table 2. Intraobserver agreement of vascular index according to intrinsic factors of the breast lesions

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>ICC</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.960</td>
<td>(0.935-0.975)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.945</td>
<td>(0.879-0.975)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.972</td>
<td>(0.936-0.988)</td>
</tr>
<tr>
<td>Pathology</td>
<td>Overall</td>
<td>87 (100)</td>
<td>(0.879-0.975)</td>
</tr>
<tr>
<td></td>
<td>Benign</td>
<td>55 (63.2)</td>
<td>(0.935-0.988)</td>
</tr>
<tr>
<td></td>
<td>Malignant</td>
<td>32 (36.8)</td>
<td>(0.944-0.988)</td>
</tr>
<tr>
<td>Size</td>
<td>&lt; 10 mm</td>
<td>37 (42.5)</td>
<td>(0.876-0.968)</td>
</tr>
<tr>
<td></td>
<td>10-20 mm</td>
<td>20 (23.0)</td>
<td>(0.937-0.968)</td>
</tr>
<tr>
<td></td>
<td>&gt; 20 mm</td>
<td>30 (34.5)</td>
<td>(0.940-0.988)</td>
</tr>
<tr>
<td>Lesion depth ratio*</td>
<td>Superficial</td>
<td>26 (29.9)</td>
<td>(0.944-0.988)</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>35 (40.2)</td>
<td>(0.876-0.968)</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td>26 (29.9)</td>
<td>(0.940-0.988)</td>
</tr>
</tbody>
</table>

Lesion depth ratio*: the value of the lesion depth divided by the breast thickness, ICC: interclass coefficient, CI: confidence interval
Table 3. Interobserver agreement of vascular index of SMI according measurement interval and BI-RADS category

<table>
<thead>
<tr>
<th>Measurement interval</th>
<th>N (%)</th>
<th>ICC</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent*</td>
<td>57 (59.8)</td>
<td>0.948</td>
<td>(0.910-0.970)</td>
</tr>
<tr>
<td>Present †</td>
<td>35 (40.2)</td>
<td>0.925</td>
<td>(0.853-0.962)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Agreement of dichotomatus division‡</th>
<th>N (%)</th>
<th>Kappa</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-mode</td>
<td>87(100)</td>
<td>0.617</td>
<td>(0.440-0.794)</td>
</tr>
<tr>
<td>B-mode + VI</td>
<td>87(100)</td>
<td>0.883</td>
<td>(0.783-0.984)</td>
</tr>
</tbody>
</table>

*Measurement of vascular index in the same day, †measurement of vascular index in different day. ‡Dichotomatus division: B-mode, benign (BI-RADS category 3), malignant (BI-RADS category 4a, 4b, 4c, 5); B-mode + vascular index, downgrade of category 4a lesions according to the cutoff value of vascular index. ICC: interclass coefficient. CI: confidence interval, SMI: superb microvascular imaging.
Table 4. Diagnostic performances of B-mode US alone and combined B-mode with VI of SMI

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer 1 B-mode*</td>
<td>96.9 (32/33)</td>
<td>52.7 (29/55)</td>
<td>&lt;0.001†</td>
<td>69.3 (61/89)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Observer 1 B-mode + VI</td>
<td>93.9 (31/33)</td>
<td>87.2 (48/55)</td>
<td>69.7 (61/88)</td>
<td>55.1 (32/58)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Observer 2 B-mode*</td>
<td>93.9 (31/33)</td>
<td>49.0 (27/55)</td>
<td>&lt;0.001†</td>
<td>65.9 (58/88)</td>
<td>0.005†</td>
</tr>
<tr>
<td>Observer 2 B-mode + VI</td>
<td>84.8 (28/33)</td>
<td>83.6 (46/55)</td>
<td>64.0 (74/88)</td>
<td>52.6 (31/59)</td>
<td>0.001†</td>
</tr>
</tbody>
</table>

Data are expressed as percentage (numbers). SMI: superb microvascular imaging, VI: vascular index of SMI, PPV: positive predictive value, NPV: negative predictive value. B-mode*: dichotomatous division of BI-RADS assessment category of the lesions based on B-mode ultrasound images; category 3 was assessed as negative and category higher than 4a was assessed as positive results, B-mode+VI: downgrading BI-RADS 4a mass in combined use of vascular index of SMI. †p-values indicate comparison of diagnostic performance of between BI-RADS category alone and the addition of VI of SMI.
A 49-year-old women with invasive ductal carcinoma. (A) B-mode sonography showing 13 mm sized microlobulated irregular hypoechoic mass in a medium depth of the left breast. (B) Each observer independently measured vascular index (VI) of a superb microvascular imaging in real-time by manually drawing a line along the margin of the mass in a still image. Measurement of VI was obtained by each observer on the same day. The vascular index value obtained by each observer was 11.1% (B, observer 1) or 12.2% (C, observer 2). Both observers assessed the mass as BI-RADS category 4b based on B-mode US. The VI of SMI obtained by each observer was over the cutoff value of 3.2%.
Figure 1B

A 49-year-old women with invasive ductal carcinoma. (A) B-mode sonography showing 13 mm sized microlobulated irregular hypoechoic mass in a medium depth of the left breast. (B) Each observer independently measured vascular index (VI) of a superb microvascular imaging in real-time by manually drawing a line along the margin of the mass in a still image. Measurement of VI was obtained by each observer on the same day. The vascular index value obtained by each observer was 11.1% (B, observer 1) or 12.2% (C, observer 2). Both observers assessed the mass as BI-RADS category 4b based on B-mode US. The VI of SMI obtained by each observer was over the cutoff value of 3.2%.

<table>
<thead>
<tr>
<th>SMI</th>
<th>ROI</th>
<th>Ratio [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pixels</td>
<td>2181</td>
<td>19602</td>
</tr>
<tr>
<td>cm²</td>
<td>0.07</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Figure

A 49-year-old woman with invasive ductal carcinoma. (A) B-mode sonography showing 13 mm sized microlobulated irregular hypoechoic mass in a medium depth of the left breast. (B) Each observer independently measured vascular index (VI) of a superb microvascular imaging in real-time by manually drawing a line along the margin of the mass in a still image. Measurement of VI was obtained by each observer on the same day. The vascular index value obtained by each observer was 11.1% (B, observer 1) or 12.2% (C, observer 2). Both observers assessed the mass as BI-RADS category 4b based on B-mode US. The VI of SMI obtained by each observer was over the cutoff value of 3.2%.

<table>
<thead>
<tr>
<th>SMI (Pixels)</th>
<th>ROI (Pixels)</th>
<th>Ratio [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2663</td>
<td>21852</td>
<td>12.2</td>
</tr>
<tr>
<td>0.09</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>
A 37-year-old woman with a left breast mass. B-mode US (A) showing an oval, angular, hypoechoic mass in the deep glandular tissue of the left breast. Both observers independently performed B-mode US and SMI on different days. The mass was assessed as BI-RADS category 4a based on real-time B-mode US by both observers (A). Superb microvascular imaging of observer 1 (B) and observer 2 (C) shows minimal Doppler signals within the mass. The VI is 1.0% by observer 1 and 2.8% by observer 2. As each VI of SMI measured by each observer is less than the cutoff value (3.2%), the final assessment of combined B-mode US and VI of SMI is negative for both observers. The mass is pathologically diagnosed as fibroadenoma on core needle biopsy.
A 37-year-old woman with a left breast mass. B-mode US (A) showing an oval, angular, hypoechoic mass in the deep glandular tissue of the left breast. Both observers independently performed B-mode US and SMI on different days. The mass was assessed as BI-RADS category 4a based on real-time B-mode US by both observers (A). Superb microvascular imaging of observer 1 (B) and observer 2 (C) shows minimal Doppler signals within the mass. The VI is 1.0% by observer 1 and 2.8% by observer 2. As each VI of SMI measured by each observer is less than the cutoff value (3.2%), the final assessment of combined B-mode US and VI of SMI is negative for both observers. The mass is pathologically diagnosed as fibroadenoma on core needle biopsy.
Figure 2C
In ROC curve analyses, AUC values are significantly higher in combined use of B-mode US and VI of SMI that those with B-mode US alone for both observers.