The Value and Limitations of Contrast-enhanced Ultrasound in Detection of Prostate Cancer

ARTA STRAZDIŅA[1], GAIDA KRŪMIŅA[1] and MĀRIS SPERGA[2]

[1]*Department of Radiology, Eastern Clinical University Hospital, Riga Stradins University, Riga, Latvia;*
[2]*State Pathology Center of Latvia, Riga, Latvia*

Abstract. *The aim of our study was to evaluate the diagnostic value of contrast-enhanced ultrasonography (CEUS) in comparision to morphological examinations of radical prostatectomy specimens and to study factors limiting the visibility of malignant lesions. Patients and Methods: Fifty patients with proven prostate cancer (PV) were examined transrectally using, grey-scale, power Doppler (PD) and CEUS (pulse-inversion mode, low mechanical index) shortly before prostatectomy. The results were compared with morphological findings. The influence of tumour size, localization and grade on tumour visibility was studied. Results: A total of 72 prostate cancer foci were found at pathologic evaluation. Grey-scale imaging demonstrated 34 (47.2%), power Doppler 37 (51.4%) and CEUS 44 (61.1%) of these foci. No lesion less than 1 cm in size was detected. Statistically significant correlation was established between the visibility of of tumour in CEUS and the size of a focus (r=0.610, p=0.001). Sensitivity of CEUS in detection of peripheral gland tumours was 63.3%, of lesions invading both peripheral and central gland 83.3%, and of centrally located tumours 27.8%. In comparision, sensitivity of grey-scale imaging was 53.3%, 70.8% and 5.6%, respectively. CEUS detected 35.5% of low-grade and 80% of intermediate-grade tumours; the corresponding results of grey-scale imaging were 16.1% and 70%, respectively. Statistically significant correlation was detected (r=0.459: p=0.001) between visualization capabilities of CEUS and the malignant grade of prostate cancer. Conclusion: CEUS improves prostate cancer detection. Sensitivity of CEUS is lower in cases of small low-grade tumours, centrally located lesions and large infiltrating prostate tumours.*

Correspondence to: Arta Strazdina, Department of Radiology, Latvian Oncology Center, Eastern Clinical University Hospital, Riga Stradins University, 4, Hipokrata Str., Riga LV1079, Latvia. E-mail: arta.strazdina@inbox.lv

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Prostate cancer (PC) is the second most frequent malignant disease in the male population throughout the world and and the seventh most common cause of death due to male malignancies in the countries of the European Union (1). Much attention is paid to the early detection of PC. PC diagnosis is based on results of prostate-specific antigen (PSA) testing, digital rectal examination and ultrasound-guided biopsy of the prostate. Every part of the diagnostic triad has its drawbacks. Rising serum PSA is a non-specific symptom and indicates damage of the prostate, but not a specific prostate disease (2-4). Digital rectal examination is of low sensitivity because most tumours found nowadays are small; only owing to the method's relatively high specificity, is it still being used as a diagnostic test for PC (5-6).

Visualization of PC is affected by the nonspecific appearance of PC foci (7). The classical appearance of PC is assumed to be that of a hypoechogenic nodule in the peripheral zone of the prostate, although biopsy of such a node will reveal PC in only 17-57% of cases (8-10). On the other hand, up to 32% of all tumours are isoechogenic with the surrounding parenchyma (11). This has led to diagnostic transrectal ultrasonography as a diagnostic tool for PC being discredited and today it is mostly used to guide the biopsy needle to the appropriate anatomical regions of the prostate (12). In an attempt to improve the effectiveness of prostate biopsies, many biopsy strategies have been developed which aim to carry out up to 20 or more biopsies per single session (13-14). However, this results in a substantial increase of complication rates and patient discomfort. To replace extensive biopsy protocols by a limited number of targeted biopsies, new investigatory methods with high sensitivity and specificity are being studied. One such direction is based on the characteristic of neoangiogenesis of PC exploited in contrast-enhanced ultrasound (CEUS) examinations. Previous studies showed improvement in detection of PC using CEUS, however, in most cases, the value of CEUS was evaluated based on the morphological results of prostate biopsy (15-17). Unfortunately standard sextant biopsies fail to detect up to 15-35% of PC (11, 18). To evaluate the true sensitivity of CEUS and to establish factors limiting PC

visualization, we studied patients with morphologically proven PC scheduled for radical prostatectomy.

Patients and Methods

Between May, 2008 and February, 2009, 50 patients (mean age 63.0±5.79 years, mean PSA value 9.31±4.96 ng/ml) with biopsy or transurethral resection proven prostate cancer scheduled for radical prostatectomy were enrolled in the study and underwent grey-scale, power Doppler and CEUS examinations. The interval between the examinations for study purposes and surgery did not exceed two weeks, but the interval betweeen prostate biopsy or transurethral prostate resection and ultrasound examinations was more than 2 months. The study did not include patients who had had or were to undergo preoperative radiotherapy, nor patients who received hormonal treatment.

Ultrasound images were obtained with a TOSHIBA Aplio system (Toshiba Medical Systems, Japan) using a 5-10 MHz rectal biplane and a 3.6-8.8 MHz endocavital end-fire probe. All examinations were carried out by one radiologist with 10 years experience in transrectal ultrasonography. Examinations were performed in the transverse (axial) plane. Grey-scale images were obtained with tissue harmonic frequencies. For power Doppler examinations, technical parameters were used allowing visualization of slow flow in tiny blood vessels with a colour gain level at which the colour background 'noise' is absent. For CEUS examinations, pulse-inversion mode with a low mechanical index (0.1-0.2) was used. The contrast medium (2.4 ml sulphur hexafluoride microbubble suspension at concentration of 8 μl/ml; Sono Vue, Bracco, Italy) was administered as a bolus injection through a catheter introduced into the cubital vein, followed by an immediate 10 ml saline flush. The maximum dose administered per patient was 4.8 ml. To achieve optimum enhancement, the infusion rate was maximal. The examination was archived in digital format. Ultrasound findings were recorded in a map, indicating the size and localization of cancer foci. The size of visible foci were measured in a transverse plane. The position of suspicious foci in relation to the craniocaudal axis of the prostate was established (basis, midgland, apex). In grey-scale imaging, the following findings were considered to indicate malignancy: hypoechogenic foci in the peripheral zone, asymmetric bulging or contours, disrupted glandular tissue structure with inclusions of microcalcificates. In Doppler ultrasound, foci of increased vascularization and asymmetric hypervascularized zones in the peripheral gland were considered as possibly malignant. In CEUS, foci with rapid contrast enhancement and increased focal contrast enhancement comparing to adjacent parenchyma were considered suspicious of malignancy.

Morphological investigations were performed by one pathologist with 12 years' experience in histological evaluation of prostate specimens. After slicing prostatectomy specimens into 3 mm sections, histological preparations were stained with haematoxylin-eosin following conventional methodology. Localization and size in a transverse plane of each tumour focus was recorded in a schematic three-dimensional image of the prostate. Based on localization, PC foci were defined as inner, outer gland lesions, or lesions that invaded both parts (mixed lesions). The position of the malignant foci in relation to the craniocaudal axis of the prostate was defined (basis, midgland, apex). PC foci, depending on the absolute size, were divided into 3 groups: smaller than 1 cm, from 1 to 3 cm, larger than 3 cm. Each focus was marked in each image individually, indicating its diferentiation according to its Gleason score. Using the Gleason score, lesions were subdivided into low-grade (Gleason score 2-4), intermediate-grade (Gleason score 5-7), and high-grade tumours (Gleason score 8-10). The extent of high-grade prostate intraepithelial neoplasia (HGPIN) was determined as well.

To determine the exact number and diameter of blood vessels, specimens from 49 patients also underwent immunohistochemical examination with vascular endothelial markers CD34 and CD31 according to generally accepted methodology. The number of blood vessels was counted at 200 times magnification in 0.27 mm^2 field of view and recorded using a digital camera (Nikon DS-Fi1 of NikonEclipse 80i microscope with NIS-Elements BR 2.30). The number of vessels was determined in each Gleason differentiation component zone of each tumour focus and in the HGPIN zone.

The influence of tumour size, localization, grade and HGPIN on tumour visibility was studied. For statistical analysis of correlation, Spearman's signed rank test was performed and odds ratio calculated. Van Leuven independent sample *t*-test was used to estimate the relationship between tumor malignancy grade and the number of vessels. To compare CEUS results with grey-scale imaging, the Wilcoxon test was used. In all tests, $p<0.05$ was considered to indicate statistical significance.

Results

Pathological examination of 50 prostatectomy specimens revealed 72 malignant foci. A total of 21 (29.2%) of PC foci were smaller than 1 cm, 27 (37.5%) were 1-3 cm in size and 24 (33.3%) foci were larger than 3 cm. The majority of lesions (30, 41.7%) were located in the peripheral gland, nearly a third of tumours (24, 33.3%) invaded both the peripheral and central gland and the rest of the foci (18, 25%) were inner gland tumours. Most (98.6%) PC foci were of low (31, 43%) or intermediate (40, 55.6%) malignancy grade. A high-grade tumour was found in only one case.

The number of blood vessels in various Gleason differentiation areas was established for 49 patients whose surgical specimens underwent immunohistochemical examination with PSA and vascular endothelial markers CD34 and CD31. It was found that the number of blood vessels among various Gleason differentiation zones differed. Excluding groups with a small number of cases (Gleason 1, n=1; Gleason 5, n=1) from the calculations, it was found that in Gleason 3, the number of vessels on average exceeded the number of vessels in Gleason 2 zone by 12.4 vessels, while in Gleason 4, on average, the number of blood vessels exceeded the number of vessels in Gleason 3 differentiation zone by 8.59. It was calculated that the number of blood vessels in different Gleason differentiation areas was statistically significantly different and there was a positive correlation between the Gleason differentiation grade and the number of blood vessels (t=0.782, $p=0.001$, Van Leuven test). Morphological investigation of the specimens showed that all (100%) foci of clinically significant cancer evolved from a background of wide

Table I. *Diagnostic capability of ultrasonographic techniques (number of tumours=50).*

US techniques revealing the dominant focus of PC	Number of foci revealed	Diagnostic sensitivity
Grey-scale US	30	60%
PD	34	68%
CEUS	40	80%
Only CEUS	7	14%
Grey-scale, PD and CEUS	42	84%
Not visible with any of the methods	8	16%

US: Ultrasonography; CEUS: contrast-enhanced US; PD: power Doppler imaging.

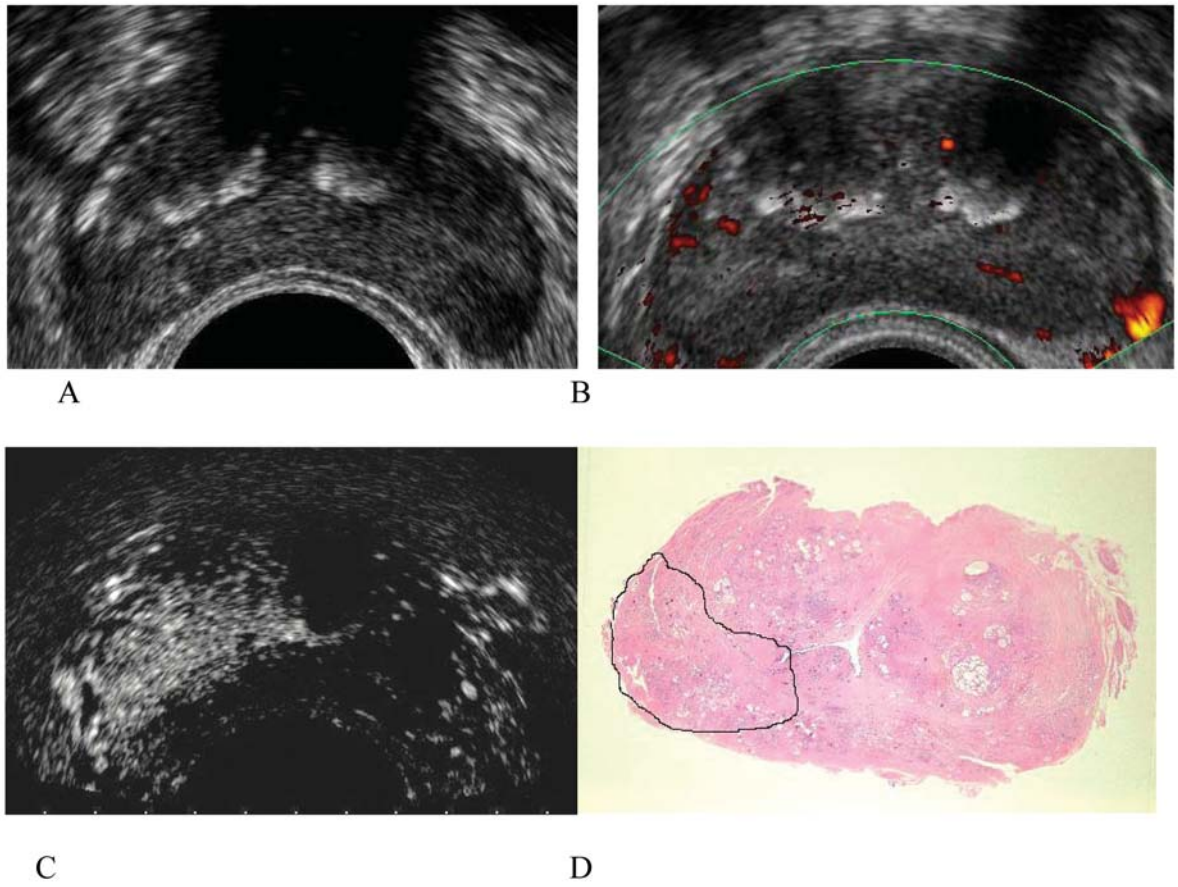

Figure 1. *Role of CEUS in diagnostics of prostate cancer. A: Transrectal ultrasound examination in grey-scale mode; no sonographical signs of malignancy can be seen. B: Inconclusive zone of enhanced vascularization in the right lobe in the vicinity of the lateral contour. C: After intravenous administration of contrast medium, a wide zone of enhanced vascularization in the right lobe is visible of a size corresponding to the morphologically identified tumour spread. D: Boundaries of the corresponding section of prostate cancer are marked in the histotopogram.*

HGPIN. In the HGPIN zone the vascular count ranged from 10 to 53, with an average of 30.21±8.36.

Baseline grey-scale imaging visualized 47.2% (34/72) of foci. An additional 3 tumours of the peripheral zone were found using power Doppler imaging, resulting in 51.4% sensitivity. CEUS revealed a total of 44 (61.1%) cancer foci; 39 of them were located in the peripheral zone or invaded both the peripheral and central gland. Six additional hypervascular lesions were found in the peripheral gland with no cancer cells at morphology consisting of prostatitis and HGPIN. This results in a positive predictive value (PPV) for peripheral and mixed

tumours of 86.7%. None of the 21 morphologically proven foci less than 1 cm in size were found using grey-scale, power Doppler and CEUS imaging. Seventeen out of 27 (63.0%) PC foci of 1-3 cm size were detected by grey-scale imaging, with power Doppler imaging 18 (66.7%) tumours of this size were detected, but using CEUS detected 25 (92.5%) of these tumours. In tumours larger than 3 cm in size (n=24), the sensitivity of grey-scale, power Doppler imaging and CEUS was practically the same at 70.8% (17/24), 79.2%, and 79.2% (19/24) respectively. Overall, statistically significant correlation was established (r=0.610, p=0.001, Spearman's rank test) between the size of a focus and visualization capabilities in CEUS.

The best results with all methods were achieved in visualization of tumours invading both the inner and outer gland (n=24): 70.8% (17) of PC foci were found with grey-scale imaging, 75% (18) using power Doppler imaging and 83.3% (20) by CEUS, which is attributed to the larger size of these tumours. More than half of the 30 outer gland (peripheral zone) tumours were found by grey-scale and power Doppler imaging (16, 53.3% and 17, 56.7%, respectively). CEUS revealed 19 out of 30 (63.3%) pure peripheral gland tumours. The worst diagnostic results were obtained for centrally (inner gland) located tumours. Of 18 centrally localized tumours, only 1 (5.6%) focus was seen using grey-scale, 2 (11.1%) using power Doppler and 5 (27.8%) using CEUS.

The ability to visualize the tumours was dependent on their malignancy. In grey-scale and power Doppler imaging 5 (16.1%) and 6 (19.4%) out of 31 low-grade tumours were found. The use of CEUS improved the PC detection rate nearly twofold: 11 (35.5%) low-grade PC foci were discovered. Using grey-scale imaging, 28 (70%) out of 40 intermediate-grade tumours were found, using power Doppler (30, 75%) and CEUS (32, 80%) improved detection of tumours. The only high-grade tumour was visible by all imaging methods. Statistically significant Spearman's rank correlation was detected (r=0.459: p=0.001) between visualization potential of CEUS and the malignant grade of PC.

To assess the diagnostic capabilities of each technique for detecting the dominant (largest) node of PC, each patient was assessed for visualization of one dominant node. If any of the methods showed several nodes, the major one was chosen.

The difference between the number of blood vessels in tumour and in the HGPIN zone around the tumour was assessed and it was estimated whether the difference in the number of blood vessels affected the visualization of foci in CEUS examinations. In cases where the number of blood vessels in the tumour node was less than the vascular count in the HGPIN zone, the capability to reveal PC foci was more than twofold smaller than in the reverse case (odds ratio, OR = 2.388, p=0.005).

Discussion

Grey-scale transrectal ultrasonography is still the most widely used imaging method to guide PC biopsies. Due to the nonspecific appearance of prostate cancer, diagnostic sensitivity of grey-scale examinations is insufficient. In our study, grey-scale imaging detected 47.2% (95% CI=0.361 to 0.586) of PC foci. Similar results are presented by others evaluating the ability of grey-scale imaging to detect morphologically identified PC foci (19). The use of power Doppler imaging in our study slihtly improved cancer detection rate: the overall sensitivity of the method achieved was 51.4% (95% CI=0.400 to 0.625). The poor results of both grey-scale and power Doppler imaging lead us to conclude that the methods cannot be used instead of guided biopsy, nor can they replace systematic protocol biopsy. Similar conclusions have been drawn by other researchers who analysed the results of biopsy specimens from foci of sonographically different appearance (20). Our study shows that the ultrasound contrast agent enables significantly improved imaging. Use of CEUS increased cancer detection to 61.1% (95% CI=0.495 to 0.715), with a PPV for peripheral gland lesions and tumours invading both the peripheral and central gland of 86.7%. Overall sensitivity of CEUS in comparision to grey-scale imaging was siginificantly better (p=0.008, Wilcoxon test). Our results correlate with those of other studies reporting the success of using CEUS (16, 21). Excellent performance was reported by Taymoorian and colleagues (22) who estimated diagnostic sensitivity of 100% and specificity of 48%. Similarly, according to the results of Aigner and colleagues (23), diagnostic sensitivity was 100% and negative predictive value was 99.8%. Our results are similar with diagnostic sensitivity of 92.5% for medium-sized (1-3 cm) peripheral zone foci. On a per patient basis, out of 50 patients, in 40 (80%) cases PC was found using CEUS, in 7 (14%) patients PC was found only with CEUS. However, in 10 patients (20%), we failed to detect cancer using CEUS.

By reviewing digitally archived examinations and comparing them with morphological findings, we retrospectively assessed what factors restrict the visualization of PC foci in CEUS. We found that visualization of the tumour is dependent on its size. We were not able to detect tumours whose size was less than 1 cm in pathological specimens; also the visualization of large tumours (>3 cm) was limited. Interestingly, half (22/44) of the tumours detected by CEUS were in fact microscopically larger in size than was estimated by imaging. When assessing the size of tumour in morphology, we did not take into consideration the shrinkage of specimens which occurs during processing. This means that tumour sizes *in vivo* were even greater than reported by morphology. For therapeutic decisions, when the volume of the tumour needs to be known, calculations of

tumour volume based on its sonographically visible size parameters could lead to underestimation of tumour volume. Nonvisualization of small tumours in CEUS is determined by a number of objective factors, first of all the neovascularity of PC. Development of neoangiogenesis in tumour was studied by Folkman and Cotran (24). They determined that small tumours up to 2 mm are practically avascular. A demonstrable increase in the density of blood vessels is shown only by tumours which have reached size of 1 ml. In addition, it should be noted that blood vessels which proliferate in PC are of a very small calibre (10-30 μm), and the blood flow in these vessels is slow (25). Small-sized foci in most cases are of low malignancy and, accordingly, have a lower density of small blood vessels, which, presumably, is not enough to generate signals visible in CEUS (26). In general, the larger tumours were better detected by CEUS. Seitz and colleagues (27) have established that larger tumours are more easily visualized. Nevertheless, we missed a significant proportion (5 out of 24) of tumours larger than 3 cm. This phenomenon could be explained by the nature of tumour angiogenesis – in early stages of tumour development the vascular volume increases, but as the node increases, the relationship between the tumour volume and the total blood volume decreases (28). Retrospective analysis of pathological protocols revealed that in most cases, nodes of large size infiltrated either the whole gland or all the peripheral zone of the gland, which prevented the possibility of comparison with normal prostate tissue.

The localization of foci was also of great importance. The best visualization was established for malignancies located in the peripheral zone and for tumours of mixed localization whose mass was partially located within the peripheral gland where, on the background of the relatively poorer vascularized normal prostate peripheral zone tissue, PC foci were discernable as hypervascular nodes.

Visualization of centrally localized tumours was the worst. Difficulties in seeing tumours of this location were associated with the intensive heterogeneous enhancement which is determined by benign prostate hyperplasia. On CEUS, 5 out of 18 centrally located tumours were found. All of them were more than 2 cm in the transverse plane and coexisting with a relatively mild mostly stromal benign prostate hyperplasia with no substantial hypervascularization.

Not surprisingly, tumours of low malignancy were less visible: in 85% of cases, tumours were smaller than 1 cm. In tumours of low malignancy (Gleason score up to 4) Gleason 2 component mainly constitutes the lesion. Although the vascular density was relatively low in low-grade tumours, we found that the average number of blood vessels (33.2±11.54) was not significantly different from that in the HGPIN zone (30±8.36) which was found to coexist with cancer in all cases. In our opinion, this could be one of the reasons which explains the poor visualization of low-grade tumour in CEUS. The average Gleason score for PC foci which were discernable with CEUS was 5.1, for foci not revealed in CEUS was 4.4. Our results suggest that the majority of tumours found with CEUS are larger and with a higher degree of malignancy than those which were not detected with CEUS (p=0.004). Similar conclusions are found in several other publications (16, 29).

Conclusion

CEUS improves sensitivity of PC detection. The highest diagnostic sensitivity for PC was achieved in clinically significant tumours located in the peripheral zone of the gland. CEUS is not suitable for detecting small low-grade tumours, which is of somewhat less clinical importance. Diagnostic sensitivity of CEUS, however, decreases for tumours of clinically relevant size when they are located in the central part of the gland or diffusely infiltrate the prostate. As such large tumours may remain undetected, we do not recommend that systematic biopsies be replaced by targeted CEUS-guided biopsies. CEUS could improve cancer detection in patients with elevated PSA serum levels and previous negative biopsy.

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