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Characterization of Adnexal Masses Using Contrast-Enhanced Subharmonic Imaging:

A Pilot Study

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Abstract

Objectives—This pilot study evaluated whether contrast-enhanced subharmonic imaging (SHI) could be used to characterize adnexal masses before surgical intervention.

Methods—Ten women (with 12 lesions) scheduled for surgery of an ovarian mass underwent an SHI examination of their adnexal region using a modified LOGIQ E9 scanner (GE Healthcare, Waukesha, WI) with an endocavitary transducer, in which digital clips were acquired by pulse destruction-replenishment SHI across the lesions. Time-intensity curves were created offline to quantitatively evaluate SHI parameters (fractional tumor perfusion, peak contrast intensity, time to peak contrast enhancement, and area under the time-intensity curve), which were compared to pathologic characterizations of the lesions.

Results—Of the 12 masses, 8 were benign, and 4 were malignant. A qualitative analysis of the SHI images by an experienced radiologist resulted in diagnostic accuracy of 70%, compared to 56% without contrast, whereas an inexperienced radiologist improved from 50% to 58% accuracy, demonstrating the benefit of SHI. A quantitative analysis of SHI parameters produced diagnostic accuracy as high as 81%. Peak contrast intensity was significantly greater in malignant than benign masses (mean $\pm$ SD, 0.109 ± 0.088 versus 0.046 ± 0.030 arbitrary units; $P = .046$). Malignant masses also showed significantly greater perfusion than benign masses ($24.79\% \pm 25.34\%$ versus $7.62\% \pm 6.50\%$; $P = .045$). When the radiologist reads were combined with the most predictive quantitative SHI parameter (percent perfusion), diagnostic accuracy improved to 84% for the experienced radiologist and 96% for the novice radiologist.

Conclusions—Results indicate that SHI for presurgical characterization of adnexal masses may improve the determination of malignancy and diagnostic accuracy, albeit based on a small sample size.

Keywords

adnexal masses; contrast agents; diagnostics; subharmonic imaging

Ovarian cancer is the seventh most commonly diagnosed cancer in women worldwide, with approximately 240,000 new cases diagnosed and 152,000 deaths each year (representing a 64% mortality rate).¹ Ovarian cancer is also the fifth most common cause of cancer death in women in the United States, and an estimated 1 per 71 women in the United States will develop ovarian cancer in their lifetime.^{2,3} If caught early enough that the disease is confined to the ovary (stage I), patients require less morbid surgical intervention, have a substantially improved quality of life, and, most importantly, have a 5-year survival rate of approximately 90%.^{2,4,5} Unfortunately, roughly 75% of new diagnoses are late-stage cancers, bringing the mortality rates up as high as 80%.⁵ The prevalence of late-stage diagnoses clearly highlights the inadequacy of conventional endovaginal ultrasound (US) imaging and pelvic examinations as the first line in detecting adnexal masses.^{6–8} Additionally, once detected, many adnexal masses are deemed clinically indeterminate on first-line US imaging, and follow-up magnetic resonance imaging (MRI), and computed tomography (CT) cannot always definitively characterize them as benign or malignant.^{9,10} Therefore, up to 80% of presenting patients undergo surgery out of an abundance of caution, in response to the aforementioned mortality rates for late-stage disease.^{11–13} Thus, there is a clear clinical need for earlier and accurate characterization of adnexal masses to improve patient survival.

Imaging techniques can play a critical role in improving detection and diagnosis of ovarian masses, especially in the assessment of angiogenesis and blood perfusion in the lesion.^{6,7,14,15} One of the earliest changes that differentiates cancerous tissues from normal tissues is tumor angiogenesis.^{16,17} Additionally, the morphologic characteristics of these angiogenic vessels can serve as predictors of malignancy in cancerous masses,^{18,19} including those in the adnexal region.^{20,21} Angiogenic vessels form a substantial portion of the mass of malignant lesions (up to 10% of the total tumor volume),²² providing a strong opportunity for noninvasive imaging to improve on the classification of these adnexal lesions compared to purely anatomic imaging modes. Although contrast-enhanced MRI and CT can be up to 90% accurate in classifying adnexal masses that were deemed indeterminate with first-line US,^{6,23} these secondary modalities are costly and use contrast agents associated with marked adverse reactions, and CT also exposes patients to substantial ionizing radiation. However, contrast-enhanced ultrasound (CEUS) imaging has great potential to provide clinically relevant information related to measuring angiogenesis and blood flow in adnexal lesions. Specifically, CEUS imaging using gas microbubbles greatly improves the ability to visualize tumor angiogenesis and quantify blood flow within tumors, including adnexal masses, without the marked adverse reactions and contraindications of MRI and CT contrast agents.^{14,24–26} Several studies have shown that CEUS imaging can be useful in classification of adnexal masses as benign or malignant,^{14,26–29} and characterizing blood flow kinetics in these masses.³⁰ However, these techniques fall short of clinically viable accuracy in diagnosis, likely because of image degradation and a reduction in blood-to-tissue contrast in tissue.³¹

In an effort to address this clinical need, we have been developing a contrast-specific imaging modality known as subharmonic imaging (SHI). Subharmonic imaging transmits the US signal at twice the resonance frequency ($2f_0$) of the ultrasound contrast agent (UCA) and receives at half of the transmit frequency (f_0), which allows for excellent suppression of tissue echoes relative to other US contrast modes.^{32,33} Although other modes, such as contrast pulse sequencing, pulse inversion, and flash-replenishment, can be used for tissue suppression, SHI combines the ability to specifically suppress tissue echoes while also providing quantitative data. Our group, as well as others, have performed extensive SHI feasibility studies.^{25,34–47} We have also demonstrated that SHI can detect the slow, small-volume blood flow associated with tumor angiogenesis in a first-in-humans study of women with breast lesions.^{34,40–42} Moreover, UCAs are pure intravascular tracers and enable CEUS to depict tumor vascularity differently from MRI, which uses gadolinium chelates as contrast agents. Given these advantages, CEUS using SHI could help address the critical clinical need by providing a more accurate imaging technique at earlier stages and may ultimately improve patient survival. Therefore, the objective of this pilot study was to evaluate whether contrast-enhanced SHI could help characterize adnexal masses before surgical intervention.

Materials and Methods

Patient Recruitment and Clinical Pathologic Evaluation

Twenty-eight women scheduled for surgery of suspicious ovarian lesions at Thomas Jefferson University between August 2017 and August 2018 who met the inclusion criteria for this Institutional Review Board–approved study were approached to participate. Twelve women agreed to enroll and signed informed consent to participate in the study and undergo a contrast-enhanced SHI examination of the adnexal region before surgery. Two women declined to participate and withdrew consent before contrast agent administration, leaving a total of 10 participants who completed the study. Inclusion criteria included a diagnosis of an adnexal mass, a plan for surgical resection of the adnexal mass, age of at least 21 years, and clinical stability. Premenopausal patients had to have a negative pregnancy test result to be enrolled in the study, since pregnancy was one of the exclusion criteria for study participation. Other exclusion criteria included pulmonary hypertension, cardiac shunts or unstable cardiopulmonary conditions, a current systemic chemotherapy regimen, clinical instability or terminal illness with a life expectancy of less than 1 month, and a history of an anaphylactic allergy to UCAs. The tumor marker cancer antigen 125 (CA-125) and risk of malignancy index (RMI) were determined by pathologic evaluation. After surgery, excised lesions were classified by clinical pathologic evaluation as part of the standard of care.

Contrast-Enhanced SHI Evaluation

Contrast-enhanced SHI scanning was performed on a LOGIQ E9 scanner (GE Healthcare, Waukesha, WI) equipped with an IC5–9-D endocavitary transducer, using a 3-pulse coded excitation SHI mode with a transmit frequency of 7.0 MHz and receiving at 3.5 MHz. The use of coded excitation will have marked improvements in the signal-to-noise ratio due to suppression of tissue signals.⁴⁸ Imaging was optimized on an individual basis, using a mechanical index of less than 0.18 in all cases (range, 0.10–0.18; average, 0.13). Patients first received a 1.5 mL intravenous bolus injection of Definity (Lantheus Medical Imaging,

North Billerica, MA), while digital clips of the lesion in the area with the most flow seen using power Doppler were acquired for up to 5 minutes after injection. Patients then rested for 10 minutes to ensure contrast clearance before receiving an infusion of 1.5 mL of Definity diluted in 25 mL of sterile saline over 5 minutes, when digital clips were acquired during flash-replenishment SHI across the lesion, including the same areas that were previously imaged. These sequences consisted of destructive US pulses at an average mechanical index of 0.6 (range, 0.5–0.8), which ruptured the UCA within the imaging plane, followed by nonlinear SHI at a lower intensity (mechanical index of 0.07) to allow monitoring of the UCA reperfusion into the lesion. An average of 6 flash-replenishment sequences per lesion were collected, with at least 4 per lesion, as the number of sequences collected varied on a case-by-case basis.

Time-intensity curves (TICs) and parametric maps were generated offline with MATLAB software (The MathWorks, Natick, MA) to quantitatively evaluate SHI parameters from the flash-replenishment sequences. These curves estimate perfusion over the adnexal lesion by calculating the slope of the curve from the time contrast was first visualized to the peak intensity (PI). Data from TICs were used to calculate the estimated fractional tumor perfusion (PER), PI, area under the time-intensity curve (AUC), and time to peak contrast enhancement (TTP), which is defined as the time from contrast agent infusion to the point at which the maximum pixel intensity is reached.^{25,40} The TIC was also fit with a 2-parameter exponential recovery curve: $VI = \alpha(1 - e^{-\beta t})$, where VI represents video intensity; α (dB) represents the asymptotic plateau correlative of the microvessel cross-sectional area; and β (mm/s) represents the blood velocity.^{49–51} The product $\alpha \times \beta$ is an estimate of perfusion or blood flow per tissue unit (mL/(s $\times$ mg)).

Additionally, a qualitative assessment of the SHI images was performed by 2 radiologists, 1 who was experienced with CEUS (>10 years of experience) and 1 who was not (around 6 months of experience). The radiologists were blinded to the pathologic classifications of the lesions and were given both grayscale and contrast-enhanced SHI clips for evaluation. After reviewing each case, the radiologists provided a qualitative score for diagnosis based purely on their assessment of the images using a 5-point visual analog scale, with 1 representing benign and 5 malignant, and their confidence in that diagnosis (on a percent scale).

Statistical Analysis

The statistical analysis was performed with Stata version 15 software (StataCorp, College Station, TX), using *t* tests ($\alpha < .05$) to compare the data between response groups. Receiver operating characteristics (ROC) curves were used to determine diagnostic accuracies as the areas under the ROC curves.⁵² Reverse stepwise logistic regression was used to combine qualitative and quantitative results to test for improved accuracy. Results were collected in triplicate, and error was reported as the standard deviation.

Results

Clinical Evaluation of Adnexal Masses

The study patients ranged in age from 34 to 76 years, with an average age of 55.5 years. Two patients withdrew their consent and did not complete the study, whereas 10 patients completed their US scans. Of these 10 patients, 2 had 2 adnexal lesions, for a total of 12 lesions analyzed in this study. Clinical pathologic classification determined that 8 (67%) of the lesions were benign, and 4 (33%) were malignant. Benign lesions were classified as mucinous cystadenoma (3), hydrosalpinx (2), mature cystic teratoma (1), a mixed epithelial neoplasm (1), and endometriosis (1). Malignant lesions were classified as carcinosarcoma (2), serous adenocarcinoma (1), and adenocarcinoma from colonic metastasis (1). In the 2 patients presenting with 2 lesions, the pathologic finding was consistent for both lesions (ie, both were benign, or both were malignant). The tumor marker CA-125 and RMI were collected for 8 of the 12 lesions, including 5 of the 8 classified as benign and 3 of the 4 classified as malignant. The CA-125 levels in benign lesions (mean $\pm$ SD, 17.46 ± 8.42 nL) were statistically similar to levels in malignant lesions (56.20 ± 75.17 nL; $P = .27$). The CA-125 had diagnostic accuracy of 60% in this pilot study. There was also no statistical difference in the RMI between benign and malignant lesions (104.00 ± 95.67 versus 505.67 ± 676.65 ; $P = .22$). The diagnostic accuracy for the RMI was 73%.

Quantitative Analysis of Contrast-Enhanced SHI

Representative images of lesions classified as malignant and benign are shown in Figure 1. Malignant lesions (Figure 1, A and C) typically presented with hyperechoic regions that showed increased blood flow on SHI, whereas benign lesions (Figure 1, B and D) typically presented with hypoechoic or anechoic regions that indicated fluid-filled cysts.

The outcomes of the quantitative SHI analysis are summarized in Table 1, and representative TICs are shown in Figure 2. The PI was significantly greater in malignant than benign masses (0.11 ± 0.09 versus 0.05 ± 0.03 arbitrary units; $P = .046$). Malignant masses also showed significantly greater PER than benign masses ($24.79\% \pm 25.34\%$ versus $7.62\% \pm 6.50\%$; $P = .045$). There were no significant differences between benign and malignant lesions in the TTP ($P = .52$) or AUC ($P = .06$). Additionally, the 2-parameter exponential recovery model did not yield any significant differences between benign and malignant masses for any of the 3 parameters ($P = .72$ for α ; $P = .19$ for β ; and $P = .07$ for $\alpha \times \beta$).

Diagnostic accuracy was calculated for each SHI parameter. The fraction of the lesion showing perfusion had the highest diagnostic accuracy at 81%. The rest of the parameters ranged in accuracy from 52% (TTP) to 79% (model parameter α). All diagnostic accuracies are presented in Table 1.

Radiologic Scoring of CEUS and SHI Clips

The performance of the radiologists on the qualitative assessment of the contrast-enhanced SHI clips demonstrates the importance of familiarity and experience with CEUS. The diagnostic confidence of the experienced radiologist significantly increased when reviewing the SHI ($86\% \pm 28\%$) compared to grayscale only ($68\% \pm 23\%$; $P = .042$). There was no

change in the diagnostic confidence for the novice radiologist between SHI ($86\% \pm 15\%$) and grayscale ($83\% \pm 15\%$; $P = .27$). The ROC curves associated with precontrast and postcontrast diagnostic confidence for both radiologists are shown in Figure 3.

There was also no difference in diagnostic confidence between the experienced and novice radiologists when reviewing the SHI clips ($P = .50$). Additionally, as shown in Figure 4, the qualitative analysis of the SHI images by the experienced radiologist resulted in a diagnostic accuracy of 70%, compared to 56% without contrast, whereas the novice radiologist only saw an 8% improvement (from 50% to 58%; Figure 4A). When the radiologist reads were combined with the most predictive quantitative SHI parameter (PER), diagnostic accuracy improved to 84% for the experienced radiologist and 96% for the novice radiologist (Figure 4B); this difference was not significant ($P = .32$). However, given that both radiologists saw improvement in diagnostic accuracy once the quantitative parameter (PER) was included in the ROC model, these results suggest that the PER parameter adds diagnostic value.

Discussion

Only 10% to 20% of all ovarian lesions surgically excised are malignant,⁵³ highlighting the necessity for a more definitive preoperative classification via imaging. Such a modality could increase the preoperative confidence for differentiating benign from malignant lesions, therefore diminishing the number of indeterminate lesions that necessitate surgery for classification.

This work represents, to our knowledge, the first study to investigate the use of endovaginal contrast-enhanced SHI in women scheduled to undergo surgery for an adnexal mass as a potential tool for characterizing the malignancy of the lesion. We demonstrated that contrast-enhanced SHI, particularly the quantitative parameters derived from subharmonic TICs, could achieve diagnostic accuracies of up to 81%. Additionally, the diagnostic accuracy of the experienced radiologist improved by 14% with the addition of contrast-enhanced SHI, suggesting that it is a valuable tool for clinical adaptation, albeit in a small sample size. Our findings suggest that noninvasive endovaginal contrast-enhanced SHI may become a clinical imaging modality for evaluating adnexal masses with the potential to reduce both the cost and risk to the patient while also improving diagnostic accuracies. In this study, we specifically evaluated SHI. It is conceivable that replacing or combining SHI with other tissue suppression methods, such as amplitude modulation (similar to contrast pulse sequencing) or pulse inversion, would improve results further. However, establishing this will require further experiments.

The quantitative analysis of the contrast-enhanced SHI images showed that malignant adnexal masses had a significantly greater perfusion than those classified as benign. We expected that malignant tumors would show tumor angiogenesis and increased blood flow,^{16,17,19} and our findings that these characteristics were identifiable with endovaginal contrast-enhanced SHI are supported by other studies using CEUS imaging.^{15,29}

We also found that clinical screening factors for malignancy were inconclusive in distinguishing benign from malignant masses in our limited study population; however, there

was a large deviation in the malignant group. Cancer antigen 125 levels are only clinically relevant for later-stage ovarian cancer, as this serum marker lacks sensitivity in early-stage disease.^{54,55} One patient with a malignant mass presented with a CA-125 level of 143 U/mL, was staged as IIB high-grade serous ovarian cancer, and is now in remission, whereas another patient with malignancy presented with a CA-125 level of 12.8 U/mL and has since died of the disease. However, the average CA-125 level for patients with benign lesions was 19.3 U/mL, with only 1 patient with a level of less than the 12.8 U/mL observed for a patient with malignancy. These cases highlight the insufficiency of CA-125 as a predictor of malignancy in ovarian masses.

The RMI is calculated from the CA-125 level as well as the patient's age, menopausal status, and clinical impression,⁵⁶ so inherently a lack of a difference in CA-125 levels between the pathologic classifications would suggest that the RMI would also be similar. We suspect that differences in the CA-125 level and RMI would appear between malignant and benign masses with increased sample sizes, as comparing 3 malignant lesions versus 5 benign lesions is hardly an ideal comparison. However, we did find that the RMI had higher diagnostic accuracy at 73% than CA-125 levels alone (60%), suggesting that the other factors used in calculating the RMI score (including a US score)^{55,56} provide a better overall assessment of the lesion. Given the high diagnostic accuracy provided by contrast-enhanced SHI, this modality could possibly be incorporated into the RMI calculation in the future, providing an even better US score and potentially further improving clinical classification of adnexal masses based on this modified RMI.

One limitation to this pilot study was the small sample size, with only 10 patients completing the study at a single medical center. Therefore, we cannot definitely determine whether the observed differences between benign and malignant adnexal masses, as measured with contrast-enhanced SHI, can serve as an effective diagnostic tool. However, it is encouraging that all of the significant findings support this trend. Further investigations, with larger sample sizes at multiple centers, are necessary to determine whether contrast-enhanced SHI evaluation of adnexal masses could be a noninvasive, real-time, quantitative factor for determining malignancy and the need for surgical intervention (at least in high-risk populations⁵³). Also, although *in vitro* and animal *in vivo* studies show that other UCAs are also effective in intermittent destruction-replenishment CEUS perfusion imaging,^{57,58} we limited our pilot study to only use the Definity contrast agent. Definity represents 1 of only 3 UCAs that are commercially available and Food and Drug Administration approved for use in humans in echocardiography, and we have previously had success with off-label use of Definity.^{25,40,59}

The potential clinical impact of these findings is promising, as there is no definitive noninvasive method for determining malignancy in ovarian lesions. Coupled with clinical standard-of-care evaluations, contrast-enhanced SHI for presurgical characterization of ovarian masses may improve the determination of malignancy, reducing the cost and risk to patients while improving diagnostic accuracy, albeit based on a small sample size.

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Abbreviations

AUC	area under the time-intensity curve
CA-125	cancer antigen 125
CEUS	contrast-enhanced ultrasound
CT	computed tomography
MRI	magnetic resonance imaging
PER	tumor perfusion
PI	peak intensity
RMI	risk of malignancy index
ROC	receiver operating characteristic
SHI	subharmonic imaging
TIC	time-intensity curve
TTP	time to peak contrast enhancement
UCA	ultrasound contrast agent
US	ultrasound

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Figure 1. Representative SHI images from study patients. **A**, Precontrast image of a lesion later classified as malignant by pathologic evaluation. **B**, Precontrast image of a lesion later classified as benign by pathologic evaluation. **C**, Subharmonic image of the malignant lesion shown in **A**. **D**, Subharmonic image of the benign lesion shown in **B**.

Figure 2.

Representative TICs from study patients. **A**, Initial wash-in of contrast within a lesion later classified as malignant by pathologic evaluation. **B**, Initial wash-in of contrast within a lesion later classified as benign by pathologic evaluation. **C**, Representative flash-replenishment sequence from the malignant lesion shown in **A**. **D**, Representative flash-replenishment sequence from the benign lesion shown in **B**. Note that the y-axis values are scaled to the data in each curve for better visualization. ROI indicates region of interest.

Figure 3. Receiver operating characteristic curves for diagnostic confidence for both novice (R_nov) and experienced (R_exp) radiologists before (BL for baseline) and after contrast-enhanced SHI.

Figure 4.

Receiver operating characteristic curves for diagnostic data. **A**, Analysis of the experienced radiologist (R_exp), novice radiologist (R_nov), and highest quantitative SHI parameter (PER). **B**, Combination of PER with radiologist reads.

Table 1.

Summary of the Quantitative SHI Analysis

Finding	TTP, s	PI, AU	PER, %	AUC, AU	α , dB	β , mm/s	$\alpha \times \beta$, mL/s/mg
Benign (n = 8)	142.79 $\pm$ 122.26	0.05 $\pm$ 0.03 ^a	7.62 $\pm$ 6.50 ^b	0.72 $\pm$ 0.50	0.76 $\pm$ 1.97	0.10 $\pm$ 0.07	5.84 $\times 10^{-3} \pm 5.73 \times 10^{-3}$
Malignant (n = 4)	140.35 $\pm$ 22.45	0.11 $\pm$ 0.09 ^a	24.79 $\pm$ 25.34 ^b	1.61 $\pm$ 1.30	0.16 $\pm$ 0.06	0.14 $\pm$ 0.08	1.89 $\times 10^{-2} \pm 2.19 \times 10^{-2}$
Diagnostic accuracy, %	52	72	81 ^c	75	79	71	75

AU indicates arbitrary units.

^a $p = .046$.

^b $p = .045$.

^c Most predictive quantitative SHI parameter.