Correlation Between Color Power Doppler Sonographic Measurement of Breast Tumor Vasculature and Immunohistochemical Analysis of Microvessel Density for the Quantitation of Angiogenesis

Wei Tse Yang, MBBS, FRCR, Gary M. K. Tse, MBBS, FRCPC, Peggo K. W. Lam, MPhil, Constantine Metreweli, MBBChir, FRCR, Jenny Chang, MD, PhD

Objective. To record the correlation between color power Doppler sonographic measurement of breast tumor vasculature and immunohistochemical analysis of microvessel density for the quantitation of angiogenesis. ***Methods.*** Women with palpable breast masses scheduled for excision biopsy were scanned with two- and three-dimensional color power Doppler sonography before and after the administration of a sonographic contrast agent. Vessel counts were performed on two- and three-dimensional sonographic images before and after contrast agent administration. All tumors were surgically removed and underwent immunohistochemical analysis for microvessel density assessment. The sonographic measure of tumor vascularity was correlated with microvessel density. ***Results.*** Pathologic examination showed 43 breast cancers and 14 benign breast masses. Higher microvessel density was noted in malignant than benign breast masses ($P <$ .0005). Color power Doppler sonographic measurement of tumor vessel number showed a significant positive correlation with tumor size ($P <$ .05) and progesterone receptor negativity ($P <$ .05). A significant positive correlation was observed between microvessel density and the number of intratumoral blood vessels assessed by both two- and three-dimensional color power Doppler sonography ($P <$ .05). Regression models showed three-dimensional color power Doppler sonography to have a significantly higher correlation with microvessel density when compared with two-dimensional color power Doppler sonography at baseline ($P <$ .005). The administration of a sonographic contrast agent did not improve correlation with microvessel density. ***Conclusions.*** A significant correlation was shown between color power Doppler sonographic measurement of tumor vascularity and microvessel density by immunohistochemical analysis. Further improvement in Doppler sonographic techniques to map capillary vessel flow should be explored to improve the current association with pathologic findings. ***Key words:*** angiogenesis; breast neoplasms; sonography, Doppler studies.

Abbreviations
CPD, color power Doppler; ER, estrogen receptor; IHC, immunohistochemical; MR, magnetic resonance; MVD, microvessel density; PR, progesterone receptor; 3D, three-dimensional; 2D, two-dimensional

Received May 14, 2002, from the Department of Diagnostic Radiology and Organ Imaging (W.T.Y., C.M.) and Center for Clinical Trials and Epidemiological Research (P.K.W.L.), Chinese University of Hong Kong, Prince of Wales Hospital, and Department of Anatomical and Cellular Pathology, Prince of Wales Hospital (G.M.K.T.), Shatin, New Territories, Hong Kong; and Breast Care Center, Baylor College of Medicine, Houston, Texas (J.C.). Revision requested June 13, 2002. Revised manuscript accepted for publication June 26, 2002.

This study was supported in part by the University Grants Council direct grant for research, Chinese University of Hong Kong project code 2040743.

Address correspondence and reprint requests to Wei Tse Yang, MBBS, FRCR, Division of Diagnostic Imaging, Section of Breast Imaging, M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Box 57, Houston, TX 77030-4095.

Angiogenesis (also known as neovascularization) is the formation of new capillaries from the existing vascular network and is essential for all tumor growth and dissemination.[1] Tumors that have increased angiogenesis include breast cancer, hepatocellular carcinoma, thyroid carcinoma, melanoma, and renal cell carcinoma. Recent studies have shown that intratumoral microvessel density (MVD) is an important prognostic marker of survival in breast cancer[2,3] and for prediction of the likelihood of systemic metastases.[4] More importantly, vascular density has been shown to decrease in responders to standard chemotherapy[5] and angiogenesis inhibitors.[6] In breast cancer, higher histologic capillary density has been found to correlate with a higher rate of recurrence, independent of other prognostic factors.[7]

To date, there are no standard validated methods for direct measurement of tumor neovascularization. Tumor capillary density, measured as "vascular hot spots" after immunohistochemical (IHC) factor VIII–related or CD31 antigen staining, has been tested as an indicator of angiogenesis in paraffin-embedded specimens[7] and is the yardstick of angiogenesis measurements. These IHC methods are limited because of a random sampling error secondary to intratumoral heterogeneity and the invasiveness of repeated biopsies for the monitoring of response to therapy.[8,9] Hence, it would be important to develop a simple and noninvasive in vivo technique to measure angiogenesis for the prediction of prognosis and for monitoring the response to systemic treatment by sequential repeated measurements.

So far, in vivo imaging techniques have focused on the use of contrast-enhanced magnetic resonance (MR) imaging for characterization of tumor microvasculature. The feasibility of contrast-enhanced MR imaging as a measure of angiogenesis has been explored by several techniques and has shown a correlation with histologic capillary density.[9–12] Nonetheless, MR imaging is time-consuming, expensive, and impractical for routine application.

Although there have been reports on the correlation between Doppler sonography and tumor neovascularization,[13,14] to the best of our knowledge, to date there are no studies in the literature reporting a correlation between pathologic MVD and two-dimensional (2D) and three-dimensional (3D) color power Doppler (CPD) sonography with a contrast agent. The aim of this study was to correlate CPD sonographic measurement of breast tumor vascularity with measurements from IHC analysis of MVD, both before and after administration of contrast material.

Materials and Methods

Study Population

Fifty-seven women age 19 to 87 years (mean, 52 years) with 57 breast masses who were scheduled for excision biopsy were recruited into the study. In vivo measurement of breast tumor vascularity was performed semiquantitatively by CPD sonography. All masses were surgically removed, and MVD by IHC analysis was assessed by a single pathologist (G.M.K.T.). This study received local ethics committee approval, and informed consent was obtained from all patients.

Imaging and CPD Sonography

Before histologic diagnosis, all women prospectively underwent sonography by 1 breast radiologist (W.T.Y.) who was blinded to the patient history. Gray scale sonography followed by CPD sonography was performed on the breast mass with a 5- to 12-MHz linear array transducer with a 6-MHz Doppler operating frequency on an HDI 3000 or HDI 5000 unit (Philips Ultrasound, Bothell, WA). Optimized power angiographic parameters were set at a pulse repetition frequency of 750 to 1000 Hz, a low wall pass filter, medium persistence, high sensitivity, and the use of dynamic motion differentiation. Color power Doppler gain was optimized with an increase in gain until the color box was filled with uniform low-level blue noise with a minimal yellow power signal detected (≈75%–85% gain).[15–17] Two-dimensional color power Doppler sonography was performed, followed by 3D CPD cine loop imaging. Acquisition involved capturing frames of CPD images over 10 seconds at a medium slice thickness (7 slices). The linear array transducer was swept slowly across the tumor mass with a steady pace and constant light pressure to avoid inadvertent compression of vessels and a possible flash artifact. Images were obtained before and after injection of a microbubble echo enhancer. A first-generation galactose-based contrast agent, SH U 508A (Levovist; Schering AG, Berlin, Germany), which contains microbubbles that are maintained during passage through the pulmonary vascular system, was adminis-

tered to provide systemic enhancement.[18,19] The recommended dose of 10 mL of contrast agent at a concentration of 300 mg/mL galactose was injected as a bolus at approximately 2 mL/s via a 19-gauge plastic cannula inserted into the brachial vein and flushed with 10 mL of saline. For evaluation of the contrast agent, the video recording was started 30 seconds before the injection and continued for 5 minutes or the time when the signal intensity of the CPD sonographic signal was judged subjectively to return to the baseline level.

Image Acquisition and Evaluation (Vessel Counts)

The most vascular regions of each tumor were captured on hard copy laser images and magneto-optical disks as well as recorded on videotape for each lesion in 2D and 3D displays both before and after contrast agent administration. For each breast mass, CPD sonographic interrogation was performed, and the number and pattern of vessels were noted. The vessel distribution was subjectively assessed according to modified previously published criteria[20] as follows: (1) peripheral vessels, 1 or more blood vessels along the margin of a mass with minimal branching; (2) central vessels, persistent focal color flow signal within the lesion, not extending to the margins and without substantial branching; and (3) side-branching vessels, persistent reproducible color flow signals that branched from any peripheral or central vessel.

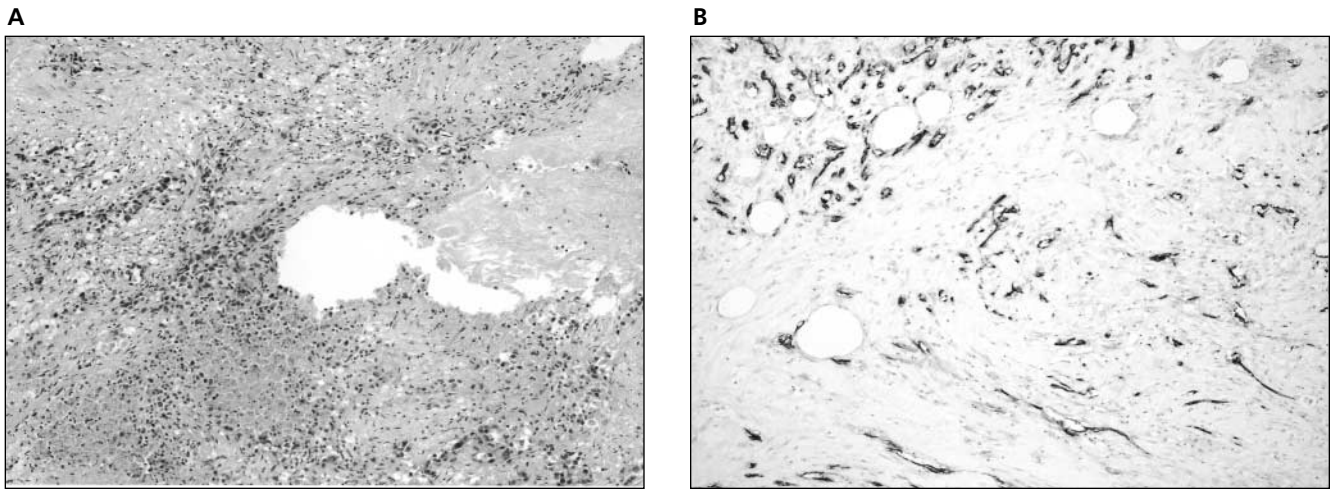
Pathologic Examination

Routine histologic analysis of breast tumors was assessed with hematoxylin-eosin stains. For all cancers, the histologic types were classified according to World Health Organization criteria. Tumor grading was performed according to modified previously published criteria.[21] Tumor size and nodal status were also evaluated. The tumor hormonal receptor status (estrogen receptor [ER], progesterone receptor [PR], and c-erbB2 [HER-2neu] expression) was determined immunohistochemically (ER, 1:100, DakoCytomation Denmark A/S, Glostrup, Denmark; PR, 1:100, Zymed Laboratories, Inc, South San Francisco, CA; and c-erbB2, 1:150, DakoCytomation Denmark A/S).

Pathologic Estimation of MVD

For intratumoral MVD, one 4-μm section that included the most representative part of the lesion (Fig. 1A) was selected for staining with CD31 (1:50, DakoCytomation Denmark A/S) by the standard avidin-biotin method. The stained section was initially scanned at low power to pick up the hot spots with a higher density of CD31-positive cells within the tumor, and 5 high-power fields (×400; field area, 0.19 mm^2) were counted. All positively stained discrete cells or cell clusters with or without visible lumina were counted as 1 microvessel (Fig. 1B).

Figure 1. A, Photomicrograph of invasive ductal carcinoma (hematoxylin-eosin, original magnification ×100). **B**, CD31 IHC analysis of the same breast specimen (original magnification ×400). All dark areas are CD31-positive vascular structures.



Statistical Analysis

The data obtained with 2D and 3D CPD sonography and MVD were compared with the final histologic findings. Pathologic size, nodal status, histologic type, histologic grade, and hormonal receptor status were assessed for all breast cancers. Clinicopathologic correlation with MVD and sonographic measurements of tumor vessel number was evaluated by a 2-sample *t* test. Pearson correlation coefficients were computed between pathologic MVD and sonographic measurement of breast tumor vascularity. Regression models were used to study differences in correlation between 2D and 3D and between precontrast and postcontrast CPD sonography.

Results

A total of 57 breast masses (43 malignant and 14 benign) were studied. The 43 malignant lesions included 34 infiltrating ductal cancers, 4 infiltrating lobular cancers, 2 colloid cancers, 1 apocrine carcinoma, and 2 cases of ductal carcinoma in situ. The 14 benign lesions included 8 fibroadenomas, 3 cases of fibrocystic change, 2 intraductal papillomas, and 1 phyllodes tumor. The mean size was 2.6 cm (range, 1–6 cm) for cancers and 3.2 cm (range, 0.7–10 cm) for benign lesions. Five patients did not undergo axillary dissection. These included 2 patients with ductal carcinoma in situ and 3 frail women 84, 86, and 87 years of age, respectively, with infiltrating ductal carcinoma who underwent wide local excision without axillary surgery.

Mean (SD) MVDs were 19.5 (5.8) for malignant lesions and 12.7 (3.1) for benign lesions ($P < .0005$; Table 1). Mean 2D precontrast, 3D precontrast, and 3D postcontrast CPD sonographic measurements of tumor vessel number were significantly higher in malignant than in benign masses ($P < .005$, .0005, and .05, respectively; Table 1).

Table 1. Association of MVD and Sonographic Measurements of Tumor Vessel Number With Pathologic Findings in 57 Breast Masses

	Malignant (n = 43)		Benign (n = 14)		
Findings by Examination	**Mean**	**SD**	**Mean**	**SD**	***P***
Microvessel density	19.5	5.8	12.7	3.1	<.0005
2D precontrast sonography	7.6	6.9	3.1	3.0	.002
2D postcontrast sonography	15.1	11.0	8.9	8.2	.057
3D precontrast sonography	11.4	7.7	4.3	4.3	<.0005
3D postcontrast sonography	19.3	12.7	11.1	7.7	.038

There was no association between MVD and clinicopathologic parameters (Table 2). Color power Doppler sonographic measurement of tumor vessel number showed a significant positive correlation with pathologic size (all $P < .05$) and a negative correlation with PR (all $P < .05$; Table 3).

For all breast masses, a significant correlation was expressed between MVD and CPD sonographic measurements of tumor vessel number ($P < .05$) both at baseline and after contrast agent administration (Table 4). The scatterplots between 2D and 3D CPD sonography before and after contrast agent administration are shown in Figures 2 and 3. Regression models showed a significantly higher R^2 value for 3D compared with 2D CPD sonography at baseline ($P = 0.005$; Table 5). Contrast agent administration did not improve the correlation with MVD.

Discussion

Breast cancer is the most common female malignancy and is ideally suited for Doppler sonography because of its superficial location and easy penetrability with the transducer, making it a suitable model for the study of in vivo angiogenesis. This neovascularization penetrates the lesion from its periphery and consists of thin-walled blood vessels that lack a muscular layer and often show chaotic anastomoses and shunts.[22–24] The multiplicity of vessels, their disordered pattern, and the arteriovenous shunts of these tumor vessels give rise to flow that can be detected as high-velocity signals with a distinctive rasping sound on continuous wave or pulsed Doppler sonography.[25] These "tumor flow signals" have been detected in carcinomas in the breast and thyroid as well as in primary hepatocellular and renal cell carcinomas.[26–29] Recent developments with the use of color Doppler and CPD sonography have shown a predictive value of tumoral color Doppler signals in determining the nature of breast lesions.[20,30–35] The clinical role of Doppler sonography in breast cancer discrimination is not fully accepted, however. Wilkens et al[36] gave a balanced summary of the mixed but generally positive results with color flow imaging in the breast as of 1998. These methods have been further explored with the addition of microbubble sonographic contrast agents. The use of contrast-enhanced sonography increased the sensitivity and specificity to

Table 2. Association of MVD With Clinicopathologic Characteristics in 43 Breast Cancers

Characteristic	n	Mean	SD	*P*
Pathologic size, mm				
1–20	20	18.6	6.6	.350
>20	23	20.3	5.0	
Histologic grade				
1–2	28	18.9	6.1	.907
3	15	19.1	4.8	
Nodal status				
Negative	22	18.7	5.3	.096
Positive	16	21.7	5.2	
ER				
Negative	12	21.1	5.0	.344
Positive	28	19.1	6.3	
PR				
Negative	14	20.7	4.7	.453
Positive	26	19.2	6.5	
c-erb-B-2				
Negative	23	19.2	6.3	.462
Positive	6	21.4	5.7	

100% in differentiating the vascularity between benign and malignant primary breast lesions.[37] The microbubble sonographic contrast agent Levovist consists of specially manufactured galactose microparticles for suspension in sterile water. It acts as a dose-dependent acoustic scatterer because of the multiple interfaces between the microbubbles and the surrounding fluid. These microbubbles are less than 4 μm in diameter and can therefore cross the lung bed and increase the echogenicity of blood by 10 to 20 dB, thereby enhancing the signal-noise ratio in color Doppler imaging. Levovist also contains a trace of palmitic acid, which improves the intravascular stability of the microbubbles, thereby providing a prolonged effectual blood pool–enhancing agent for an average duration of 6 min. By this mechanism, sonographic imaging with contrast enhancement provides a reliable measurement of blood pool vascularity of tumors.

This report shows significantly higher MVD and CPD sonographic measurements of tumor vessel number in malignant compared with benign breast masses, reinforcing the potential of sonography in differentiating between malignant and benign breast disease. Although a previous report showed no significant correlation between MVD and intratumoral blood flow velocity assessed by color-coded Doppler imaging,[14] the results of this study show a weak but significant correlation between CPD sonograph-

Table 3. Association of Sonographic Measurements of Tumor Vessel Number With Clinicopathologic Characteristics in 43 Breast Cancers

		2D Precontrast Sonography			2D Postcontrast Sonography			3D Precontrast Sonography			3D Postcontrast Sonography		
Characteristic	n	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	*P*
Pathologic size, mm													
1–20	20	4.7	4.3	.006	10.2	8.0	.004	7.7	6.0	.004	12.6	7.4	.001
>20	23	10.1	7.7		19.5	11.5		14.4	7.8		24.8	13.5	
Histologic grade													
1–2	28	6.7	6.7	.275	13.3	10.8	.262	9.8	7.8	.124	15.6	10.8	.035
3	15	9.3	7.6		17.4	11.2		13.8	7.7		24.3	14.0	
Nodal status*													
Negative	22	8.8	8.1	.555	16.1	11.7	.693	11.9	8.2	.917	20.5	13.6	.989
Positive	16	7.4	6.0		14.7	10.0		12.1	7.3		20.6	12.2	
ER†													
Negative	12	11.3	7.9	.049	20.3	10.5	.101	15.1	6.8	.078	26.2	14.6	.043
Positive	28	6.5	6.2		14.0	10.8		10.4	7.7		17.3	11.1	
PR†													
Negative	14	12.8	8.4	.006	23.4	12.5	.005	16.1	7.5	.008	26.8	14.7	.011
Positive	26	5.4	4.4		11.9	7.5		9.5	6.8		16.2	10.0	
c-erb-B2‡													
Negative	23	7.8	7.1	.082	16.5	10.5	.396	12.8	7.9	.433	21.1	12.3	.770
Positive	6	13.8	8.3		20.8	11.8		15.7	7.8		22.7	8.2	

*Nodal dissection was not performed in 5 patients.
†ER and PR status was not determined in 3 patients.
‡c-erb-B2 status was not determined in 14 patients.

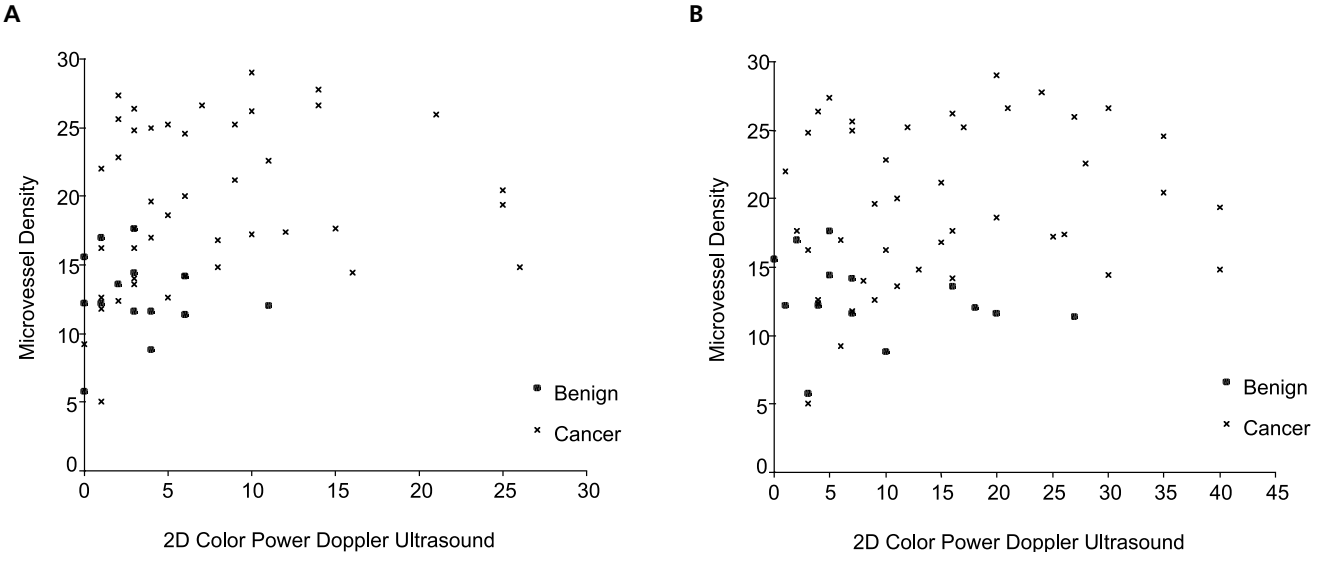
Table 4. Pearson Correlation Coefficients Between MVD and Sonographic Measurements of Tumor Vessel Number in 57 Breast Masses

Examination	Malignant		Benign		All	
	r	*P*	r	*P*	r	*P*
2D precontrast sonography	0.195	.209	–0.041	.889	0.296	.025
2D postcontrast sonography	0.221	.154	–0.227	.435	0.268	.043
3D precontrast sonography	0.365	.018	0.248	.437	0.469	<.0005
3D postcontrast sonography	0.289	.063	–0.038	.906	0.352	.009

ic measurement of the tumor vessel number and pathologic MVD in breast tumors. Color power Doppler sonography is 3 to 5 times more sensitive than color Doppler sonography in the detection of flow signals, because it measures the total integrated Doppler power spectrum, thereby imaging the amplitude of blood flow rather than the direction or velocity, as in conventional color or pulsed Doppler sonography.[15,16] This suggests that high-frequency sonographic studies of angiogenesis may best be directed at the vessel number and morphology rather than blood flow velocity, because velocity indices at current levels of transducer sensitivity and equipment technology may not accurately reflect the pathophysiologic characteristics of angiogenesis. Correlation with MVD was significantly higher with 3D than with 2D CPD sonography. Three-dimensional sonography permits a more global perspective of tumor vessel morphologic characteristics, thus permitting greater accuracy of vessel mapping and improved association with tumor angiogenesis. This can potentially contribute to breast cancer treatment, particularly in the evaluation of the response to medical therapy with antiangiogenic agents.

Previous studies have shown no association between MVD and conventional prognostic indicators, including lymph node status and lesion size.[10,14,38] CPD sonographic vascularity, however, showed a significant positive correlation with lesion size and PR negativity in this study. The exact reasons for these heterogeneous findings are not clear, but this preliminary positive correlation between sonographic measurement of the tumor vessel number and tumor biological characteristics may increase the role of sonography in prognostication of breast disease, with the potential that CPD sonographic vascularity may become an independent prognostic marker.

Figure 2. Scatterplot showing correlation between MVD and 2D CPD sonographic measurement of tumoral vascularity before (**A**) and after (**B**) contrast agent administration.



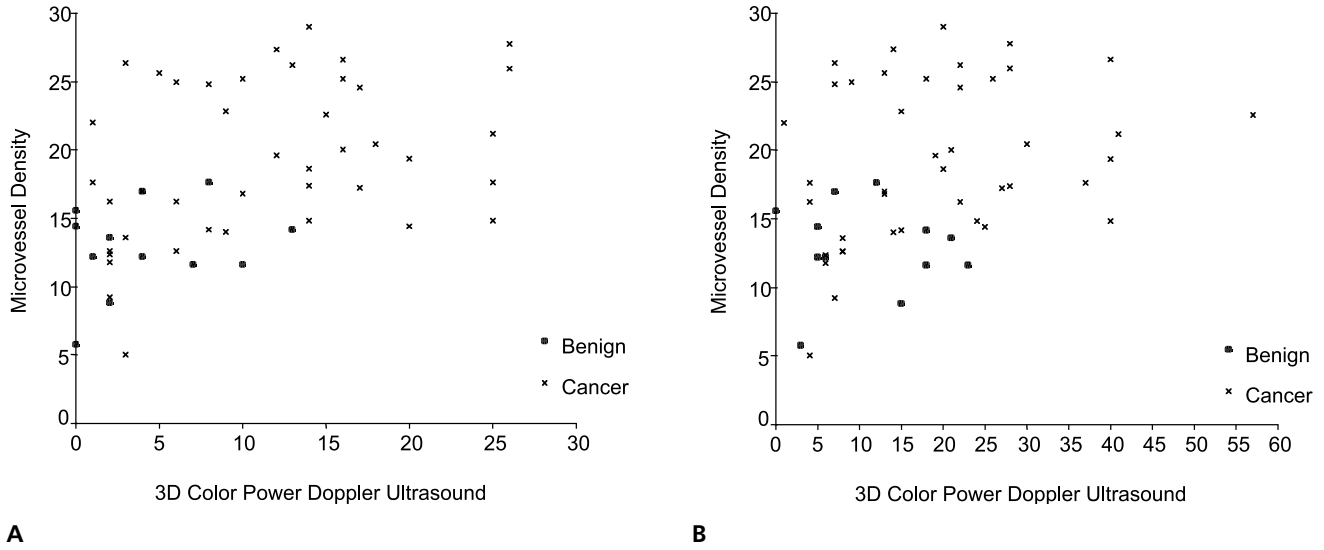


Figure 3. Scatterplot showing correlation between MVD and 3D CPD sonographic measurement of tumoral vascularity before (**A**) and after (**B**) contrast agent administration.

Interestingly, the administration of the sonographic contrast agent did not improve the correlation with MVD. This may be related to a limitation known as the "blooming" artifact that occurs after administration of current contrast agents. This artifact appears as excessive contrast enhancement beyond the anatomic boundaries of vessels, which may impede vessel count. Newer contrast agents are being developed together with improved Doppler techniques, including contrast-enhanced harmonic and pulse inversion imaging, which have been extensively investigated in the interrogation of liver lesions by lower-frequency transducers.[39–42] This technique improves lateral resolution and increases the signal-noise ratio, thus decreasing image-degrading noise and flash artifacts when compared with the conventional power Doppler model. Therefore, these new and specially developed techniques may open a highway for improved small-vessel flow imaging.

These preliminary results encourage the continued development of sonographic techniques for mapping and depiction of tumor vascularity. If successful, this technique may serve as a means for correlation of vessel density, prognosis, and possibly treatment response and thus may provide a new and useful method for assigning individual patients to appropriate therapeutic regimens. Although a positive correlation was not obtained in this small study between MVD and prognosis, many reports in the literature suggest that a strong positive correlation between MVD and sonography will be applicable for predicting the prognosis and response to standard systemic treatment and antiangiogenic agents, not only for breast cancer but also for a wide range of other malignancies, notably hepatocellular carcinoma, melanoma, and renal cell carcinoma. Sonography can be further used to study variation in angiogenesis between different metastatic sites (e.g., liver metastases and nodal involvement) and possibly to give valuable insight into understanding the biological mechanisms behind tumor resistance to systemic therapy.

Table 5. Regression Model to Detect Differences in R^2 values for 2D and 3D Sonography and Precontrast and Postcontrast Sonography

Variables in the Model	Change in R^2, %	*P* for the Change
2D precontrast vs 3D precontrast sonography	15.4	.002
2D postcontrast vs 3D postcontrast sonography	3.4	.163
2D precontrast vs 2D postcontrast sonography	0.1	.768
3D precontrast vs 3D postcontrast sonography	0.4	.593

References

1. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990; 82:4–6.
2. Bosari S, Lee AK, DeLellis RA, Wiley BD, Heatley GJ, Silverman ML. Microvessel quantitation and prognosis in invasive breast carcinoma. Hum Pathol 1992; 23:755–761.
3. Gasparini G, Weidner N, Bevilacqua P, et al. Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinoma. J Clin Oncol 1994; 12:454–466.
4. Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res 1974; 34:997–1004.
5. Protopapa E, Delides GS, Revesz L. Vascular density and response of breast carcinomas to mastectomy and adjuvant chemotherapy. Eur J Cancer 1993; 29A:1391–1393.
6. O'Reilly MS, Holmgren L, Shing Y, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 1994; 79:315–328.
7. Horak ER, Leek R, Klenk N, et al. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet 1992; 340: 1120–1124.
8. Dvorak HF, Sioussat TM, Brown LF, et al. Distribution of vascular permeability factor (vascular endothelial growth factor) in tumors: concentration in tumor blood vessels. J Exp Med 1991; 174:1275–1278.
9. Van Dijke CF, Brasch RC, Roberts TP, et al. Mammary carcinoma model: correlation of macromolecular contrast-enhanced MR imaging characterizations of tumor microvasculature and histologic capillary density. Radiology 1996; 198:813–818.
10. Stomper PC, Winston JS, Herman S, Klippenstein DL, Arredondo MA, Blumenson LE. Angiogenesis and dynamic MR imaging gadolinium enhancement of malignant and benign breast lesions. Breast Cancer Res Treat 1997; 45:39–46.
11. Parker GJ, Suckling J, Tanner SF. Probing tumor microvascularity by measurement, analysis and display of contrast agent uptake kinetics. J Magn Reson Imaging 1997; 7:564–574.
12. Buckley DL, Drew PJ, Mussurakis S, Monson JRT, Horsman A. Microvessel density in invasive breast cancer assessed by dynamic Gd-DTPA enhanced MRI. J Magn Reson Imaging 1997; 7:461–464.
13. Lee WJ, Chu JS, Houng SJ, Chung MF, Wang SM, Chen KM. Breast cancer angiogenesis: a quantitative morphologic and Doppler imaging study. Ann Surg Oncol 1995; 2:246–251.
14. Peters-Engl C, Medl M, Mirau M, et al. Color-coded and spectral Doppler flow in breast carcinomas: relationship with the tumor microvasculature. Breast Cancer Res Treat 1998; 47:83–89.
15. Rubin JM, Bude RO, Carson PL, Bree RL, Adler RS. Power Doppler US: a potentially useful alternative to mean frequency-based color Doppler US. Radiology 1994; 190:853–856.
16. Bude RO, Rubin JM. Power Doppler sonography [editorial]. Radiology 1996; 200:21–23.
17. Young LK, Yang WT, Chan KW, Metreweli C. Hepatic hemangioma: quantitative color power US angiography: facts and fallacies. Radiology 1998; 207:51–57.
18. Cosgrove D. Ultrasound contrast enhancement of tumours. Clin Radiol 1996; 51(suppl 1):44–49.
19. Schlief R. Galactose-based echo-enhancing agents. In: Goldberg BB (ed). Ultrasound Contrast Agents. London, England: Martin Dunitz; 1997:75–82.
20. Raza S, Baum JK. Solid breast lesions: evaluation with power Doppler US. Radiology 1997; 203:164–168.
21. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer, I: the value of histological grades in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19:403–410.
22. Folkman J, Merler E, Abernathy C, Williams G. Isolation of a tumor factor responsible or angiogenesis. J Exp Med 1971; 133:275–288.
23. Schor AM, Schor SL. Tumor angiogenesis. J Pathol 1983; 141:385–413.
24. Strickland B. The value of arteriography in the diagnosis of bone tumors. Br J Radiol 1959; 32:705–713.
25. Wells PT, Halliwell M, Skidmore R, Webb AJ, Woodcock JP. Tumour detection by ultrasonic Doppler blood-flow signals. Ultrasonics 1977; 15: 231–232.

26. Schoenberger SG, Sutherland CM, Robinson AE. Breast neoplasms: duplex sonographic imaging as an adjunct in diagnosis. Radiology 1988; 168:665–668.

27. Bamber JC, Sambrook M, Minassian H, Hill CR. Doppler studies of blood flow in breast cancer. In: Jellins J, Kobayashi T (eds). Ultrasonic Examination of the Breast. Chichester, England: John Wiley & Sons; 1983:371–378.

28. Ramos IM, Taylor KJW, Kier R, Burns PN, Snower DP, Carter D. Tumor vascular signals in renal masses: detection with Doppler US. Radiology 1988; 168: 633–637.

29. Shimamoto K, Sakuma S, Ishigaki T, Ishiguchi T, Itoh S, Fukatsu H. Hepatocelluar carcinoma: evaluation with color Doppler US and MR imaging. Radiology 1992; 182:149–153.

30. Cosgrove DO, Bamber JC, Davey JB, McKinna JA, Sinnett HD. Colour Doppler signals from breast tumors. Radiology 1990; 176:175–180.

31. Cosgrove DO, Kedar RP, Bamber JC, et al. Breast diseases: color Doppler US in differential diagnosis. Radiology 1993; 189:99–104.

32. Carson P, Fowlkes J, Roubidoux M, et al. 3D Doppler image signal quantification of breast masses. Ultrasound Med Biol 1998; 24:945–952.

33. Sehgal CM, Arger PH, Rowling SE, Conant EF, Reynolds C, Patton JA. Quantitative vascularity of breast masses by Doppler imaging: regional variations and diagnostic implications. J Ultrasound Med 2000; 19:427–440.

34. Bhatti PT, LeCarpentier GL, Roubidoux MA, Fowlkes JB, Helvie MA, Carson PL. Discrimination of sonographic breast masses using frequency shift color Doppler imaging in combination with age and gray scale criteria. J Ultrasound Med 2001; 20:343–350.

35. Taylor KJW, Merritt C, Piccoli C. Ultrasound as a complement to mammography and breast examination to characterize breast masses. Ultrasound Med Biol 2002; 28:19–26.

36. Wilkens T, Burke B, Cancelada D, Jatoi I. Evaluation of palpable breast masses with color Doppler sonography and gray scale imaging. J Ultrasound Med 1998; 17:109–115.

37. Kedar RP, Cosgrove D, McCready VR, Bamber JC, Carter ER. Microbubble contrast agent for color Doppler US: effect on breast masses: work in progress. Radiology 1996; 198:679–686.

38. Mehta TS, Raza S. Power Doppler sonography of breast cancer: does vascularity correlate with node status or lymphatic vascular invasion? AJR Am J Roentgenol 1999; 173:303–307.

39. Burns PN, Wilson SR, Simpson DH. Pulse inversion imaging of liver blood flow: improved method for US characterizing focal masses with microbubble contrast. Invest Radiol 2000; 35:58–71.

40. Choi BI, Kim TK, Han JK, et al. Vascularity of hepatocellular carcinoma: assessment with contrast-enhanced second-harmonic versus conventional power Doppler US. Radiology 2000; 214:381–386.

41. Halpern EJ, McCue PA, Aksnes AK, Hagen EK, Frauscher F, Gomella LG. Contrast-enhanced US of the prostate with Sonazoid: comparison with whole-mount prostatectomy specimens in 12 patients. Radiology 2002; 222:361–366.

42. Forsberg F, Piccoli CW, Liu JB, et al. Hepatic tumor detection: MR imaging and conventional US versus pulse-inversion harmonic US of NC100100 during its reticuloendothelial system-specific phase. Radiology 2002; 222:824–829.