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Contrast-enhanced Ultrasonography of the Prostate with Sonazoid

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Objective: The diagnosis of prostate cancer is based on the results of ultrasonography-guided needle biopsy of the prostate, but cancer foci are often not visible in conventional transrectal ultrasonography. Sonazoid is a new microbubble contrast agent. The purpose of our study was to compare areas of contrast material enhancement in the prostate at ultrasonography with whole-mount radical prostatectomy specimens to determine if the use of Sonazoid improves the detection rate of prostate cancer.
Methods: Fifty patients with biopsy-proven cancer of the prostate who were scheduled to undergo radical prostatectomy were recruited for this study. The day before the operation, each patient was evaluated with ultrasonography at baseline and again during intravenous infusion of Sonazoid. A map of ultrasonography findings was created prospectively at the time of imaging. Following radical prostatectomy, independent mapping of the pathologic results was performed and the maps were compared.
Results: Ultrasonography evaluation at baseline demonstrated that at least one focus of cancer was identified in 20 of the 50 subjects (40.0%). Meanwhile at least one cancer focus was enhanced in 31 of the 50 patients (62.0%) when Sonazoid was used. The combination of baseline grayscale imaging and contrast-enhanced imaging allowed identification of at least one focus of cancer in 40 patients (80.0%). Contrast-enhanced ultrasonography can improve sensitivity, especially for the detection of large cancer, peripheral zone cancer and highly malignant cancer.
Conclusions: Our study has demonstrated significantly improved detection of prostate cancer with the combination of baseline grayscale imaging and contrast-enhanced imaging compared with conventional ultrasonography techniques only, and this technique may be applicable to targeted biopsy.

Key words: Sonazoid – ultrasonography – prostate cancer – contrast medium

INTRODUCTION

The diagnosis of prostate cancer is based on the results of ultrasonography (US)-guided needle biopsy of the prostate. Currently, at least 10 biopsy cores are recommended for routine use in the prostate cancer guidelines provided by the European Association of Urology (1). Because cancer foci are often not visible at conventional transrectal US systematic biopsy, which is the standard method, clinically important cancers can be missed. Therefore, the improvement of prostate cancer detection is an important topic of diagnostic imaging. Detection and localization of prostate tumors during transrectal US would enable the urologist to perform a targeted biopsy.

Over the past few years, microbubble contrast-enhanced sonography has been introduced as a promising tool that can improve prostate cancer detection. Levovist® (Schering, Berlin, Germany) was used on targeted biopsy and the detection rate for targeted biopsy cores was reported to be significantly better than for systematic biopsy cores (2–4). However, the microvascular bed scanning time is limited

with a high mechanical index contrast US mode because of the rapid consumption of contrast material, and continuous scanning and real-time evaluation of blood flow are unavailable. New microbubble contrast agents, which could be used at a lower mechanical index and would have longer enhancement duration, were then developed, and the visualization of prostate cancer has been further improved using this technique. Presently, the most widely used transrectal US contrast agent is SonoVue® (Bracco, Milan, Italy), which has shown significantly higher positive biopsy core rates (5–7). Large single-institutional studies were also performed using Imagent® (Imcor, San Diego, CA, USA) (8) and Definity® (DuPont Pharmaceuticals, Billerica, MA, USA) (9).

These new contrast agents have not been approved by the Pharmaceutical and Medical Devices Agency in Japan and thus cannot be used. Only Sonazoid® (GE Healthcare, Oslo, Norway) has been approved for use in liver imaging. However, there have only been a few reports on Sonazoid for prostate imaging (10,11), and the analysis was insufficient. The purpose of the present study was to compare areas of contrast material enhancement in the prostate at US with whole-mount radical prostatectomy specimens to determine if the use of Sonazoid improves the detection rate of prostate cancer.

PATIENTS AND METHODS

Institutional review board approval was obtained for this study, and all patients provided written informed consent. Patients with biopsy-proven cancer of the prostate, who were scheduled to undergo radical prostatectomy, were recruited between April 2008 and March 2009. A total of 56 patients were recruited for the study. Patient age ranged from 51 to 75 years, with a mean of 64 years. Six of these patients underwent neoadjuvant hormonal therapy prior to the study for a mean of 6 months (range, 4–10 months), and their contrast enhancement on the US was very poor. Analysis was therefore performed on the 50 remaining patients who did not receive neoadjuvant hormonal therapy and were treated only with surgery.

The day before the operation, each patient was evaluated with US at baseline and again during intravenous infusion of Sonazoid. Imaging was performed in the transverse plane with a slow sweep of the transducer from the base to the apex. The US equipment used was a ProFocus® (B-K Medical ApS, Herlev, Denmark) with a transrectal probe (Type 8818). Normal grayscale imaging was performed at baseline at the fundamental frequency. Sonazoid is a lipid-stabilized suspension of perfluorocarbon microbubbles with a median diameter of 2.4–2.5 μm. Sonazoid was provided in vials that were reconstituted to yield 2 ml of liquid with a concentration of 10 μl of microbubbles per milliliter. One milliliter of this contrast material was infused as a bolus at a rate of 1 ml/s with a 22-gauge cannula placed in the antecubital vein, and flushed with 10 ml normal saline, though the dose was subsequently increased to yield the desired level of enhancement. The scanner was set in contrast harmonic imaging mode with a transmitting frequency of 4 MHz. The acoustic power was set at a mechanical index of 0.2 and the dynamic range was fixed at 70 dB. The depth of focus was set at 1.5 cm, and images were delivered at 12 frames/s. In some cases, we used a flash replenishment technique, involving bursting bubbles with a high mechanical index of 1.9, to examine suspicious lesions repeatedly. A nodule with intense increase in signal enhancement with a contour was considered a positive lesion, whereas nodules with unchanged or only slight signal enhancement were considered negative. However, judgment of the enhancement level was based on subjective visual impression of findings before and after injection. US examination was performed by a single experienced urologist (K.M.). A map of the US findings was created prospectively at the time of imaging.

Following radical prostatectomy, the entire gland was cut in 4 mm sections perpendicular to the urethra from the apex to the base. Apical and bladder neck shaves were cut radially. The slides were stained with hematoxylin and eosin. Each section was examined for cancer location and capsular penetration. Gleason score was assigned according to the 2005 International Society of Urological Pathology consensus (12). The outlines of each tumor focus on each slide were marked with a pen. The greatest dimension of the largest single focus of tumor from all sections of the tumor was determined by marking both ends of the tumor with a pen and measuring this distance directly from the glass slide. Independent mapping of the pathology results was performed, and all pathologic diagnoses were made by a different urological pathologist (A.H.). The two maps of US findings and pathology results were then compared.

The χ^2 test was used to examine difference in sensitivities, with findings of $P < 0.05$ considered significant.

RESULTS

The mean prostate-specific antigen (PSA) of the patients was 7.8 ng/ml (range, 2.8–24.8) and the mean prostate volume was 27.8 ml (range, 9.9–104). The results of pathologic examination revealed 104 cancer foci in the 50 prostate glands, consisting of 63 foci in the peripheral zone (PZ) and 41 foci in the transitional zone (TZ). The cancers were located in the PZ alone in 19 (38.0%) of the 50 cases and in the TZ alone in 8 (16.0%) of the 50 cases. In the remaining 23 subjects (46.0%), cancer foci were seen in both the PZ and TZ in the pathologic examination.

Figures 1 and 2 show representative images of grayscale US and contrast-enhanced US, compared with pathological slides. Figure 1a demonstrates the baseline sonogram in a 74-year-old man with an elevated PSA level of 8.2 ng/ml, who did not have any positive findings although a nodule

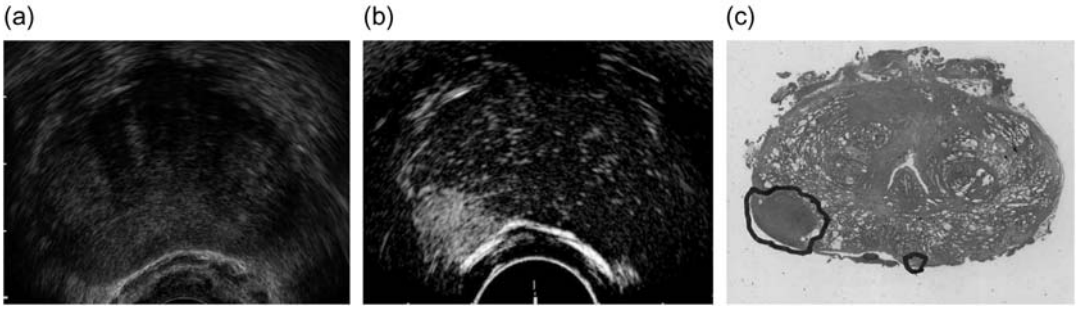

Figure 1. (a) A 74-year-old man with an elevated prostate-specific antigen (PSA) level of 8.2 ng/ml who had a palpable prostate nodule on the right lobe by rectal examination. Grayscale sonogram showed no focal lesion with hypoechogenicity. (b) Contrast-enhanced sonogram showed strongly increased vascularity in the right lateral lesion, suggesting the presence of prostate cancer. (c) Pathological examination revealed that the tumor was a Gleason 4 + 3 adenocarcinoma with capsular penetration, which proved to be in agreement with the enhancement of the lesion.

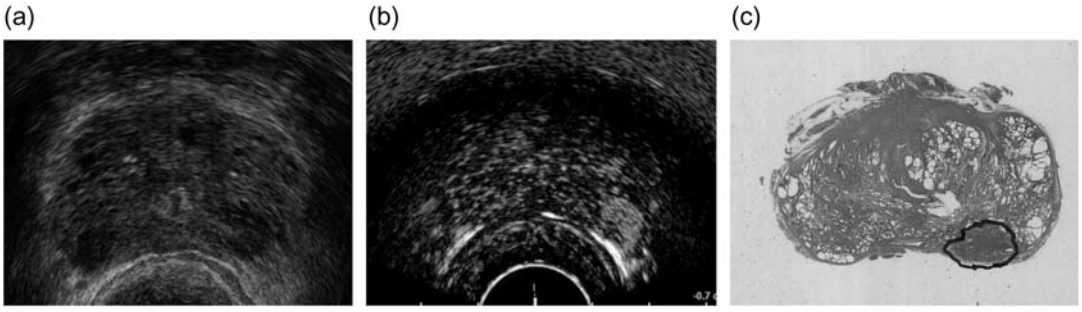

Figure 2. (a) A 72-year-old man with an elevated PSA level of 7.2 ng/ml. Digital rectal examination revealed that a nodule was palpable only on the left lobe. Transverse grayscale ultrasonography (US) image showed hypoechoic lesions on both the right and left lateral lesions. (b) Malignancy was suspected due to the rapid enhancement of the left side. (c) Histologic study demonstrated there was a Gleason 4 + 5 adenocarcinoma in the left lateral lesion with capsular penetration.

was palpable on the right lobe during rectal examination. About a half minute after the infusion of Sonazoid, intense enhancement of the right lobe appeared (Fig. 1b), which proved to be in agreement with the pathological slide (Fig. 1c). Pathologic examination revealed that the tumor was a Gleason 4 + 3 adenocarcinoma with capsular penetration. In Fig. 2a, grayscale US showed bilateral hypoechoic lesions in a 72-year-old man with an elevated PSA of 7.2 ng/ml. Digital rectal examination revealed that a nodule was palpable only on the left lobe. Figure 2b is a transverse sonogram obtained during bolus administration of Sonazoid, which showed focally increased enhancement of the left PZ. Histologic study demonstrated that there was a Gleason 4 + 5 adenocarcinoma in the same lesion with capsular penetration (Fig. 2c).

US evaluation at baseline demonstrated that 21 of the 104 cancer foci (20.2% sensitivity), including 11 foci in the PZ and 10 foci in the TZ, could be detected as hypoechoic lesions. At least one cancer focus was identified at baseline in 20 of the 50 patients (40.0%). Contrast-enhanced US demonstrated 32 foci (30.8% sensitivity) with increased contrast enhancement, including 27 in the PZ and 5 in the TZ. At least one cancer focus was enhanced by using Sonazoid in 31 of the 50 patients (62.0%), although five false positive lesions were enhanced by Sonazoid. The combination of baseline grayscale imaging and contrast-enhanced imaging allowed identification of 43 of the 104 cancer foci (41.3% sensitivity), including 30 in the PZ and 13 in the TZ. The sensitivity of combined imaging was significantly superior to that of baseline grayscale imaging ($P < 0.001$). At least one cancer focus was identified in 40 of the 50 patients (80.0%). Contrast-enhanced US demonstrated 32 cancer foci with increased contrast enhancement, and their mean size was 11.9 $\pm$ 5.1 mm (range, 24–4 mm), which was larger than that of the contrast-enhanced US negative 72 cancer foci (8.2 $\pm$ 5.6 mm; range, 21–2 mm).

Contrast-enhanced US can improve sensitivity for the detection of cancers, especially in the PZ as mentioned above. On the other hand, contrast-enhanced US was not sufficiently helpful in the detection of cancers within the TZ. It detected only 5 (12.2%) of a total of 41 TZ cancer foci, compared with 27 (42.9%) out of 63 PZ cancer foci. The difficulty with detection of TZ cancers is probably related to the intense, heterogeneous enhancement pattern associated with benign prostatic hyperplasia (13). However, TZ cancer foci that were large in size were detectable with contrast-enhanced US. Contrast-enhanced US detected five cancer foci in the TZ whose sizes were 21, 20, 17, 14 and 9 mm. Grayscale US did

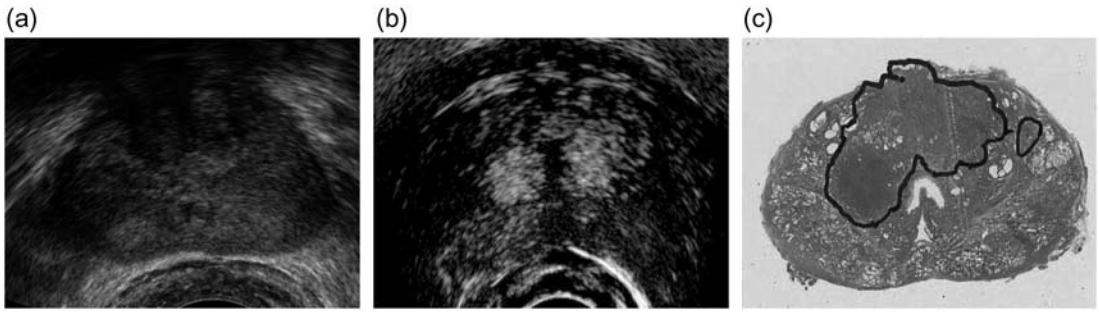

Figure 3. (a) A 65-year-old man with an elevated PSA level of 8.0 ng/ml whose clinical stage was T1c. Baseline grayscale image demonstrated no hypoechoic mass. (b) Contrast-enhanced US showed strong enhancement on the bilateral transitional zone (TZ) lesions. (c) Pathological diagnosis was TZ cancer with a Gleason score of 3 + 4, but capsular invasion was not observed.

Table 1. Percentage of patients whose cancers (at least one cancer focus) were detectable

	Baseline grayscale US	Contrast-enhanced US	Combination
Total 50 patients	20 patients (40.0%)	31 patients (62.0%)	40 patients (80.0%)
Capsular penetration (17 patients)	9 patients (52.9%)	12 patients (70.6%)	16 patients (94.1%)
Gleason score ≥4 + 3 (25 patients)	14 patients (56.0%)	18 patients (72.0%)	23 patients (92.0%)

US, ultrasonography.

not show any positive lesions in a 65-year-old man with an elevated PSA of 8.0 ng/ml whose clinical stage was T1c (Fig. 3a). Contrast-enhanced US showed strongly increased vascularity in bilateral TZ, suggesting the presence of prostate cancer (Fig. 3b), and the pathological results confirmed the US findings (Fig. 3c). The Gleason score was 3 + 4, but capsular invasion was not observed.

Highly malignant potential cases accompanying capsular penetration or showing a high Gleason score tended to be easy to find by the combination of baseline grayscale imaging and contrast-enhanced imaging. The results of the pathologic studies showed that capsular penetrations were observed in 17 (34.0%) out of 50 cases. In these 17 cases, at least one focus of cancer was identified in nine subjects (52.9%) on the grayscale sonogram, and in 12 patients (70.6%) on the contrast-enhanced sonogram. The combination imaging enabled us to detect at least one cancer focus in 16 patients (94.1%). The distribution of the Gleason score was as follows: 6 cases were classified as Gleason 6, 34 as Gleason 7, 5 as Gleason 8 and 5 cases as Gleason 9. Among the 25 cases whose Gleason scores were ≥4 + 3, at least one focus of cancer was identified at baseline in 14 patients (56.0%). By using Sonazoid, at least one cancer focus was enhanced in 18 patients (72.0%). The combination of baseline grayscale imaging and contrast-enhanced imaging allowed identification of at least one cancer focus in 23 patients (92.0%). These sensitivity data are summarized in Table 1.

No adverse events were observed in any patient and no complications resulted from the medication or US examinations.

DISCUSSION

We believe that the main strength of our study was the comparison of US findings with whole-mount slides from radical prostatectomy specimens. Pathologic evaluation of radical prostatectomy specimens allows detection of all cancer foci, not just those cancers found by needle biopsy. On the basis of independent prospective pathologic and US interpretations, we are able to compute the true sensitivity for the detection of cancerous lesions.

The ideal number of biopsies to be performed in order to detect prostate cancers remains a controversial topic. It was initially recommended that US-guided prostate biopsies obtain 6 cores, 3 from each side of the prostate gland, and this recommendation was later changed to 10–12 cores, on the basis of data showing that the more extensive biopsies resulted in the detection of 30% more cancers than the conventional sextant biopsy (14). Nevertheless, prostate biopsy is still associated with significant false negative findings, and it is still possible to miss significant cancers. A significant percentage of those men who had a persistent suspicion of cancer would be diagnosed with prostate cancer by saturation needle biopsy (>20 cores) (15,16). However, saturation biopsy is still associated with increased cost and complications (15,17). The issue of whether taking more biopsy cores results in the detection of more tumors with lower-risk characteristics remains controversial (18). Detection of insignificant cancer should also be taken into account.

Considering these weak points of saturation biopsy, targeted biopsy under contrast-enhanced US is desirable and

thought to be a promising procedure for detecting prostate cancer. We expect that the complication rates, including hematuria, urinary retention and infection, would be almost the same as for conventional systemic needle biopsy, because the procedure only requires a few additional targeted biopsy cores from the enhanced lesions. Furthermore, Sonazoid is known to be a fairly safe agent because it has been used for liver mass imaging in Japan since 2007 and no severe complications have been reported. No adverse events were observed in any of the 56 patients in the present study who received Sonazoid injections.

Studies of microvessel density within the prostate have demonstrated a clear association of increased microvessel density with the presence of carcinoma (19), metastases (20), stage of disease (21–23) and disease-specific survival (24,25). This neovascularity results in more blood flow, although much of the flow is in small vessels. However, the microvessels that proliferate in prostate carcinoma are undetectable with conventional transrectal Doppler US because of the limited spatial resolution of US equipment and slow flow in these vessels. However, intravascular ultrasound contrast agents can enhance the back-scattered echo from blood flow in small vessels. Recent studies have also suggested that contrast-enhanced US was more sensitive for the detection of clinically significant prostate cancer. Mitterberger et al. (26) performed contrast-enhanced color Doppler targeted biopsies plus 10-core systematic biopsies in 690 patients suspected of having prostate cancer. Contrast-enhanced color Doppler targeted biopsies detected significantly higher Gleason scores compared with systematic biopsies. In our study as well, Table 1 demonstrates that clinically significant cancer (capsular penetration cases and/or high Gleason score cases) was slightly more enhanced, which is thought to be a result of their high microvessel density.

The disadvantages of contrast-enhanced US are the extra time and additional costs involved. Preparing Sonazoid and the investigation itself takes ~5 min. If applied to a targeted biopsy, including preparation of the contrast medium, the venous administration and the handling time of extra biopsies, the total needle biopsy time would be increased by another 5–10 min. It also requires the additional cost of a high-end US machine with contrast-specific software installed to suppress signal from the background tissue, leaving only the signal from the microbubbles, although the US machine models for Sonazoid are becoming increasingly popular for liver imaging in Japan. Furthermore, this technique is operator dependent and there is a definite learning curve. We believe that contrast-enhanced US using Sonazoid is safe and effective for detecting prostate cancer, although it has the drawbacks of extra investigation time, additional costs and a learning curve. Therefore, the use of contrast-enhanced US for a targeted biopsy may not be suitable for a routine prostate needle biopsy of a new case, but rather should be adopted for repeat biopsy in patients with an elevated PSA level and negative biopsy results, who may have a significant cancer that should not be overlooked.

CONCLUSION

In conclusion, the present findings have demonstrated significantly improved detection of prostate cancer with the combination of baseline grayscale imaging and contrast-enhanced imaging compared with conventional US techniques only, and that the procedure may be applicable to targeted biopsy.

Conflict of interest statement

None declared.

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