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Quantitative nonlinear contrast-enhanced ultrasound of the breast

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Abstract

Breast cancer is the most frequent type of cancer among women (25% of all cancers). The angiogenic process that fuels the growth of tumors is a potential early indicator for differentiating between malignant and benign tumors. Recently, the use of microbubble based contrast agents in combination with ultrasound imaging has allowed for the development of contrast agent specific imaging modes that provide visualization of tumor neovascularity. Contrast-enhanced Doppler, harmonic and subharmonic imaging are some of the imaging modes that have been investigated for visualizing and quantifying the vascularity in breast tumors.

Keywords

Breast cancer; contrast enhanced ultrasound; subharmonic imaging

Background

Breast cancer was the second leading cause of cancer related deaths among women (40,290 estimated deaths) in the United States in 2015 [1]. Mammography, which is the primary imaging modality for screening breast cancers, has been shown post biopsy to produce a very high false-positive rate (65% – 90%) [2, 3]. Ultrasound imaging is used extensively as a supplementary imaging modality for examining breast tumors [2, 4, 5]. The ability to produce high-resolution images in real-time and easily differentiate between cystic and solid tumors (as compared with mammography) gives ultrasound imaging an edge over other breast imaging modalities.

There is a wealth of experimental evidence that suggests that tumor growth beyond 2–3 mm³ is dependent on angiogenesis [6, 7]. Tumor angiogenesis is the development of a new vascular network from pre-existing vessels [7–9]. This process is one of the early indicators differentiating cancerous from normal tissue. Tumor growth and metastasis are angiogenesis-dependent [7]. A tumor must continuously stimulate the growth of new capillary blood vessels for the tumor itself to grow. Furthermore, the new blood vessels

embedded in a tumor provide a gateway for tumor cells to enter the circulation and to metastasize to distant sites [7]. In order to visualize this angiogenic process, Doppler imaging techniques (power and color) have been studied [10–13]. However, these studies produced mixed results in terms of characterizing the tumors as benign or malignant, due to a low sensitivity to depict the slow blood flow (<1 mm/s) and small vessels (<100 μ m) associated with breast cancer neovascularity [10–13]. Contrast enhanced ultrasound imaging provides an alternate means to image vascularity.

Ultrasound contrast agents

Ultrasound contrast agents (UCA) consist of gas filled microbubbles that are stabilized with an outer shell. These bubbles range in diameter from 1–8 μ m, which allows them to be injected intravenously, travel through the capillaries and in turn, the entire vascular system [14–21]. These microbubbles are different in terms of their compressibility ($\approx 20,000\times$) and density ($\approx 900\times$) relative to their surrounding medium (blood and tissue) [20]. This leads to an acoustic impedance difference causing ultrasound signal backscattering and therefore vascular enhancement on the ultrasound images (up to 25 dB) [14]. This paper reviews select studies that have used contrast-enhanced ultrasound imaging in the field of breast imaging.

Contrast-enhanced Doppler imaging techniques

Early qualitative work for imaging vascularity in breast lesions was performed using Doppler imaging with the addition of UCA in order to improve the Doppler sensitivity to blood flow in small vessels (<100 μ m). In 1996, Kedar et al. studied the effect of UCAs on color Doppler examinations of 34 patients identified with breast masses [22]. These exams were evaluated for increase in intensity of Doppler signals and changes in vascular patterns before and after injection of UCA. It was observed that post UCA injection, there was greater and longer signal enhancement in the malignant lesions compared to the benign ones. Analysis of vascular morphology showed that the cancers displayed additional vessels and an increase in vascular tortuosity.

Madjar et al. conducted a similar but larger multicenter study in 2000, including 220 patients, 40 of whom had breast lesions [23]. Any changes in Doppler signal intensity before and after injection of UCA's were evaluated. The study similarly concluded that the use of UCA improved the quality of Doppler flow signals and was able to improve the differential diagnosis for both malignant and benign lesions. While these initial studies were promising [24, 25], similar studies performed by other groups using contrast-enhanced Doppler examinations showed an overlap between vascular characteristics for malignant and benign lesions [26, 27].

In order to more fully appreciate the tortuous nature of vascularity in malignant lesions, Forsberg et al. conducted a contrast-enhanced three-dimensional (3D) power Doppler imaging study of breast lesions [28]. In this study the ability to visualize vascularity using contrast-enhanced 3D power Doppler imaging was compared to 2D power Doppler (pre and post UCA injection) as well as baseline 3D power Doppler. Figure 1 shows the 2D and 3D

power Doppler images acquired before and after UCA injection for a benign and malignant case. A total of 50 lesions were imaged using all four modes and the area under the ROC curve for diagnosis of cancer was evaluated. Overall, mammography showed the highest area under the ROC curve among all imaging modes (0.86) followed by contrast-enhanced 3D power Doppler (0.76). When using logistic regression to combine results, mammography together with all the ultrasound modalities produced an area under the ROC curve of 0.96 for diagnosis of cancer, and this was significantly higher than all other imaging combinations ($p < 0.05$) except mammography and 3D baseline (which showed a trend towards significance). However, these studies were also based on Doppler imaging techniques and performed only qualitative evaluations of the tumor vascularity.

Contrast-enhanced non-linear imaging techniques

While contrast-enhanced Doppler imaging techniques have shown an improvement in the visualization of lesion vasculature, their ability to quantitatively differentiate between malignant and benign breast lesions has not been conclusive. This is in part due to the lack of clear delineation between the UCA (vascular flow) and surrounding tissue signals. In order to improve this delineation, various groups have been focusing on developing and quantifying contrast-specific non-linear imaging techniques. When insonated, the UCA microbubbles produce non-linear oscillations and therefore, non-linear frequency components in the received signal relative to the transmitted signal (f_0). These non-linear components consist of higher harmonics ($n \cdot f_0$), ultraharmonics ($[2n + 1] \cdot f_0/2$) and subharmonics ($f_0/2$). Most commercial contrast-imaging packages are based on the ultrasound systems receiving signals at higher harmonics (so called harmonic imaging; HI), with the expectation of suppressing tissue signals at these higher frequencies.

However, research has shown that tissue is also able to generate non-linear harmonic signals and consequently complete suppression of tissue signals by performing contrast-specific imaging (i.e., receiving) at higher harmonics is not feasible in practice [18, 29]. The generation of second and higher harmonics is a continuous process, which occurs to varying degrees for all levels of acoustic excitation. However, the subharmonic emission (at $f_0/2$) only occurs if the applied acoustic excitation crosses a certain acoustic pressure threshold [18]. These subharmonic emissions ($f_0/2$) are specific to the UCA and are not generated from the tissue. By receiving signals at the subharmonic frequency, it is possible to isolate the UCA from the surrounding tissue. The resulting subharmonic image (SHI) is able to achieve improved contrast-to-tissue ratios (roughly six-fold or more) relative to HI via increased suppression of tissue signals (while providing greater depth penetration due to the lower frequency) [30]. Several groups have been able to independently validate the generation of UCA subharmonic signals and the feasibility of SHI for a variety of clinical applications [31–36].

A number of groups have documented the ability to perform *in vivo* SHI in animals [32, 37–39]. However, SHI in humans is a relatively new development. In terms of breast imaging, Forsberg et al. performed the first human study using SHI [40]. The objective of this study was to prospectively compare the accuracy of SHI with that of regular B-mode ultrasound, power Doppler (with and without UCA) and mammography for the diagnosis of breast

cancer. Histopathology results were used as the reference standard. The study included 14 women with 16 breast lesions that were imaged using a Logiq 9 scanner (GE Healthcare, Milwaukee, WI), which was modified to perform SHI with a transmit frequency of 4.4 MHz and a receive frequency of 2.2 MHz (optimized for the UCA). Routine clinical breast imaging is usually around the 10–16 MHz range. Biopsy of the lesions resulted in 12 benign and 4 malignant masses. All lesions imaged with mammography were scored as 3 or 4 on the BIRADS scale. In terms of ultrasound imaging, SHI resulted in almost complete suppression of tissue signals, in both baseline and contrast-enhanced imaging. SHI also improved the visualization of vascular morphology relative to contrast-enhanced power Doppler via better tissue suppression as demonstrated in Figure 2.

A comparison of sensitivity, specificity and accuracy for each of the imaging modes is listed in Table 1. The specificity of each of the ultrasound modes was significantly greater than that of mammography ($p < 0.04$). Baseline ultrasound imaging and SHI achieved the highest accuracy (81%) in the study, but only SHI showed a trend toward significant improvement over mammography. The area under the ROC curve for the diagnosis of breast was higher for SHI (0.78) than any of the other modes tested (although not significant). Furthermore, the degrees of vascularity observed with baseline and contrast-enhanced Doppler correlated with those observed with SHI ($r^2 = 0.40$, $p < 0.009$). SHI was also able to visualize more vascular morphology compared to baseline power Doppler without the blooming artifacts associated with contrast-enhanced power Doppler. The study concluded that, based on the limited sample size, SHI appeared to improve the diagnosis of breast cancer relative to conventional ultrasound imaging and mammography.

Based on the data available from the first-in-humans SHI breast study [40], Dave et al. developed a novel automated motion compensation algorithm to produce cumulative maximum intensity (CMI) images for SHI [41, 42]. The images constructed using CMI-SHI provided detailed vascular structures and overall better perceived image quality (Figure 3). The area under the ROC curve for CMI-SHI was higher than all the other imaging modes (0.90) and this was significantly better than mammography ($p = 0.031$) [42]. While, these studies highlighted the improvement that SHI provided to imaging the vascularity in breast lesions, they were primarily qualitative in nature.

Eisenbrey et al. investigated the use of various skeletonization algorithms for defining breast lesion architecture using CMI-SHI images and subsequently quantifying breast vasculature [43]. Skeletonization of the vasculature could potentially reduce complex patterns to single line structures, while still maintaining critical information such as vessel length, vessel density, number of bifurcations and other topological features [44]. In that study, the performance of the three different algorithms (sequential thinning, parallel thinning and distance transformation) was tested *in vitro*. The sequential thinning algorithm was selected for and applied to the CMI-SHI breast lesion images for skeletonization (Figure 4). Vascular skeletonization was successful in 13 out of 16 lesions. Quantifying the number of vessel bifurcations and the average vessel length resulted in both a higher number of bifurcations (9.8 ± 8.18 vs. 6.9 ± 6.50) and longer average vessels (88.9 ± 79.10 vs. 63.2 ± 45.65 pixels/vessel) for malignant compared to benign lesions; although these differences were not statistically significant. This was a first step towards quantifying the vascularity in breast

lesions using SHI. Another important marker of malignancy is the blood flow kinetics in the lesion. Malignant lesions tend to have more chaotic and tortuous vascularity compared to benign lesions leading to variations in their flow patterns [45, 46]. Another study by Eisenbrey et al. used UCA flow within breast lesions to develop parametric contrast maps [47]. By using SHI they were able to achieve near complete suppression of tissue signals and delineate the vascularity (i.e., the UCA flow). For each lesion, parametric maps were generated based on a pixel-by-pixel basis of the time to peak contrast signal (TTP), estimated perfusion (EP) and area under the curve (AUC). An example showing the EP parametric maps is presented in Figure 5. Subsequently, a region of interest (ROI) was selected for each case corresponding to the breast lesion and an average value for each parameter (TTP, EP and AUC) was generated over the entire lesion. The lesions were grouped according to pathology and parametric values were compared. Figure 6 shows the EP compared between the different lesions. Carcinomas were found to have significantly higher rates of perfusion relative to benign lesions (0.10 ± 0.03 vs. 0.05 ± 0.02 IU/s; $p = 0.001$). No significant variations were detected using TTP or AUC ($p > 0.6$). Although the sample size of this study was very limited (14 patients), quantification of vascularity in breast lesions using SHI was shown to be feasible and flow parameters (specifically perfusion) were useful in characterizing breast lesions.

Recently, Sridharan et al. attempted to quantify the vascular heterogeneity in breast lesions using contrast-enhanced 3D HI and 3D SHI [48]. This study included 138 women who were identified with breast lesions as part of their clinical assessment. Biopsy results were used as the reference standard and this resulted in a total of 99 benign and 35 malignant lesions. Post contrast-examination, UCA flow was observed for 68 lesions in 3D SHI and only 8 lesions in 3D HI (due to higher signal attenuation and therefore weaker response from UCA compared to SHI). Overall, vascularity was better appreciated in 3D SHI compared to 3D HI due to better suppression of surrounding tissue signals as seen in Figure 7.

Volumetric maps of UCA flow within the lesion were created and vascular intensity plots developed (based on the relative change of UCA intensity from baseline to peak) for the whole lesion. These maps were split in to central and peripheral sections as a means of quantifying the vascular heterogeneity across the lesion. Overall, for benign lesions, the central sections showed significantly increased vascular activity relative to the peripheral sections (1.71 ± 0.96 vs. 1.13 ± 0.79 dB, $p < 0.001$). For malignant lesions, however, no significant differences were observed between the central and peripheral sections indicative of more vascular spread and the heterogeneous nature of vasculature in malignant breast lesions (1.66 ± 1.39 vs. 1.24 ± 1.14 dB, $p = 0.24$). This study demonstrated the feasibility of using contrast-enhanced 3D SHI to quantify vascular heterogeneity in breast lesions.

Conclusion

In this paper we have reviewed some of the research in the field of contrast-enhanced ultrasound for imaging breast lesions. Most of the early studies provided only a qualitative assessment of the advantages of using UCAs for imaging vascularity in breast lesions. However, some of the more recent studies have been able to quantify this neovascularity. The use of UCAs in combination with ultrasound for breast imaging is a relatively new

application with a lot of clinical potential. Researchers are developing new contrast-specific ultrasound imaging techniques for visualizing and quantifying breast lesion vascularity. Contrast-enhanced ultrasound is a promising imaging modality for generating quantifiable measures of vascularity that could potentially help characterize breast lesions.

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Figure 1a.

Ductal carcinoma in situ imaged using 2D power Doppler (a) before and (b) after injection of UCA. Same lesion imaged using 3D power Doppler (c) before and (d) after injection of UCA. Example of a fibroadenoma imaged using 2D power Doppler (e) before and (f) after injection of UCA. The same fibroadenoma imaged using 3D power Doppler (g) before and (h) after injection of UCA. Reproduced from [28]

Figure 2.
A fibroadenoma imaged using (a) baseline 2D power Doppler and (b) contrast-enhanced SHI. Reproduced from [40]

Figure 3. Comparison of (a) the best single frame SHI of benign ductal microcalcifications with CMI-SHI images generated using (b) manual technique (c) no threshold (d) a threshold set to the mean value of the reliability parameter and (e) threshold set to 85% of the mean reliability parameter. Reproduced from [42]

Figure 4.

Vascular skeletonization performed on CMI-SHI images of (a) benign ductal microcalcifications (b) malignant papillary carcinoma (c) benign cyst (d) lymph node (e) fibroadenoma and (f) and benign cyst. Reproduced from [43]

Figure 5.
Parametric images showing the estimated perfusion (EP) for three different types breast lesions, (a) fibroadenoma (b) subdermal scar and (c) invasive papillary carcinoma. Reproduced from [47]

Figure 6.
Box plot comparing the estimated perfusion (EP) data for each different type of lesions classification. Reproduced from [47]

Figure 7.
Contrast-enhanced (a) 3D HI and (b) 3D SHI of an invasive ductal carcinoma.
Reproduced from [48]

Table 1.

Comparison of the sensitivity, specificity and accuracy of the different imaging modalities used for imaging breast lesions. Reproduced from [40].

	grayscale	power Doppler	power Doppler with contrast	SHI	mammo
sensitivity [%]	50 (2/4)	50 (2/4)	75 (3/4)	75 (3/4)	100 (3/3)
specificity [%]	92 [*] (11/12)	92 [*] (11/12)	75 [‡] (9/12)	83 [*] (10/12)	20 (2/10)
accuracy [%]	81 (13/16)	81 (13/16)	75 (12/16)	81 [§] (13/16)	38 (5/13)

^{*}
 $p = 0.008$ vs. mammography

[‡]
 $p = 0.03$ vs. mammography

[§]
 $p = 0.07$ vs. mammography