Riga Stradin’s University
Faculty of Medicine

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Value of Ultrasound in Diagnosis of Prostate Cancer

Summary of Thesis
Submitted for the Degree of Doctor of Philosophy in Medicine
Subspeciality: Radiology

Scientific Supervisor:
Habilitated Doctor of Medicine, Professor
Gaida Krūmiņa

ESF
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Riga, 2011
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1. Introduction

Topicality of the Research

Today prostate cancer (PC) is the second most common malignant disease in men. It is predicted that the incidence of prostate cancer will continue to grow. Despite the fact that the 5-year survival rates continue to improve, prostate cancer still remains a serious health problem. Expressions of prostate cancer constantly change. Tumours found nowadays, in PSA era, are smaller, with an earlier stage and a lower degree of malignancy than 20 years ago, but their aggression level is very different. Abilities to predict tumour biological aggressiveness are still limited.

Since prostate cancer is more and more often detected and treated in younger men, the predicted survival of which after treatment is 20-30 years, special attention is required for the clinical and radiological assessment of specific treatment outcomes and consequences. Among the developed and leading world countries the trend in cancer patient nursing in the new millennium is a therapy which is individual, patient-specific, with minimal risk of complications and would provide maximum possible therapeutic effect (Hricak, 2007).

In case of prostate cancer, this approach requires gaining precise information on the parameters characterizing tumours, which, in turn, allows selection of optimal treatment approach. Currently, different approaches and solutions are present in clinical practice and research literature, for example, observation, postponing the initiation of specific therapy (Wilt, 2009; Oliffe, 2009), androgen ablation (Seidenfeld, 2000, Loblaw, 2007), radical surgery (radical prostatectomy (Barre, 2007) or laparoscopic prostatektomija (Soderdahl, 2005; Kaipia, 2007), as well as different types of radiation therapy: external radiation therapy (Sandler, 2006; Pollack, 2000), brachytherapy (Machtens, 2006, Ash, 2000) and combination of the
methods mentioned above. Recently the choice of treatment options has been improved with several local ablation therapy methods: cryoablation (De La Taille, 2000), focused ultrasound ablation (Chaussy, 2001; Aus, 2006), radiofrequency ablation (Patriarca, 2006).

Informativity and specificity of visual prostate cancer imaging is still insufficient, there is no sufficiently high positive correlation between radiological findings from conventional US and presence of malignant tissue in surgery or biopsy specimens (de la Rosette, 2001). Alongside with positron emission tomography (PET) (Liu, 2001; Oyama, 2002), computed tomography (CT) (Engeler, 1992; Rørvik, 1998) and magnetic resonance (MRI) (Mullerad, 2005; Wang, 2006) options of the newest ultrasonography (US) technologies are evaluated as well (Gilligan, 2002; Thompson, 1997). A promising direction in diagnosis of prostate cancer is associated with assessment of neoangiogenesis typical to prostate cancer which is put into practice in Doppler and contrast-enhanced US (CEUS) examinations. Up to 85% of case PC is a multifocal process, while histological structures of PC foci often differ in terms of their various malignant cell differentiation. From the clinical point of view it is very important to obtain morphological material from the focus of the highest degree of malignancy. Since tumours of higher malignancy grade are usually characterized by higher density of microvascularisation, in assessment of such foci vascular imaging methods which allow performing visually guided biopsies of limited number would be of essential importance to detect clinically significant prostate cancer. Thus, it would be possible to avoid unnecessary biopsies, which is particularly important in patients with negative first-time biopsy results and an advanced elevation of prostate specific antigen (PSA) level.
Objectives of the Research

To investigate and compare value as well as accuracy of various ultrasound diagnostic methods in diagnosis of prostate cancer according to tumour size, location, grade of malignancy and neoangiogenesis in tumour tissues.

Tasks of the Research

1. To characterize the gray scale (B mode) US diagnostic potential in diagnosis of PC, depending on tumour size, localization and degree of malignancy.
2. To establish the value of energy Doppler mode in detecting of prostate cancer.
3. To approbate US contrast media in transrectal prostate ultrasound (TRUS) examinations and to assess diagnostic potential of this method.
4. To evaluate and to compare the accuracy and the diagnostic value in correlation with morphological findings for the B mode US, Doppler US and contrast enhanced US. To study factors limiting the visibility of malignant lesions.
5. To develop and recommend the optimal algorithm of ultrasound examination for patients with suspected PC.

Hypotheses Proposed

1. Ultrasound methods of vascular visualization - contrast enhanced US and Doppler examinations improve diagnostic capabilities and accuracy of early detection of prostate cancer;
2. Visualization abilities of PC depend on tumour size, localization and malignancy, characterized by a different amount of blood vessels in different malignancy areas in the tumour.
3. The highest radiological sensitivity of contrast enhanced US and Doppler US methods is achieved in the group of high-grade malignancy prostate cancers with typical neoangiogenesis in the tumour tissues.

**The Scientific Novelty and Practical Significance of the Study**

Prostate cancer is a common oncologic disease in men, which varies widely with regard to the degree of aggressiveness. Therefore, in addition to a precise topographic characterization, it is very important in a radiological examination to gain additional significant functional evidence on eventual biological potential of the tumour.

Although during the last decade a significant progress in primary radiological diagnostic of prostate cancer has been achieved, the options of currently used clinical - laboratory and diagnostic imaging techniques are limited and the results are not optimal. The study comprehends new options for radiological diagnosis of prostate cancer and detection of its anaplasia, basing on visual characteristics of tumoural neoangiogenesis.

1. A new technology – CEUS has been approbated for the first time in Latvia.
2. A comparative assessment of diagnostic sensitivity among different US techniques and a systematic summary of the results has been performed, correlation with clinical and morphological data has been carried out.
3. Evidence has been gained that the use of ultrasonographic vascular visualization methods - CEUS and Doppler examinations improves diagnostic sensitivity of high-grade prostate cancers group with typical neoangiogenesis in the tumour tissues.
4. Practical recommendations for transrectal prostate examination have been developed.

**Structure and Volume of the Thesis**

The Thesis is written in Latvian, in the classical form, containing sections of introduction, literature review, material and methods as well as analysis of the gained results and discussion, conclusions, bibliography and appendix. The amount of work is 135 pages. Analytically illustrative and statistical material is presented in 40 figures and 45 tables. List of literature includes works of 309 authors in the period from 1912 to 2009.

**2. Material and Methods**

**2.1. Material of the Study**

The study was carried out in Riga Eastern Clinical University Hospital, Latvian Oncology Center from January 2007 to November 2009. The patients enrolled in the Study were from the Urology Division, with biopsy-proven prostate cancer scheduled for radical prostatectomy. The interval between the examinations for study purposes and surgery did not exceed two weeks, but 97% of cases the operation was made 1-2 days following the examinations. The study did not include patients with carried-out or planned preoperative radiotherapy nor patients who received hormonal treatment. Morphological examinations of prostatectomy specimens were performed in State Pathology Center of Riga Eastern Clinical University Hospital.

Statistical data processing was carried out in Riga Stradin's
University, Department of Physics. Ethics Board of Riga Stradin's University was apprized of the study plan and design and issued authorization to perform the study. Consent from patients to participate in the study was received.

Study comprised 99 patients aged from 47 to 72 with the average age of 63.07 ± 5.68. All patients in preoperative period underwent various transrectal ultrasonography examinations. 50 patients from the total 99 (average age 63.00 ± 5.79, the average PSA value 9.31 ± 4.96) underwent grey scale (B mode), Doppler US and CEUS examinations; 49 patients (average age of 63.14 ± 5.62, the average PSA value of 9.33 ± 4.97) underwent only gray scale (B-mode) and power Doppler examinations. There was neither statistically significant age difference of patients in both groups (t = 0.124, p = 0.901), nor in PSA levels (t = 0.028, p = 0.978). Overall prostate-specific antigen (PSA) levels in the blood of patients varied from 3.2 to 30.3 ng/mL with the average level of 9.32 ± 4.94 ng/mL. The largest group of patients was the one with total PSA levels in the range from 4 to 10 ng/ml (60.6%) within the range of 10-20 ng/ml - 31.3%.

2.2. Transrectal Ultrasound Protocol and Evaluation of Sonographic Findings

The ultrasound system used for transrectal ultrasonoscopes was TOSHIBA Aplio XG (SSA-790A) (Toshiba Medical Systems, Japan) with 5-10 MHz rectal biplane and 6-10 MHz endocavital end-fire probe. Investigations were performed by one radiologist with a 10 year experience in transrectal ultrasonography.

Examinations in gray scale and power Doppler mode for all patients were performed in transverse (axial) and longitudinal (longitudinal) planes. Gray scale imaging were carried out using tissue harmonic frequencies within range from 7.5 to 10 MHz with the dynamic range of 55 dB. Energy Doppler examinations were carried out using technical parameters, which allows to visualize
slow flow in tiny blood vessels, choosing low pulse repetition frequency (PRF), within 400-500 Hz range and such color gain level at which the color background “noise” is absent.

For CEUS examinations there was used pulse-inversion mode with low mechanical index (0.1-0.2). The contrast medium (2.4 ml Sulphur hexafluoride microbubbles suspension (Sono Vue, Bracco, Italy) with concentration of 8 microlitres/ml) was administered as a bolus injection through a catheter, introduced into the cubital vein. The maximum dose administered per patient was 4.8 ml. Immediately after the intravenous contrast 10 ml of physiological saline was introduced into the vein. To achieve optimum enhancement degree the infusion rate was maximal. The CEUS examination was performed within 15-20 minutes.

The examination was archived in a digital format.

In the B mode with use of harmonic frequencies, the following signs were considered to be malignant:

- hypoechogetic foci in the peripheral zone,
- asymmetric bulging or contours,
- disrupted glandular tissue structure with inclusions of microcalcificates

In energy Doppler examinations the following signs were considered to be malignant:

- foci of increased vascularisation,
- asymmetric hypervascularized zones in the peripheral gland.

In CEUS examinations the following signs were considered to be malignant:

- foci of increased vascularisation,
- asymmetric hypervascularized zones,
Each focus was characterized by the following parameters: 1) size, 2) localization. The size of the focus was suggested to be its maximum size in the transverse plane images.

Depending on the location of foci, they were divided into:
- peripheral zone (outer gland),
- central zone (inner gland),
- tumours of mixed-localization.

The data were collected in a special prostate examination card with a schematic image of the prostate. All the foci detected in US examination were marked in the card, indicating their size and localization.

2.3. Morphological Examination of Radical Prostatectomy Specimens

Morphology investigations were performed by one doctor - pathologist with a 12 year experience in histological evaluation of prostate specimens. After fixation of the specimen in 10% solution of buffered formalin the size of the prostate was determined in three dimensions and the prostatic tissue was split in 3 mm thick slabs taking into account the anatomical structure of the prostate (McNeal zonal anatomy).

Histological preparations were stained with hematoxilin-eosin following conventional methodology. During histological investigation of specimens the histological type of a tumour was determined according to the WHO classification of prostate cancer and diagnostic criteria as well as the degree of malignancy according to Gleason scale. For each focus individually the minimum and maximum Gleason number from 1-5 was determined, and the sum of these figures represented the assessment of a focus
according to the Gleason scale.

Extension of a tumour was plotted in a schematic three-dimensional image of a prostate. Each focus was marked in the picture individually, indicating its differentiation according to the Gleason scale. Also extension of high-grade prostate intraepithelial neoplasia (HGPIN) was determined.

To determine the exact amount and diameter of blood vessels specimens from 49 patients also underwent immunohistochemical examination with prostate specific antigen – vascular endothelial marker CD34 and CD31 according to generally accepted methodology. Immunohistochemical staining results were considered positive assessing intensity of intracytoplasmatic brown color. Findings were divided into positive and negative reactions.

The number of blood vessels was counted in Windows XP environment at 200 times magnification in 0.27 mm² field of view using digital camera (Nikon DS-Fi1 of NikonEclipse 80i microscope with NIS-Elements BR 2.30) Alongside with the counting of blood vessels also measurements of vascular diameter were conducted. The amount and the diameter of blood vessels was determined in the characterising Gleason differentiation zone of each tumour focus. In specimens from 42 patients also amount and diameter of blood vessels were determined in HGPIN zone.

Results of radical prostatectomy specimens were summarized in a morphological card. It consisted of:

1. Schematic three-dimensional image of the prostate in which localization as well as size of tumour foci and HGPIN zone were outlined;
2. A textual description of the morphological finding and the histological diagnosis;
3. Data on the amount and diameter of blood vessels in tumour areas of various differentiation as well as in HGPIN zone.
2.4. Assessment of Correlatation Between Findings of Transrectal US and Morphological Changes

After the apparent change in the US and morphological findings were documented, both cards were compared. Compliance of visualised with different US methods foci to morphological findings was assessed determining whether the focus is / is not sonographically visible. Afterwards the foci, depending on the absolute value of their morphology observed size, were divided into 3 groups (1 - smaller than 1 cm, 2 - from 1 to 3 cm, 3 - larger than 3 cm). Following the Gleason sum of the tumour foci these foci were divided into low-grade (Gleason score 2-4), intermediate-grade (Gleason score 5-7), and high-grade tumours (Gleeson score 8-10).

Capability to visualize tumour foci, depending on their size, location, malignancy grade, amount and diameter of tumour blood as well as background (HGPIN) changes, were evaluated.

2.5. Methods of Statistical Analysis of Results

The aim of data statistical analysis was, using appropriate statistical methods, to assess absolute and relative distribution of malignant foci among the study groups, based on their size, location, degree of malignancy, Gleason score as well as the amount of blood vessels and to find the relation of abovementioned parameters visualization potentialities for malignant tumour foci.

The data were recorded in the computer program Microsoft Excel table and converted to the computer program SPSS Statistics 18.0 of the company PASW (ASV) for further data processing.

For the data statistical processing there were used common descriptive and conclusive statistical methods.

In all cases, the statistical significance level of the hypothesis,
p<0.05 was considered as the basis to reject the corresponding null hypothesis and to accept of alternative hypothesis.

3. Results

3.1. Morphological Characterization of Cancer Foci

In total 194 tumour foci were found to 99 patients. Multifocal process was identified to 44 patients (44.4%), with the number of cancer foci varying from 2-7. The absolute size of the malignant foci was within range from 0.2 to 5.5 cm (M = 1.74 ± 1.39 cm).

Dividing the PC foci into groups of size <1 cm, from 1-3 cm, >3 cm, it was found that the highest number was in the small foci group of sizes up to 1 cm (40.7%) and almost as much in the group of sizes from 1 to 3 cm (40.2%).

Nearly 2/3 (62.9%) of foci were located in the peripheral gland zone, the rest of the foci distribution in the central part and with mixed localization was almost equal (18% and 19.1%).

The most common Gleeson differentiation figures of observed morphology in tumour foci were 2+2 (43.8%), followed by 2+3 (25.3%) and 3+3 (15.5%).

In terms of numbers the majority were moderate malignancy grade tumours (50.5%), followed by low-malignancy grade tumours (45.4%), which together accounted for 95.9% of all cancers.

Malignancy grade among foci of different sizes differs significantly: the majority of small foci (75%) were of low malignancy degree, while the number of large tumours with low degree of malignancy was small (11%). In assessing the odds ratio (OR) between the foci of less than 1 cm and the other two groups (foci size ≥1 cm) in relation to the malignancy degree of foci it was calculated that the chance that a focus of size <1 cm will have a
low degree of malignancy is 9.06 times higher than the possibility that a focus of size >1cm will have a low degree of malignancy (95% CI 4.59 to 17.80, p=0.05). Testing the statistical hypothesis on relationship between foci size and the degree of malignancy with chi-square test, it was established that the distributions differ statistically reliably ($c^2$=51,052: df 4: p = 0.001) and the correlation is moderate ($r$=0.459: p = 0.001), estimating the Spearman’s rank correlation.

The number and diameter of blood vessels in various Gleason differentiation areas was established for patients (49) whose surgery specimens underwent immunohistochemical examination with prostate specific antigen - vascular endothelial marker CD34, and it was found that the number of blood vessels among various Gleason differentiation zones differs. Excluding from the calculations groups with a small number of cases (Gl 1 n =1, Gl 5 n=2), it was found that in Gleason 3 differentiation zone the amount of blood vessels on average exceeds the Gleason 2 zone for 12.3 vessels, while in the Gleason 4 differentiation zone, on average, the number of blood vessels exceeds the amount in Gleason 3 differentiation zone for 13.1 greater than 3. It was calculated that the amount of blood vessels in different Gleason differentiation areas is statistically significantly different and shows a positive correlation between the Gleason differentiation number and the amount of blood vessels. Statistically significant differences in the diameter of blood vessels in various Gleason differentiation areas were not observed, and there was no correlation between vascular diameter and Gleason differentiation score.
3.2. Ultrasonography Findings

3.2.1. Visualization of Prostate Cancer in the Grey Scale (B) Mode

Performing TRUS examinations B mode, from 194 foci verified morphologically 83 foci were discerned by US; 111 foci were not detected. The diagnostic sensitivity of the test was 42.8% (95% CI 36.0 to 49.8%).

Within each size group the opportunity to discern foci in the B mode was different (see Figure 1). The best visualization was in the group of size > 3cm - 73% of foci were detected (27 of 37), followed by the group of foci with size from 1-3 cm - diagnostic sensitivity equals 60.25% (47 out of 78). In the foci group of size <1 cm just 11.4% of foci (9 of 79) were discernable. In determining the odds ratio of visualization in relation to foci size, it was estimated that the probability to discern a focus of size ≥ 1 cm is 4.81 times greater than the chance to discern a focus which is less than 1 cm (95% CI 2.1 to 10.63, p=0.005).

In total statistically significant correlation was established (r=0.519, p=0.001) between the size of a focus and visualization capabilities in B mode.

![Figure 1. Visualization of PC foci in B mode depending on the size](image-url)
The relationship between focus localization and visualization potentialities in B mode was analyzed, and established that visualization is better for foci located in the peripheral gland – number of visible foci was 57 of 122 (46.7%) as well as foci of mixed localization - 25 of 37 foci (67.6 %) were visible. The potentialities to visualize foci of central localization appeared to be very weak – only 1 out of 35 foci (2.9%) was detected. Testing the statistical hypothesis on potentiality to visualize foci size in relationship to their localization with chi-square test, it was established that the distributions differ statistically reliably ($\chi^2=32.643$: df 2: p=0.001) but do not correlate ($r=0.065$: p=0.0369). Calculations of the odds ratio showed that generally probability to visualize foci located peripherally and foci of mixed localization foci is 36 times greater than the probability to establish a centrally localized PC focus (95% CI 0.3 to 20.7, p=0.05).

Also correlation analysis between visualization possibilities in B mode and the degree of malignancy foci was performed. In total, visualization of low-grade malignancy foci were poor – it was possible to visualize only 16 of 88 foci (18.1%), however medium-grade and high-grade malignancy foci were significantly more visible, 62.2% and 75.0% (corresponding to 61 from 98 and 6 out of 8 foci).

Analyzing the results of different size foci groups, it was established that in two major foci groups all six high-grade malignancy PC foci were discerned (100%). 1-3 cm large tumour of intermediate-grade malignancy were visualized in 69.3% (34 of 49 foci), but foci of intermediate-grade malignancy larger than 3 cm - in 74.2% of cases (23 foci out of 31).

Testing the statistical hypothesis of potentialities of visualization in B mode in relation to the degree of malignancy with chi-square test, it was established that the distributions differ statistically reliably ($\chi^2 = 32.643$: df 2: p=0.001) and there is a statistically significant intermediate Spearman’s rank correlation ($r = 0.454$: p = 0.001).
3.2.2. Characterization of Prostate Cancer Vascularization in Energy Doppler Mode

Performing TRUS examinations in energy Doppler (ED) mode, 83 foci (42.8\%) were visualised from 194 foci which were morphologically verified as hypervascular nodes or asymmetrical hypervascularisation areas, however 111 foci (57.2\%) were not established. The diagnostic sensitivity of the test is 42.8\% (95\% CI 36.0 to 49.8\%).

Within each size group the opportunity to discern foci in the ED mode was different (see Figure 2). The best visualization was in the group of size larger than 3cm - 78.4\% of foci were detected (29 of 37), followed by the group of foci with size from 1-3 cm, discerned in - 60.3\% of cases (47 out of 78). In the foci group of size smaller than 1 cm just 8.9\% of foci (7 of 79) were detected. In total statistically significant correlation was established (r=0.574, p=0.001) between the size of a focus and visualization capabilities in ED mode, Spearman’s rank correlation.

Figure 2. Visualization of PC foci in ED mode depending on the size
Analyzing the relationship between focus localization and visualization capabilities in ED mode, it was established that visualization is better for foci located in the peripheral gland – the rate of established foci was 46.7% (57 of 122), as well as foci of mixed localization which were visualized in 67.6% of cases (discerned 25 from 37). The visualization of a focus localized in the central zone was successful in one case from 35 (2.9%).

Testing the statistical hypothesis on potentiality to visualize foci in ED mode size in relationship to their localization with chi-square test, it was established that the distributions differ statistically reliably ($\chi^2 = 32.643$: df 2: $p=0.001$) but do not correlate ($r = 0.065$: $p = 0.0369$), Spearman’s rank correlation.

Calculating the odds ratio it was established that the overall probability to discern a centrally localized PC focus is 2.8% of the probability that a focus of peripheral and mixed localization will be visualised (95% CI 0.3 to 20.7, $p=0.05$).

Also correlation analysis between visualisation potentialities in ED mode and the degree of malignancy foci was performed. In total, visualization potentialities of low-grade cancers were poor – it was possible to visualize only 16 of 88 foci (18.1%), however medium-grade and high-grade cancers were significantly more visible, 61 out of 98 foci and 6 out of 8 foci, respectively (diagnostic sensitivity accordingly was 62.2% and 75%).

Analyzing the results of different-sized foci groups, it was established that in two major foci groups all six high-grade PC were discerned (100%). 1-3 cm large tumour of intermediate-grade were visualized in 69.4% (34 out of 49 foci), but foci of intermediate-grade larger than 3 cm - in 80.6% of cases (24 foci out of 31).

Testing the statistical hypothesis of capabilities of visualisation in ED mode in relation to the degree of malignancy by chi-square test, it was established that the distributions differ statistically reliably ($c^2 = 40.312$: df 2: $p=0.001$) and there is a statistically significant intermediate Spearman’s rank correlation ($r=0.454$: $p=0.001$).
3.2.3. Results of Approbation of Contrast Enhanced US, Value of Contrast Enhanced US in Prostate Cancer Diagnostics

50 patients underwent CEUS examination. Intravenous US contrast medium (Sono Vue) administration was well tolerated. Only one patient experienced transient dizziness. Tumour enhancement type was established analyzing digitally archived CEUS series. In cases where a tumour was apparent in ultrasound, pathological changes were visible as more echogenic foci or asymmetrical hyperechogenic tissue bands on background of less enhanced parenchyma. Echogenicity difference between normal prostate tissue and pathological changes remained for 8-10 seconds. Afterwards the tissue adjacent to the tumour became isoechogenic with the tumour focus. In 5 cases (10%) also earlier washout from the alternated zone was observed. In most cases, simultaneously with the tumour intensive enhancement of the transition zone (central gland) with BPH-specific hyperplastic knots. Echosignals caused by the contrast medium in tissue could be visible for approximately 2-3 minutes. The test was conducted within 15 - 20 minutes.

In the CEUS group 72 cancer foci were morphologically found.

Foci distribution groups based on size, location, degree of malignancy and amount of blood vessels in tumours of different malignancy grade are displayed in tables 1-3.
Table 1. Distribution of foci according to their size in the group of patients examined by CEUS

<table>
<thead>
<tr>
<th>Group of foci size</th>
<th>Number</th>
<th>%</th>
<th>95% CI from</th>
<th>95% CI to</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 cm</td>
<td>21</td>
<td>29.2</td>
<td>19.9</td>
<td>40.5</td>
</tr>
<tr>
<td>1–3 cm</td>
<td>27</td>
<td>37.5</td>
<td>27.2</td>
<td>49.1</td>
</tr>
<tr>
<td>&gt;3 cm</td>
<td>24</td>
<td>33.3</td>
<td>23.5</td>
<td>44.8</td>
</tr>
<tr>
<td>Total number</td>
<td>72</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The number of PC foci was almost equal in all three size groups, with a slight predominance (37.5%) of the group of medium size (1-3 cm).

Table 2. Distribution of foci according to their localization in the group of patients examined with CEUS

<table>
<thead>
<tr>
<th>Localisation of foci</th>
<th>Number</th>
<th>%</th>
<th>95% CI from</th>
<th>95% CI to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral zone</td>
<td>30</td>
<td>41.7</td>
<td>31.0</td>
<td>53.2</td>
</tr>
<tr>
<td>Central zone</td>
<td>18</td>
<td>25.0</td>
<td>16.4</td>
<td>36.0</td>
</tr>
<tr>
<td>Mixed localization</td>
<td>24</td>
<td>33.3</td>
<td>23.5</td>
<td>44.8</td>
</tr>
<tr>
<td>Total number</td>
<td>72</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to localization the highest number of tumours was present in the peripheral zones nodes, followed by tumours of mixed localization.
Table 3. Distribution of foci according to their malignity degree in the group of patients examined with CEUS

<table>
<thead>
<tr>
<th>Malignity degree</th>
<th>Number</th>
<th>%</th>
<th>95% CI from</th>
<th>to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (Gleason score 2-4)</td>
<td>31</td>
<td>43</td>
<td>32.3</td>
<td>54.6</td>
</tr>
<tr>
<td>Intermediate (Gleason score 5-7)</td>
<td>40</td>
<td>55.6</td>
<td>44.1</td>
<td>66.5</td>
</tr>
<tr>
<td>High (Gleason score 8-10)</td>
<td>1</td>
<td>1.4</td>
<td>0.25</td>
<td>7.5</td>
</tr>
<tr>
<td>Total number</td>
<td>72</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Most (98.6%) of PC foci were of low (43%) or intermediate (55.6%) malignancy grade. A high-degree malignancy tumour was found in only 1 case.

With transrectal prostate CEUS examination, from 72 morphologically verified foci in CEUS 44 foci were discernable; 28 foci were not detected. The diagnostic sensitivity of the test was 61.1% (95% CI 49.5 to 75.4%).

Within each size group the opportunity to discern foci in the CEUS mode was different (see Figure 3). The best visualization was in the group of medium size foci (1-3 cm) in which 25 foci out of 27 were discerned (92.5%), followed by the group of foci larger than 3 cm which were discerned in 19 cases out of 24 (79.2%).

Foci smaller than 1 cm were not discerned in any case. In total statistically significant correlation was established (r=0.610, p=0.001) between the size of a focus and visualization capabilities in CEUS.
Analyzing the relationship between focus localization and visualization possibilities applying CEUS it was found that the best discernable foci are the ones of peripheral localization – the total rate of visualized nodes was 63.3% (19 out of 30), slightly worse differentiation was observed for foci of mixed localization - 54.0% (20 out of 37). Visualization of centrally localized PC foci was poor – only 27.8% of foci (5 out of 18) were detected.

Testing the statistical hypothesis on potentiality to visualize foci size in relationship to their localization with chi-square test, it was established that the distributions differ statistically reliably ($\chi^2=13.465$: df 2: $p = 0.001$) but do not correlate ($r =0.154$: $p = 0.196$), Spearman’s rank correlation.

Also correlation analysis between visualisation potentialities in CEUS and the degree of malignancy foci was performed. In total, visualization capabilities of low-grade cancer foci were lower - 35.5% of foci (11 of 31) were discerned, however foci of intermediate-grade malignancy were significantly better visible - in 80% (32 of 40). Also the only high-grade malignancy found morfologically was detected.
in CEUS. Statistically significant intermediate Spearman's rank correlation was detected \( r=0.459; p=0.001 \) between visualisation potentialities of CEUS and malignancy grade of PC.

### 3.3. Comparison of US Methods

A comparison was made among percentage rates of Gray scale (B mode), energy Doppler US (ED mode) and CEUS. In the assessment of potentialities of B and ED treatment the diagnostic results of all patients (99 patients, 194 foci) were included, however the diagnostic results for the CEUS method represented findings from the contrast group patients (50 patients, 72 foci).

<table>
<thead>
<tr>
<th>Method</th>
<th>Diagnostic sensitivity (%)</th>
<th>95% CI from</th>
<th>to</th>
</tr>
</thead>
<tbody>
<tr>
<td>B mode (n=194)</td>
<td>42.8</td>
<td>36.0</td>
<td>49.8</td>
</tr>
<tr>
<td>ED mode (n=194)</td>
<td>42.8</td>
<td>36.0</td>
<td>49.8</td>
</tr>
<tr>
<td>CEUS (n=72)</td>
<td>61.1</td>
<td>49.5</td>
<td>75.4</td>
</tr>
</tbody>
</table>

The sensitivity of B and ED modes over the whole number of foci was low (42.8%). In total, the use of CEUS allowed to reveal by 18.3% PC foci more than using B or ED mode US examinations.

Diagnostic results to large extent were influenced by the size of foci (see Figure 4.) - foci smaller than 1 cm were not detectable with CEUS and in B and ED modes they were seen in a very small number (11.4% and 8.9%). Visualization of the foci greater than 1 cm with ED method was just slightly better than using a routine gray scale B mode examination (64.3% and 66.1%), while the results from CEUS also in this size group were better by more than 20% (86.3%).
Figure 4. Diagnostic sensitivity of US methods depending on the size of PC foci

All test results also were influenced by localization of foci (see Figure 5). Foci of mixed localization had the best visualization with all the examination methods (67.6% for B as well as ED mode, 83.3% for CEUS), which is attributed to larger sizes of tumours in this group. Examinations in B and ED modes allowed to reveal almost half (46.7%) of all nodes localized peripherally, however the results from the group of CEUS in this foci group were significantly better (63.3%). Unlike exams in B un ED modes, which visualized centrally localized tumour nodes only in a few cases, with CEUS method 27.8% foci were revealed.
Figure 5. Diagnostic sensitivity of US methods depending on PC foci localisation

All examination methods allowed to reveal foci located in the peripheral zone and foci of mixed localization more often when sizes of the nodes were larger. In the group of foci size from 1 to 3 cm B and ED modes allowed to reveal respectively 65% and 66.7% of foci in the peripheral zone, but CEUS method from 17 (94.1%) foci of such localization and size. Visualization of nodes less than 1 cm was significantly worse, regardless of localization of tumours. Although in all the diagnostic results of centrally located tumours are regarded as poor, in both groups of major foci (≥ 1 cm) CEUS allowed to reveal 5 lesions of 6 (83.3%) as opposed to B and ED modes (11.1%).

Malignancy degree of foci influenced sensitivity of all examination methods (see Figure 6).
Foci of low-grade malignancy were detectable in B and ED mode only in 18% of cases, while CEUS revealed almost twice bigger number of nodes (35.5%). Results were significantly better in the group of foci ≥ 1 cm: in B mode, low-grade tumour nodes were revealed in 37.9% of cases, in ED mode, and with CEUS 41.4% and 78.5%, respectively.

In all size groups cancers of intermediate and high-grade were better visualize by B and ED modes, however the best results were in the group of intermediate-grade malignancy foci of size 1-3 cm, where the CEUS was able to reveal all 16 (100.0%) tumours (in B and ED mode 69.4% of the corresponding group of foci size). Among the study population high-grade malignancy tumours were rare and all nodes of 1 cm and larger were revealable with all examination methods.

To assess the diagnostic capabilities of detecting the dominant (largest) node of prostate cancer with various US methods, it was investigated at how many patients and with which US techniques PC was revealed in the CEUS group. Each patient was assessed
visualization potentialities of one dominant node. If any of the methods showed several nodes, the major of them was chosen.

Table 5. Diagnostic possibilities using a variety of US techniques in the CEUS group (n=50)

<table>
<thead>
<tr>
<th>US techniques and their combinations which allow to reveal the dominant focus of PC</th>
<th>Number of foci revealed</th>
<th>Diagnostic sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>B mode US</td>
<td>30</td>
<td>60%</td>
</tr>
<tr>
<td>ED mode US</td>
<td>34</td>
<td>68%</td>
</tr>
<tr>
<td>At least one of both methods</td>
<td>36</td>
<td>72%</td>
</tr>
<tr>
<td>CEUS</td>
<td>40</td>
<td>80%</td>
</tr>
<tr>
<td>Only CEUS</td>
<td>7</td>
<td>14%</td>
</tr>
<tr>
<td>B, ED techniques and CEUS</td>
<td>42</td>
<td>84%</td>
</tr>
<tr>
<td>Not visible with any of the methods</td>
<td>8</td>
<td>16%</td>
</tr>
</tbody>
</table>

In order to assess diagnostic potentialities of PC foci of clinically significant size (≥ 1 cm) using a variety of US techniques it was compared how many PC foci ≥ 1 cm in the CEUS group were revealed with any of US techniques (see Table 6.)

Table 6. Diagnostic of PC foci of clinically significant size in CEUS group (n=51)

<table>
<thead>
<tr>
<th>US techniques and their combinations which allow to reveal the dominant focus of PC</th>
<th>Number of foci revealed</th>
<th>Diagnostic sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>B mode US</td>
<td>38</td>
<td>74.5%</td>
</tr>
<tr>
<td>ED mode US</td>
<td>36</td>
<td>70.6%</td>
</tr>
<tr>
<td>CEUS</td>
<td>40</td>
<td>86.3%</td>
</tr>
</tbody>
</table>
3.4. Possibilities of Visualization of PC Foci in Terms of Difference Between Amount and Diameter of Blood Vessels in Areas of Tumour and the Background Lesion - High-Grade Prostate Intraepithelial Neoplasia (HGPIN).

Morphological investigation of the specimen showed that all (100%) foci of clinically significant cancer have evolved on the background of wide high-grade prostate intraepithelial neoplasia (HGPIN) – precancer lesion. Immunohistochemically the number and diameter of blood vessels were assessed in HGIN zone in 42 patients. The number and diameter of blood vessels were assessed in the zone of highest grade by Gleason scale also in each of the 58 PC foci of clinically significant size (> 1 cm) which were revealed in these patients. In HGIN zone the vascular count ranged from 10 to 53, with an average of 30.21 ± 8.36, the diameters - from 5.36 to 44.84 micrometers with an average of 13.64 ± 8.49. The average number of blood vessels in tumour nodes in various areas of Gleason differentiation was different (see Table 7).

Table 7. Number of blood vessels in various differentiation zones of PC foci (0.27 mm² field-of-view, magnification 200 times)

<table>
<thead>
<tr>
<th>Asinsvadu skaits</th>
<th>n</th>
<th>Mazākais</th>
<th>Lielākais</th>
<th>Vidējais</th>
<th>Std</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason 1</td>
<td>1</td>
<td>43</td>
<td>43</td>
<td>43.00</td>
<td></td>
</tr>
<tr>
<td>Gleason 2</td>
<td>80</td>
<td>14</td>
<td>61</td>
<td>33.20</td>
<td>11.54</td>
</tr>
<tr>
<td>Gleason 3</td>
<td>57</td>
<td>26</td>
<td>82</td>
<td>45.60</td>
<td>16.43</td>
</tr>
<tr>
<td>Gleason 4</td>
<td>16</td>
<td>30</td>
<td>83</td>
<td>54.19</td>
<td>17.49</td>
</tr>
<tr>
<td>Gleason 5</td>
<td>2</td>
<td>71</td>
<td>120</td>
<td>95.50</td>
<td>34.65</td>
</tr>
</tbody>
</table>

30
The difference between the number of blood vessels in tumour node and in HGPIN zone was assessed and estimation whether the difference in the number of blood vessels affected the visualization potentialities of foci in power Doppler examinations. 

In cases where the number of blood vessels in the tumour node was less than the vascular count in HGPIN zone, the potentiality to reveal PC node was more than 2 times smaller than in cases of opposite blood vessels ratios (odds ratio $OR = 2.39$, $95\% CI\ 0.66$ to $8.72$, $p=0.05$)

4. Analysis of the Results and Discussion

4.1. Morphological Findings

In our study multifocal process was found in 44 patients (44.4%) of number of cancer foci ranging from 2-7. Byar and colleagues (Byar, 1972) report that PC is a multifocal process in up to 85% of cases.

Dividing the PC foci into groups of size $<1\ cm$, from $1-3\ cm$, $>3\ cm$, it was found that the highest number was in the small foci group of sizes up to $1\ cm$ (40.7%) and almost as much in the group of sizes from 1 to 3 cm (40.2%).

In the process of assessing the results it is necessary to pay attention to diagnosis of clinically insignificant cancer. In 1996 Dugan and colleagues (Dugan, 1996) argued that a clinically insignificant tumour is one for which an increase in volume would not exceed 20 ml during estimated patient survival time and the Gleason score will not exceed 6. The clinical relevance of the tumour may thus be defined by its volume, tumour volume doubling time and the patient's expected survival. Excluding low-differentiated
tumours with Gleason score 4 and 5, upper limit of the volume, to which the tumour is charged to clinically insignificant cancer, is 0.5 ml. Such a distinction, though controversial, is widely used in clinical practice (Goto, 1996; Elgamal, 1997; Douglas, 1997; Epstein, 1998; Hauttman, 2000; Brossner, 2000). In our study the group of smaller tumours included foci with a maximum size of <1cm in the transverse plane. Tumour volume with a diameter of 0.9 cm is ± 0.38 ml (4/3π R3) - hence the size criteria for the size of the foci correspond to the definition of clinically insignificant tumours. Only in 6% (6 of 99) of patients, none of all (1-4) nodes found morphologically was larger than 0.9 cm. The remaining 94% tumours of clinically insignificant size were accompanying finding of tumours of clinically relevant size.

The localisation of nodes found in the study corresponded to commonly known regularities of zonal distribution in pathological process of prostate (McNeal, 1988). In our study 18% of foci were located in the central zone of the gland, 63% were peripheral zone tumours, but the ratio of foci infiltrating both parts of the gland what is partly determined by their large sizes, was 19%.

In terms of numbers the most were intermediate grade cancers (50.5%), followed by low-grade tumours (45.4%), which together accounted for 95.9% of all cancers. The large number of low-grade tumours in our study is related to high percentage of tumours of small size. This, in turn, is determined by the research design - we investigated morphological prostatectomy specimens, including micronodules, which biopsy usually can not established. Also the small degree of high-grade tumours in our study is natural. This is explained by the protocol adopted in the clinic and proper patient selection, which does not assign radical surgical treatment for patients with low differentiated PC.

We found positive correlation between the Gleason score of a tumour and the amount of blood vessels in foci of malignant lesions - for malignancies with higher Gleason score a higher
density of microvessels is typical. Also data from other studies of density of microvessels in prostate show that increased density of blood vessels strongly correlates to malignancy degree (Bigler, 1933). In its turn in our study there was no statistical difference in diameter of microvessels within zones of various tumour differentiation. In ultrasound, in few cases sizeable blood vessels in tumour mass was discernable, suggesting that vascular gauge might be associated with tumour size.

4.2. Ultrasonography Findings and Comparison of Various US Techniques

Visualization of Prostate Cancer in the Grey Scale (B) Mode

In our study, diagnostic sensitivity of B mode ultrasonographic examination, over all foci found morphologically, including the number of clinically insignificant foci turned out to be low - 42.8% (95% CI 36.0 to 49.8). Similar results are presented also by other researchers who have evaluated visualization potentialities of morphologically identified PC foci (Rifkin, 1988). The diagnostic sensitivity became higher with increasing tumour size. If in the group of size less than 1 cm just 11.4% of the tumour foci were discerned, then 1-3 cm tumours were visible in 60.3%, but tumours larger than 3 cm – in 73% of cases.

Retrospectively assessing the sonographical appearance of PC foci we established that the nodes which were not possible to be discerned in B mode technique were isoechogenic with the surrounding parenchyma and were located either in the mass of the gland, thus not causing deformation of the contour, which could indicate location of the malignant tissue, or causing infiltration of pericapsular tissues without
alteration of the shape or causing visible asymmetry of the structure. Hypoechogenic PC foci were easier revealable, especially, if they were in the peripheral zone of the gland. We established that in the majority of cases, low-differentiated tumours appeared distinctly hypoechogenic relative to the surrounding parenchyma, as confirmed by other studies (Shinokara, 1989).

B mode examinations failed to reveal a part (27.0%) of the PC nodes, that were larger than 3 cm. There are several explanations: 1. larger tumours are characterized by heterogeneous structure and a higher proportion of echogenic structures which makes these tumours isoechochogenic with the surrounding parenchyma; 2. nodes of such size in most cases infiltrated either the whole gland or all the peripheral zone of the gland, which prevented the possibility of comparison with normal prostate tissue.

Visualization possibilities in B mode, to a large extent, were also determined by the localization of foci. In the peripheral gland PC foci of 1 cm and larger were discernable in 64.3% of cases. Foci of size <1cm were revealed only in 11.4% of cases, which, we believe, indicates that visualization of foci , to the greatest extent, is determined by their size. Capability to visualize tumours of central location in B mode investigations was poor - only 1 out of 35 PC foci was discernable. This node was larger than 3 cm. The poor visualization of foci located in the central zone is objectively justified: 1) PC foci develop in background of BPH with a large number of cases has a very heterogeneous appearance, so foci of PC are not discernable; 2) Visualization of PC is prevented by calcificates on the interface between the transition and peripheral zone, marked by the so-called “surgical capsule”.

All listed restrictions of the B mode technique leads us to conclude that the method cannot be used instead of guided
biopsy neither can it replace systematic protocol biopsies. Similar conclusions have been stated by other researchers, who analyzed the results of biopsy specimens from foci of a sonographically different appearance. Onur and colleagues (Onur, 2004) showed that although the specimens from guided TRUS biopsies reveal more PC than the systematic ones, the insufficient specificity of the method determines why such an approach can not be used as a single method for selection the biopsy site.

**Characterization of Vascularization of Prostate Cancer in Energy Doppler Mode**

In our study the diagnostic results were not improved by the Doppler technique. The diagnostic sensitivity of the test, over the whole number of morphologically identified foci was low (42.8%). Compared to the B mode examinations, ED technique managed to reveal slightly more tumours larger than 3 cm (78.4% vs 73%). Low sensitivity and specificity of ED technique have been reported in several studies (Halpern, 2000; Okihara, 2002, Ramsey, 2004). In total, data from various reports on sensitivity in color and power Doppler examinations varies considerably: respectively, 15-95% in color Doppler and 17-92% - in energy Doppler examinations. The large differences in interpretation among various researchers indicate that the method is subjective and depends heavily on hardware quality and technical characteristics. Diagnostic results are also determined by a number of objective factors, first of all neovascularity features of PC – blood vessels which proliferates in PC, are of a very small caliber (10-30 μm), their size is below the resolution limits of conventional Doppler methods and the blood flow in these blood vessels is too slow (Kay, 1998).

Another major drawback of the Doppler technique is its non-specificity – vascularization type of benign changes and
malignancies in a part of cases are similar, so a hypervascular focus can be both inflammatory and malignant.

Like in B mode techniques, also for the Doppler ultrasound visualization capabilities principally were determined by tumour size. This is due to the nature of neoangiogenesis development in tumour which was studied by Folkman and colleagues (Folkman, 1976). They determined that small tumours up to 2 mm are practically avascular. Demonstrable increase in the density of blood vessels is shown only by tumours which have reached size of 1 ml. Consequently, visualization of small tumours discernable in Doppler ultrasound as hypervascular foci is possible only in rare cases. In turn, larger tumours are characterized by necrotic changes that leads to destroying of the blood vessels nourishing the tumour, which might explain why some PC lesions strongly visible in our study in B mode sonography showed no measurable hypervascularization in Doppler ultrasound.

In our opinion, doplerographic visualization of PC nodes are also affected by the difference between the amount of blood vessels in the tumour focus and the zone of high-grade prostate intraepithelial neoplasia (HGPIN). Morphological investigation of the specimen showed that all (100%) foci of clinically significant cancer have evolved on the background of wide high-grade intraepithelial neoplasia (HGPIN) – precancer lesion. We established that in cases where the number of blood vessels in the tumour node was less than the vascular count in HGPIN zone, the potentiality to reveal PC node was more than 2 times smaller than in cases of opposite blood vessel ratios (odds ratio OR = 2.388, 95% CI from 0.66 to 8.72). In the zone of Gleason 2 grade, which is a component containing the largest percentage of low-grade tumour, the average number of blood vessels (33.2 ± 11.54) was not significantly different from the blood vessels in HGPIN zone (30 ± 8.36). This could be one of the reasons which explains the poor visualization capabilities of low-grade tumour in Doppler ultrasound.
Figure 7. Extensive HGPIN zone along all dorsal contour with marked hypervascularisation, on the background of which small PC foci are not discernable (Gleason score 4). A. B mode ultrasound image. B. Energy Doppler ultrasound image. C. Histotopogram of appropriate HGPIN (outlined in green) and PC (outlined in black) lesions.

Also, the localization of foci were of great importance. The best visualization was established to malignancies located in the peripheral zone and tumours of mixed localization whose mass partially was 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.

Centrally localized tumours were discernable only in 2.9% (1 out of 35). Like in B mode examinations a factor limiting visualization
of central zone tumours was stones and calcificates on the interface between the transition and peripheral zone, as also indicated by other authors (Takanashi 2000).

As it was expected, the Doppler examinations were more suitable for tumours of higher malignancy grades, characterized by greater number of blood vessels. In our study all nodes, including clinically irrelevant size microfoci, which in most cases are characterized by low Gleason score and relatively lower vascular amount, were recorded. This partially is the explanation of the low overall diagnostic results in our Doppler examination. However, in groups of tumours of high clinical significance (size ≥ 1 cm) Doppler ultrasound revealed all high-grade malignancy PC foci, which, of course, is of significant clinical relevance. Sensitivity of Doppler ultrasound technique in visualization of clinically significant intermediate-grade tumour groups was 72.5%. Similar results and correlation of visualization potentialities with the degree of malignancy is also found in other studies (Weidner, 1993; Louvar, 1998).

Results of Approbation of CEUS, Comparative Potentialities in Diagnostics of PC

Although the largest blood vessels nourishing a tumour in conventional Doppler examinations are well discernable, microvessels that are typical to PC in greater extent cannot be revealed with conventional Doppler examinations (Halpern 2006). Application of microbubble contrast agent is a way to discern these blood vessels (Radge, 1997). At the same time it is possible to assess dynamic characteristics of blood flow in both major and small blood vessels (Wilson 2006).

In our study for CEUS examinations we used pulse inversion method, which is a new program, designed specially for gray scale mode with low mechanical index (0.1 to 0.2). Applying the
pulse inversion method with low mechanical index, the envelopes of microbubbles do not get damaged and in large amount come into prostate, including the tiny blood vessels of malignant tissue. Moreover, circulation of the contrast medium in prostate tissue can be observed for a longer time at least 2 minutes.

The total diagnostic sensitivity of CEUS in all morphologically identified foci as compared with B mode and energy Doppler examinations was higher - accordingly 42.8% in B-mode and Doppler examinations and 61.1% - for CEUS.

We established that also for CEUS the visualization of foci were greatly related to their size. Also, Seitz and colleagues (Seitz, 2009) have established that larger tumours are of a better visualization. With CEUS we did not manage to discern foci smaller than 1 cm. Explanation of this drawback has been found by researchers who faced similar difficulties. Small-sized foci in most cases are of low malignancy degree and accordingly lower density of small blood vessels, which, presumably, is not enough to generate the signals visible in CEUS (Halpern, 2001). The best visualization potentialities in CEUS were in the group of medium-sized (1-3 cm) foci where almost all (92.5%) nodes were discernible. Foci larger than 3 cm in CEUS were remarkably less revealed (79.2%), which correlates with data from other studies (Tang, 2003). This phenomenon is explained with the nature of tumour angiogenesis – in early stages of tumour development its vascular volume increases, but, as the node increases, the relationship between the tumour volume and the total blood volume decreases.

As with the B mode and Doppler examinations, in the best visualization in CEUS were tumours of mixed localization and peripheral gland foci; the visualization capabilities also correlated with tumour size. Central zone tumours were discernable only in 27.8% of cases. Difficulties in seeing tumours of this location are associated with the intensive heterogeneous enhancement which is determined by BPH changes (Deering, 1995).
We found that the diagnostic sensitivity of CEUS is correlated with tumour malignancy grade. Low-grade tumours, which accounted for 43% of the total number of morphologically identified foci in the CEUS group were discernable only in 35.5% of cases. Low-grade malignancy foci which were not visible in CEUS in 85% of cases were smaller than 1 cm, thus complying with the definition of clinically insignificant tumours. Tumours of intermediate-grade malignancy were found in 80% of cases. In total, out of 28 PC foci not revealed by CEUS six tumours (21%) were of clinically significant size from 2.3 to 4.5 cm with a Gleason score ranging from 4-7. These results point to two major problems of CEUS: 1. the method is not suitable for detecting small low-grade tumours, which from the point of clinical importance are somewhat less important; 2. large tumours may remain undetected, which is determined by the relative reduction of the mass of tumour vascularization and in cases of infiltration into larger part of the gland or the whole gland which is a reason for lack of normal tissue for comparison. High-grade malignancy tumours were found in 100% of cases. The average sum of Gleason scores for PC foci, which were discernable with CEUS was 5.1, for foci not revealed in CEUS - 4.4. Our results suggest that the majority of tumours found with CEUS are larger and with a higher malignancy degree than those which were not detected with CEUS ($\chi^2=15,242; df=4; p=0,004$). Similar conclusions are found in several other publications (Frausher, 2002; Mittenberg, 2007).
Figure 8. Role of CEUS in diagnostics of prostate cancer. A. Transrectal ultrasound examination in grey scale mode; no sonographical signs of malignancy have been detected. B. Inconclusive zone of enhanced vascularisation in the right lobe in vicinity of the lateral contour. C. After intravenous administration of contrast medium in the right lobe a wide zone of enhanced vascularization is visible in the size corresponding to morphologically identified tumour spread. D. Boundaries of the corresponding section of prostate cancer are marked in histotopogram.

Our study shows that the diagnostic sensitivity of CEUS was 61.1%, which correlates with results from other studies reporting good results of using CEUS (Pelzer, 2005; Frausher, 2002). Excellent performance was reported by Taymoorian and colleagues (Taymoorian, 2007), who estimated diagnostic sensitivity of 100% and specificity of 48%. Similarly, according to the results of Aigner
and colleagues (Aigner, 2009), diagnostic sensitivity was 100%, negative predictive value - 99.8%. These results are similar to our results regarding diagnostic sensitivity of 92.5% for medium-sized (1-3 cm) foci. Seitz and colleagues (Seitz, 2009) compared results of preoperative CEUS with morphological findings from prostatectomy specimens and found that the visualization potentialities of the dominant PC focus is 71% and the method can not be recommend for guided biopsies. However, studies of other authors suggest that, basing on the ratio between the amount of PC positive specimens to the total number of biopsies, with the guided biopsies revealed from 3.1 (Pelzer, 2005) to 3.5 (Halpern, 2002) times more PC than performing biopsies according to the protocol.

The question whether CEUS with guided biopsies may replace randomized protocol biopsies is widely discussed in literature of radiology and urology disciplines and represents two conflicting views.

To evaluate the efficiency of the method it must be: 1) clarified whether this method can reveal clinically significant tumours 2) shown that this approach would reduce costs (Halpern, 2005). In our study from 50 patients undergoing conventional gray scale, Doppler ultrasound and CEUS., 40 cases of cancer (80%) were found using CEUS, in 7 patients from them (14%) PC was found only with CEUS. Malignancy degree of tumours revealed with CEUS was higher than that of tumours, which could not be revealed with CEUS (5.1 vs.4, 4). It allows to presume that in case of clinically significant tumour CEUS application for guided biopsies could significantly improve diagnostic possibilities in PC. Halperns and colleagues (Halpern, 2001) compared the results from protocol biopsies and CEUS-guided biopsies and concluded that the diagnostic value of CEUS-guided biopsy is similar to or even higher than in protocol biopsies. The results give rise to the conclusion that CEUS-guided biopsies give chance to find additional foci of malignancy, which would have been impossible to reveal using randomized sextant biopsies.
When calculating the cost of procedures, it appears that the CEUS-guided biopsies in limited number are more cost-effective than protocol biopsies in terms of cost of morphological analysis, however, the contrast medium makes the procedures more expensive. From a clinical point of view, it is important that reducing the amount of performed biopsy, also the number of complication decreases.

4.3. Practical Recommendations for Ultrasonography Diagnostics of Prostate Cancer

Elevated level of prostate specific antigen in serum (PSA) and/or suspicious digital rectal findings are indicators giving rise to suspicion on possible prostate cancer. The diagnosis of prostate cancer is confirmed or disproved on the basis of morphological results of prostate biopsy. Punktijas biopsijas veikšanai lietojama transrektāla prostatas ultrasonogrāfija, kura ļauj vienlaicīgi 1) vizuāli novērtēt priekšdziedzera iespējamo patoloģiju 2) precīzi kontrolēt adatas gaitu noteiktos anatomiskos apvidos. To perform prostate biopsy transrectal prostate ultrasonography should be used which makes it possible in the same time 1) to assess the eventual pathology of prostate 2) to guide the needle in the certain anatomical areas. Among ultrasonography techniques as an invalid method due to its low resolution, also percutaneous (transabdomināla) prostatic US should be dismissed.

Conventional ultrasound is the basis for gray scale (B mode) US which is to be performed with endocavitary detector, preferably, a dedicated probe for transrectal examinations, with variable frequency in range of 5-10 MHz. Image quality is improved by use of tissue harmonic frequencies. Examination is to be carried out in transverse (axial) and longitudinal (sagittal) planes, including all parts of the gland from the base of to the apex and covering the seminal vesicles.
Eventual malignancy in B mode examinations is suspected, if there is:

- gland contour asymmetry (especially bulging of dorsal and lateral contours in the apical part of the gland);
- capsule roughness as a possible symptom of infiltration;
- local changes of tissue echostructures (hypo-and hyperechogenic foci) and microcalcinites;
- disorganization of parenchymal structure and inability to clearly visualize the surgical capsule is considered to be characteristics of prostate transition zone infiltration;
- signs of tumour spread outside the prostate is the fatty infiltration between the gland and the seminal vesicles as well as structural asymmetry of seminal vesicles.

Conventional B mode ultrasonography ir advisable to supplement with power Doppler imaging. For this reason the detector must be equipped with high resolution colour and power Doppler capabilities. The examination is to be carried out using technical parameters, which allows to visualize slow flow in tiny blood vessels, choosing low pulse repetition frequency (PRF), within 400-500 Hz range and such colour gain level at which the colour background “noise” is absent.

Eventual prostate cancer in energy Doppler examinations is suggested in case of:

- foci of increased vascularization;
- asymmetric hypervascularized zones in the peripheral gland.

Establishing the diagnostic conclusion, it should be noted that local changes in tissue echogenicity and vascularization may also be related to inflammation process and hyperplastic lesion.

From a wide range of sonographic tests used in diagnosis of PC,
CEUS as a method of visualization of microvessels is considered to be the most sensitive diagnostic test for determination of PC. The protocol of CEUS examination is determined by the examination programme dedicated for CEUS and the choice of a contrast medium. The best results are achieved by the pulse inversion method with a low mechanical index (0.1 to 0.2) and the second-generation US contrast medium.

A possible prostate cancer in CEUS is considered the presence of:
- early foci of enhanced vascularization;
- asymmetric hypervascularized zones;
- early washout of the contrast medium from the focus.

Similarly to examinations performed with Doppler ultrasound, also hypervascularisation of tissues in CEUS is a non-specific sign which might be associated with benign hyperplastic changes or inflammatory process.

Before prostate biopsy, it is important to carefully assess the diagnostic results obtained with all US techniques, as well as to determine the prostatic volume. Prostate biopsy is carried out under visual control of TRUS in the sagittal plane with the needle course oriented laterally. The number of biopsies is determined by a protocol accepted in a clinic (not less than 6-10). Choosing the number of biopsies also the size of the prostate should be taken into account. The most optimal results can be supplying a randomized protocol biopsy with Doppler US and biopsy of suspicious foci in CEUS. It is necessary to point out that replacement the protocol biopsy with only guided biopsies under control of CEUS shall not be accepted because of potential risks that in such a way clinically significant tumours may remain undiagnosed. If the first time randomized biopsy is negative, for repeated biopsy in patients with elevated PSA serum levels it is recommended to apply CEUS.
Conclusions

1. The mean sensitivity of B-mode examinations in revealing of prostate cancer is low (42.8%) and depends on tumour size, location and grade. The most informative the method is in detecting tumours located in peripheral parts of the gland, sensitivity decreases in detecting small low grade tumours and centrally located cancers.

2. Feasibility of energy Doppler and B mode investigations for detecting prostate cancer are generally equivalent (42, 8%). Energy Doppler mode selectively improves detection of cancer foci larger than 3 cm for low- (75%) and intermediate-grade (80.6%) as well as disclosure of tumours located in the peripheral zone of the gland (89%).

3. Clinical tolerance of ultrasound contrast media is good. The mean sensitivity of CEUS in detecting prostate cancer is 61, 1%. CEUS improves detection of cancers located in the central part (27.8%) and disclosure of 1-3 cm large tumours (92.6%). The supreme diagnostic sensitivity of CEUS (100%) is found for high-grade prostate cancers in the group with typical neoangiogenesis.

4. The diagnostic accuracy of CEUS in detecting prostate cancer in comparison with B mode and energy Doppler is the best – the use of CEUS rises diagnostic accuracy for an average of 18.3%. With all diagnostic methods visibility of malignant lesions is limited in cases of small (<1 cm) and large (>3 cm ) tumours as well as when tumours are located centrally and is of low malignancy grade.

5. The most optimal results in prostate cancer diagnostic can be achieved when combining B mode, energy Doppler and CEUS in a particular order.


Value of Ultrasound in Diagnosis of Prostate Cancer

Publications


4. V. Lietuvietis, M. Jakubovskis, M. Sperga, A. Strazdina, L. Engele, J. Gardovskis. Prostate cancer, importance of diagnostic methods in men with the serum PSA level of 4.0-20.0 ng/ml. Riga Stradin’s University Scientific Papers (RSU Zinātniskie raksti), 2006;101-107


6. A. Strazdina, M. Sperga, G. Krūmiņa. Multifocality and Vascularization Features in Contrast Enhanced Ultrasound and Doppler Ultrasound, Riga Stradin’s University Scientific papers, (RSU Zinātniskie raksti), (accepted for publication)

Participation in the Scientific Project

Education and Science Ministry project ‘Development of Scientific Activity in Higher Education Institutions’ No RSU-ZP 07-5 grant from 2006 to 2008, investigator “Diagnostic Criteria of Contrast Enhanced Ultrasound and Doppler Ultrasound in Prostate Cancer Patients and Relationship to the Morphological Findings”

Reports in Congresses and Conferences


Value of Ultrasound in Diagnosis of Prostate Cancer


Abbreviations

B - grey scale ultrasound examination (B mode)
BPH - Benign Prostate Hyperplasia
DNA - Deoxyribonucleic Acid
DRE - Digital Rectal Examination
CI - Confidence Interval
CT - Computed Tomography
CEUS - Contrast Enhanced Ultrasonography
ED - Energy Doppler
Gl - Gleason
HGPIN - High Grade Prostate Intraepitelial Neoplasia
MRI - Magnetic Resonance Imaging
PC - Prostate Cancer
PET - Positron Emission Tomography
PRF - Pulse Repetition Frequency
PSA - Prostate Specific Antigene
TRUS - Transrectal Prostate Ultrasound
US - Ultrasonography