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Detection of Clinically Significant Prostate Cancer Using Subharmonic Imaging

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Abstract—**This study assessed the prostate cancer (PCa) detection rates of contrast-enhanced, transrectal subharmonic ultrasound imaging (SHI). This IRB-approved study enrolled 55 subjects. The initial 5 subjects were studied for SHI optimization, while the remaining 50 were evaluated with conventional grayscale, continuous color and power Doppler as well as contrast-enhanced continuous SHI, color and power Doppler and SHI combined with maximum flash replenishment. A maximum of 6 directed biopsy cores were obtained from sites of greatest asymmetrical enhancement, followed by 12 spatially distributed systematic cores. SHI time-intensity parameters, including time to peak intensity, peak intensity and estimated perfusion were also evaluated for each biopsy core. Receiver operating characteristic (ROC) curve analysis and conditional logistic regression were employed to assess the benefit of each modality and the quantitative SHI parameters. Cancer was detected in 22 of 50 subjects. Among subjects with clinically significant PCa (n=11), targeted cores were more likely to be positive (odds ratio 1.39, p=0.02). The majority of patients detected by SHI demonstrated significant PCa (5/8) and SHI remained an independent marker of malignancy in a multivariate logistic regression model (p=0.027). ROC analysis of imaging findings compared to biopsy results yielded diagnostic accuracies ranging from 0.59 to 0.80 for all imaging modalities with the highest being for quantitative subharmonic perfusion estimates. In conclusion, this first-in-humans study provides a preliminary estimate of the diagnostic accuracy of SHI for detection of clinically significant PCa (up to 80%).**

Keywords— Contrast agent, prostate cancer diagnosis, targeted biopsy, contrast enhanced subharmonic imaging

I. Introduction

Prostate cancer (PCa) is the most prevalent cancer among American men and the sixth leading cause of cancer death (7.4% of deaths) among men worldwide [1]. The American Cancer Society estimates about 174,650 new cases of PCa will be diagnosed each year in USA alone, leading to about 31,620 deaths per annum [2]. The primary diagnostic tools for detecting PCa include biochemical screening with serum prostate specific antigen (PSA) and transrectal ultrasound (TRUS) guided biopsies. However, PSA screening results in the detection of many clinically insignificant cancers, which do not progress even without aggressive therapy [3]. Also, a significant minority of clinically significant cases go undetected in patients with low PSA values.

Clinically insignificant PCa can be managed by active surveillance (AS) and does not require treatment. In contrast, patients with a Gleason score $\geqslant$7, or those with $\geqslant$50% biopsy core involvement, are more likely to progress if left untreated; these "clinically significant" cancers should be considered for aggressive therapy [4-6]. A major barrier to the widespread adoption of AS is the lack of an accurate noninvasive diagnostic test to prospectively identify patients with clinically significant PCa, whose disease will progress without definitive therapy.

A. Contrast-Enhanced Imaging of Prostate Cancer

Our group has focused on prostate imaging with microbubble-based ultrasound contrast agents (UCAs), as ultrasound is the least expensive and most commonly used modality for prostate biopsy guidance. These UCAs have diameters less than 8 μm, having a lipid, protein or a polymer shell and can traverse the entire vasculature including the capillaries [7, 8]. At higher incident pressures (>200 kPa), these UCAs exhibit nonlinear behavior [9]. This behavior is used in diagnostic imaging as the UCA's nonlinear oscillations occur over a wide range of frequencies from subharmonics ($f_0/2$) to second harmonics ($2f_0$) and ultraharmonics ($3f_0/2$) of the insonation frequency (f_0) as well as its multiples. These signals can be used to create specific contrast imaging modes, such as subharmonic imaging (SHI), harmonic imaging (HI) and superharmonic imaging, respectively [10]. Clinical studies using HI have demonstrated that enhancement of the prostate with contrast enhanced transrectal ultrasound (CE-TRUS) correlates with increased microvessel density, [11, 12] and improved detection of PCa; [13, 14] with fewer biopsy cores [15, 16].

Although CE-TRUS with HI selectively detects clinically significant PCa, overall by-patient detection of high grade PCa with targeted biopsy based upon HI alone remains inferior to a 12 core systematic biopsy. This limitation is related, in part, to difficulty discerning enhancing foci of PCa adjacent to the hypervascular transition zone. A major limitation of HI is the reduced contrast-to-tissue ratio resulting from second harmonic generation and accumulation in tissue [14]. Hence, SHI is an attractive alternative imaging mode, because of the weaker subharmonic generation in tissue relative to the significant subharmonic scattering produced by UCAs [9]. This study focused on optimizing the detection of clinically significant PCa using SHI with CE-TRUS for targeted biopsies of the prostate.

II. Methods and Materials

Fifty-five men were prospectively enrolled and provided written informed consent between February 2017 and February 2018. The initial 5 subjects were used for SHI

optimization, and the remaining 50 for prospective evaluation of PCa detection with SHI. The initial optimization involved 9 different input pulses with varying shapes as previous studies have shown that the initial slope of pulses can alter the subharmonic response from microbubbles [17, 18], All pulses were evaluated for background suppression, visualization of blood flow, and presence of artifacts by two experienced observers and the one with best image quality was selected. An endocavitary IC5-9D transducer on a modified Logiq E9 system (GE Healthcare; Waukesha, WI, USA) was used. The UCA was Definity® (Perflutren Lipid Microsphere, Lantheus Medical Imaging; N. Billerica, MA, USA). Two vials of Definity (activated per the manufacturer's instructions) were diluted in 50 ml of saline and infused (4-10 ml/min titrated to effect).

Study participants were imaged in the lithotomy position with grayscale ultrasound performed to measure gland size [19]. Next, the prostate was evaluated with grayscale, color and power Doppler as well as with contrast-enhanced color Doppler, power Doppler and SHI with/without MIP. A standard imaging sweep through the prostate was performed in the axial plane from the base of the gland to the apex for each of the imaging modalities.

The peripheral zone of each prostate was rated for suspicion of PCa at 12 sites, including the medial and lateral components of each sextant. The CE-TRUS images were interpreted by two experienced investigators in consensus using a previously validated 5-point scale from 1 indicating benign, to 5 representing malignant [20]. SHI was used to direct targeted biopsy of the prostate with up to 6 targeted biopsy specimens from suspicious sites. This was followed by a conventional systematic double sextant biopsy of the prostate by a second investigator blinded to the location of the targeted biopsies.

A. Data Analysis

Each biopsy result was categorized in two ways: for the presence of PCa, and for the presence of clinically significant cancer (i.e., Gleason score ≥7 or ≥50% core involvement by tumor).

First, systematic and targeted cores were analyzed for per patient and per core detection rates for PCa and clinically significant PCa.

Secondly, conditional logistic regression analysis was used to assess the benefit of each imaging modality for predicting PCa as well as for predicting clinically significant PCa using Stata® 15.0 (Stata Corporation, College Station, TX, USA). Statistical significance was considered at $p < 0.05$. A stepwise reverse multivariate clustered conditional logistical regression was performed to select the imaging mode, which was most significant in predicting malignancy. The regression model included ten input variables: age, PSA, PSA density, and the scores from the seven imaging modes, as well as a single binary output variable: the biopsy result.

Lastly, SHI scans from each of the patients were used to create time intensity curves (TIC) for the base, mid, and apex regions of the prostate. First, peak intensity (PI) parametric maps were created pixel by pixel by stepping though each frame of each clip. The time it took for each pixel to reach PI was recorded as the time to peak intensity (TTP) parameter. Finally, a parametric map of perfusion (PER) was created. This parameter estimates perfusion as the slope of the wash-in of the TIC [21, 22].

Receiver operating characteristic (ROC) curve analysis was performed for each of the imaging modalities as well as for the three quantitative SHI parameters to determine diagnostic accuracy for detecting PCa overall and clinically significant PCa, in particular.

III. RESULTS

The observers concluded that a rectangular pulse shape with a 3.5 MHz receiver frequency and a 2.5 MHz bandwidth was the optimal transmit pulse.

Enhancement of capsular and intraprostatic vessels was observed in all patients upon intravenous infusion of the UCA. Of the 50, a total of 49 patients (98%) demonstrated suspicious enhancement on contrast enhanced ultrasound with SHI and received targeted biopsies, see figure 1.

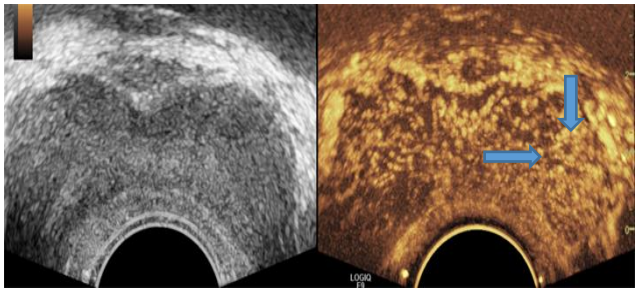

Figure 1. Grayscale and SHI at the base of the prostate obtained in a patient with prior negative MRI. Arrows point to an area of increased contrast enhancement at the left base. The targeted biopsy from this area demonstrated a Gleason 6 PCa.

A. Comparison between systematic and targeted biopsies for detecting PCa

PCa was detected in 22 of the 50 subjects (44%), and clinically significant cancer was detected in 11 of the 22 subjects (50%) using systematic biopsies. Whereas targeted biopsies diagnosed cancer in 8 subjects (16%) of which 5 were clinically significant (62.5%). Analysis of Gleason scores in the 22 patients with cancer detected by the 2 techniques failed to demonstrate any difference in Gleason scores by systematic versus directed biopsy cores ($p = 0.50$). In total 29 patients out of the 50 had a negative prior MRI within the year prior to study participation, of which 8 were found to have PCa after biopsy. In term of per core, of the systematic biopsy cores, 49 of the 600 were positive for PCa (8.2%) and 24 of 228 directed cores were positive (10.5%). The logistic odds ratio (OR) of a positive result with targeted cores compared to systematic cores in patients with cancer was 1.32. The OR for positive results for clinically significant PCa was 1.39 (23 of 600 systematic cores vs 12 of 228 targeted cores). A statistically significant higher number of patients were diagnosed with prostate cancer based upon the 600 systematic cores as compared to the 228 directed cores ($p = 0.0002$).

B. *Comparison of all imaging modalities for prediction of PCa*

Using conditional logistic regression for each imaging modality to assess its value for predicting malignancy, SHI was the most significant parameter for predicting PCa ($p = 0.001$) as well as for clinically significant PCa ($p = 0.027$), as shown in Table 1. After employing multivariate conditional logistic regression with backwards elimination, to compare SHI and other contrast enhanced imaging modalities, SHI remained as the most significant independent predictor of the presence of PCa ($p = 0.016$) and also for clinically significant PCa ($p = 0.027$).

Table 1: Conditional logistic regression giving significance value for each imaging mode

		For PCa	For clinically significant PCa
		p-value	p-value
Baseline:	Grayscale	0.361	0.103
	Color Doppler	0.055	0.065
	Power Doppler	0.003	0.033
Contrast:	Color Doppler	0.003	0.048
	Power Doppler	0.002	0.053
	SHI	0.001	0.027
	MIP-SHI	0.002	0.063

C. *SHI parameters for PCa detection*

A summary of the three SHI parameters obtained overall, as well as for clinically significant PCa and benign cores is given in Table 2. There was a significant difference in the estimated perfusion for clinically significant PCa compared to benign cases (34.5 8± 11.55 ml/min*ml vs 25.32 ± 8.26 ml/min*ml; $p = 0.003$).ROC analyses of the ability for all modalities and SHI parameters to detect all PCa as well as to detect clinically significant PCa yielded Az's from 0.59 – 0.80 as shown in Table 3. SHI demonstrated an Az of 0.73 for detection of clinically significant PCa, comparable to other CEUS imaging modes and the conventional grayscale imaging modes. The Az of the quantitative perfusion parameter for detecting clinically significant cancer was the highest (at 0.80) with a corresponding sensitivity of 0.80 [95% CI: 0.70%-0.89%] and specificity of 99% [95% CI: 99%-100%].

IV. DISCUSSION

Targeted biopsies aided by SHI demonstrated an OR of 1.32 and 1.39 relative to systematic biopsy for detection of PCa and clinically significant PCa, respectively. Previous studies employing CE-TRUS have shown an advantage over conventional TRUS, however with mixed success [23, 24]. In a previous clinical trial, 301 men were evaluated with contrast enhanced intermittent and continuous grayscale HI as well as color and power Doppler imaging and directed cores were twice as likely as systematic cores to be positive (i.e., contain PCa) [25]. However, directed biopsies missed 20% of patients with cancer (21 of 104), suggesting that systematic biopsy cannot be eliminated. Similar results were obtained in another study using a specific MIP implementation (MicroFlow Imaging or MFI). A potential advantage of MIP-SHI is the ability to visualize individual microvessels and to discern benign vs malignant vascular patterns.

As demonstrated in our multivariate regression model, SHI was an independent predictor of PCa even when combined with conventional grayscale and Doppler imaging as well as with previously employed contrast modalities. Although the PCa detection rate was lower relative to prior CEUS studies (OR of 1.39 in this study vs 2.10 in our prior study), a diagnostic accuracy of 80% was achieved for detection of clinically significant PCa with the quantitative SHI perfusion parameter. This suggests that further investigation into perfusion imaging with SHI may provide a useful method to predict the presence of significant PCa.

The lower OR in the current study as compared to our prior studies, may be related to a patient selection bias. Our urologists have begun using multiparametric MRI on many of their prostate biopsy patients. Many of the patients referred into the current study were sent because of a negative multiparametric MRI in the setting of high clinical suspicion for PCa, resulting in a study population with 29 negative MRI exams out of 50 patients. It is possible that these "MRI negative" cases of PCa are more difficult to detect with CEUS, as SHI preferentially detects more aggressive cancers that are often positive on MRI.

Our initial results indicate that SHI, and specifically parametric SHI, may be an effective follow-up screening tool for identifying clinically significant PCa in a patient population with elevated PSA or on AS. SHI can potentially reduce the number of biopsy cores for patients on AS who are subjected to annual repeat biopsies in order to detect high grade (Gleason score>7) or high volume (core involvement of >50%) PCa. However, even though there was improved detection of PCa with SHI guided TRUS, there were 13 PCa cases diagnosed only by systematic biopsy, suggesting that further research is needed to optimize SHI detection of PCa.

Table 2: SHI Parameters for targeted biopsies (biopsy result for clinically significant PCa)

	Mean Overall	Mean Positive Biopsy	Mean Negative Biopsy	p value
PI (dB)	124.31 ± 16.07	128.23 ± 21.79	124.18 ± 15.86	0.431
TTP (sec)	3.02 ± 1.51	2.58 ± 1.29	3.03 ± 1.52	0.151
PER (ml/min*ml)	25.62 ± 8.55	34.57 ± 11.55	25.32 ± 8.28	0.003

V. CONCLUSION

Contrast enhanced imaging of the prostate using SHI demonstrates excellent enhancement of the prostatic microvasculature. Directed biopsies with SHI were performed with only a third the number of cores used for systematic sextant biopsy. ROC analysis for diagnosing clinically significant PCa using quantitative SHI perfusion estimates provided an area under the curve of 0.80.

Nonetheless, many of the patients diagnosed by systematic biopsy were missed by SHI. This first in human study of SHI provides preliminary data on the diagnostic accuracy of contrast-enhanced SHI and quantitative derived parameters for detection of clinically significant PCa and may serve as the basis for future research efforts and clinical trials using SHI.

Table 3: Diagnostic Accuracies of various imaging modes given by Area under the ROC curve – Az

	Baseline			CEUS		SHI	MIP-SHI	SHI Parameters		
PCa	Grayscale	Color Doppler	Power Doppler	Contrast Color	Contrast Power			Peak Intensity	Time to Peak	Estimated Perfusion
All	0.61	0.64	0.70	0.58	0.62	0.66	0.65	0.61	0.54	0.66
Clinically Significant	0.76	0.74	0.80	0.69	0.73	0.73	0.72	0.60	0.59	0.80

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