Abstract. The aim of this study was to determine the prostate cancer detection rate of targeted biopsy using contrast-enhanced ultrasound (US) in patients with elevated prostate specific antigen (PSA) levels and previous negative biopsy. Patients and Methods: A total of 114 patients initially underwent ultrasonography using transrectal ultrasound (TRUS) and power Doppler. All the patients had at least one previous biopsy series negative for prostate carcinoma. Ninety-five of the patients were examined with a new broadband Doppler technique, advanced dynamic flow (ADF), after i.v. injection of the echo enhancer (2.4 ml, SonoVue). The systematic biopsies were also obtained supplemented by removal of two targeted tissue cores in all the patients with a suspicious area in the inflow phase or late phase. Results: Histology confirmed prostate cancer in 30 of the 95 patients. Sensitivity for the detection of prostate cancer by contrast-enhanced US was 100% and specificity was 48%. Suspicious areas were identified in 48 cases including 40 hypervascularized areas and 14 hypoechoic areas after 60 sec. Targeted biopsy identified 24 of the 30 carcinomas. Randomized octant biopsy identified only 8 of the 30 carcinomas. All 65 patients with negative histology were prostate cancer free at 12-month follow-up. The ADF US scans were degraded by fewer artifacts than the power Doppler images. Conclusion: Contrast-enhanced ADF Doppler allows reliable differentiation of prostate cancer and normal prostate tissue with a high sensitivity in patients with previous negative biopsy and fewer artifacts than power Doppler images, thus providing a good basis for targeted prostate biopsy instead of systematic biopsy.

Prostate cancer is the most common malignancy in men and its incidence is continuously increasing. As with other malignant tumors, early diagnosis improve the chances of cure. Biopsy guided by transrectal ultrasound (TRUS) is considered the gold standard for confirming the diagnosis in men with elevated levels of prostate-specific antigen (PSA) (1). However, in a subgroup of patients with elevated PSA levels, no malignancy was detected by biopsy (2, 3) or up to four biopsies had been performed before prostate cancer was detected (4). The number of men with persistently high PSA levels and previous negative biopsies has increased over the past decade, mainly because of more widespread and closer screening of serum PSA.

As a result of this situation, unnecessary biopsies are performed in healthy men while others have a poorer prognosis because the diagnosis and treatment of prostate cancer are delayed since a negative biopsy does not exclude prostate cancer. Some authors have claimed that the histological diagnosis of prostate cancer can be made by increasing the number of rebiopsies or by using saturation biopsy protocols (5-8). However, the cancer detection rates reported for repeat biopsy are only 10 to 23% (9, 10). In addition, patients experience repeat biopsy as unpleasant and painful and often need general or spinal anesthesia. Furthermore, repeat biopsy is associated with an increased risk of complications such as infection or acute bleeding (11). Several studies have shown that the development of prostate cancer is associated with metabolic changes that...
alter perfusion in tumor tissue (12, 13). TRUS is an established tool for guiding systematic needle biopsy but not for detecting suspicious areas within the prostate (14). Color Doppler US is likewise limited in detecting and localizing prostate cancer, mainly because it does not reliably detect very small vessels with slow blood flow, which are typical of tumor vascularization (15). The advent of US contrast media (USCM) has markedly improved the detection of very slow blood flow in small vessels (16).

In the study presented here, a new broadband Doppler technique (ADF, advanced dynamic flow) was used to investigate the detection of prostate cancer by contrast-enhanced US in comparison with conventional B-mode imaging, unenhanced power Doppler and histology. The aim of this investigation was to determine the prostate cancer detection rates of targeted biopsy using contrast-enhanced US in 100 patients with elevated PSA levels and previous negative biopsy.

**Patients and Methods**

All patients presenting to a specialist urological service with elevated PSA levels and previous negative biopsy series over a 24-month period (Jan. 2004 – Feb. 2006) were included in the study. All patients had at least one biopsy series negative for prostate carcinoma (range: 1 to 8, median: 2, Figure 1). A total of 114 patients initially underwent transrectal digital examination and ultrasonography using TRUS and power Doppler. Subsequently, 104 patients were examined by contrast-enhanced US. Ten patients were excluded from the contrast-enhanced examination, exclusion criteria included unstable angina pectoris, acute myocardial infarction within 2 weeks before the examination, other severe cardiac or pulmonary disease, no written informed consent and known intolerance of the echo enhancer. The 104 patients undergoing contrast-enhanced US had a median age of 66 years (range: 44 to 73 years) and a median PSA level of 10.0 ng/dl (range: 4 to 48). The study was approved by the responsible ethical committee.

**Preliminary study.** Based on a report in the literature (17) describing the potential of contrast-enhanced power Doppler US, nine patients were initially examined with this technique. These examinations and all subsequent contrast-enhanced US examinations were performed using a high-end scanner (Aplio 80, Toshiba, Otawara, Japan) and an endorectal probe with contrast capabilities (9C3, Toshiba). B-mode scanning was performed at 6-9 MHz and Doppler US at 3.6 MHz. However, the preliminary study using this technique was discontinued after examination of nine patients due to a high rate of blooming artifacts in the power Doppler examination and the short scan window (destruction of the contrast medium bubbles due to the high mechanical index in the Doppler mode). These nine patients were not included in the analysis.

**US with echo enhancer.** The remaining 95 patients (Table I) were examined with the new ADF broadband Doppler technique, after intravenous bolus injection of the echo enhancer SonoVue (Bracco-Altana, Milano, Italy). All the patients were examined by the same investigator (KT). A venous line was placed in the right cubital vein, and 2.4 ml of the echo enhancer with 5 ml physiological saline solution was administered as a bolus within 4-5 sec (standardized injection technique). The entire arterial inflow phase and washout of the USCM were stored digitally over 60 sec. Time-intensity curves

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**Table I. Patient characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>Mean (range)</th>
<th>Median</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>95</td>
<td>65.34</td>
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<tr>
<td>Age (years)</td>
<td>66 (44-73)</td>
<td>65.34</td>
</tr>
<tr>
<td>PSA (ng/dl)</td>
<td>10 (4-48)</td>
<td>13.08</td>
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<tr>
<td>Prostate volume (cm³)</td>
<td>50 (22-120)</td>
<td>55.30</td>
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<tr>
<td>No. of previous negative biopsies</td>
<td>2.4 (1-8)*</td>
<td>2.0</td>
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</tbody>
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*see Figure 1.

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**Figure 1. Number of previous negative biopsies.**

**Figure 2. Diagramm (coronal view) of USCM findings correlated with histology for localization of prostate cancer on contrast-enhanced US and histology.**
served to determine maximum inflow in the tumor and normal tissue, which were documented on single images. In addition, first inflow (on average 19.5 sec after echo enhancer administration) was documented as well as the last image after 60 sec. All the US examinations were performed at an intermediate transmit power (36%) and fairly low mechanical index (MI<0.4), which yielded an adequate image quality of B-mode scans while minimizing destruction of USCM microbubbles.

**Biopsy protocol.** Biopsies were obtained from all 95 patients after contrast-enhanced US (after 30 min) using a standard protocol. The systematic biopsy was supplemented by removal of two targeted tissue cores in all the patients with a suspicious hypervascularized area in the inflow phase or a hypoechoic area (wash-out) in the late phase. The suspicious areas were recorded on a diagram and again visualized by contrast-enhanced US before the targeted biopsies were obtained. All the biopsies were performed by the same examiner (KT) using a 7.5 MHz biplanar rectal probe (Viking 2400, B&K Medical, Germany) for guidance. All the biopsies were performed according to Hodges’ protocol (18) with the removal of 8 specimens in patients without suspicious areas in the preceding US and 10 specimens in the patients with vascular abnormalities. A 5-day course of fluoroquinolone antibiotics (ciprofloxacin 500 mg twice/day) was initiated on the day before biopsy. The PSA level in the blood, a blood count, and simple coagulation parameters were determined in all cases. Exclusion criteria for prostate biopsy were age greater than 73 years and infections, particularly urinary tract infections. None of the 95 patients was excluded. Biopsy was performed with an 18 gauge automated biopsy gun and the patient in the left lateral position according to Hodges’ protocol (18). For local anesthesia, 20 ml 2% lidocaine gel was applied rectally. No transrectal local infiltration was conducted to exclude any effect on local microvasculature. In the cases where suspicious areas were detected, two probe target biopsies were drawn from the suspicious area, followed by the biopsies according to Hodges’ protocol.

**Image analysis.** The contrast-enhanced US findings were analyzed prospectively (TF, KT, AT) in a patient-by-patient manner in all 95 cases. All hypervascularized areas with lateral asymmetry on the inflow images were classified as suspicious by consensus and documented on a diagram (Figure 2). In addition, hypoechoic areas in the outflow phase (washout) were classified as suspicious and also recorded on the diagram. Areas with inhomogeneous enhancement were classified as inconclusive findings. Homogeneous and symmetrical inflow of the echo enhancer on both sides was classified as negative for cancer. On the basis of the suspicious areas identified in this way, the entire prostate was classified as suspicious, inconclusive or negative for cancer. Suspicious lesions were localized by assigning them to the apical, intermediate, lateral, or basal zone of the prostate on the right or left side.

**Retrospective analysis.** Included only the patients with histologically proven prostate cancer in the systematic or

Figure 3. Initial B-mode ultrasonography using TRUS in a 65 years old patient with a PSA level of 14.0 ng/dl and negative previous biopsy (A). Broadband Doppler technique advanced dynamic flow (ADF) before (B) and after (D) intravenous bolus injection of the USCM. Good delineation of the 12mm hypervascularized tumor 25 sec after administration of the USCM, confirmed only by targeting biopsy. Typical blooming artifacts in the power Doppler (PD) examination after administration of the USCM (C) and destruction of the contrast medium bubbles due to the high mechanical index in the PD mode.
randomized octant biopsy. The analysis was done by consensus by two examiners (KT, TF) blinded to the histological findings who evaluated the images of initial contrast inflow, maximum inflow, and late enhancement. The respective areas were classified as suspicious, inconclusive or negative for cancer and again documented on a diagram (Figure 2). In addition, the ADF images documenting the time of maximum inflow were compared with the power Doppler images documenting maximum inflow immediately before biopsy. Both investigators assessed the occurrence of artifacts on an analogue scale of 0 to 4 (0=none, 1=mild, 2=moderate, 3=severe and 4=artifacts not analyzable).

Statistical analysis. The USCM findings and repeat biopsy results were correlated by using a cross table, from which sensitivity and specificity were calculated. Statistical significance was calculated by using the chi-square test according to Pearson. A p-value of less than 0.05 indicated a significant correlation.

Reanalysis of the cases with negative study biopsy. All the patients whose biopsies in this study were again negative were followed up for 12 months. The negative results were confirmed by follow-up PSA test, digital rectal examination, repeat biopsy, or magnetic resonance imaging in all patients. None of the patients was diagnosed with prostate cancer during the follow-up period.

Results

Prospective analysis. All 95 patients were analyzed prospectively. Histology confirmed prostate cancer in 30 of the patients and additional high-grade prostatic intraepithelial neoplasia (PIN) in 7 of them. Histological evaluation demonstrated chronic prostatitis in 74 cases (74/95) and benign prostate hyperplasia (BPH) in 48 cases (48/95). Some of the patients with prostate cancer had concomitant prostatitis (n=17), BPH (n=12), or high-grade PIN (n=6). All 30 patients with prostate cancer confirmed in biopsy showed at least one suspicious area in contrast media US. The remaining 34 cases with suspicious areas revealed diagnoses other than prostate cancer. Therefore, sensitivity for the detection of prostate cancer by contrast media US was 100%, specificity was 48%.

The three readers (TF, KT, AT) identified suspicious areas in 48 cases, 40 hypervascularized areas and 14 hypoechoic areas after 60 sec. Six of the patients had a combination of both US abnormalities. Targeted biopsy identified 24 of the 30 carcinomas and tumor diameter ranged from 0.5-3.0 cm. Randomized octant biopsy identified only 8 of the 30 carcinomas. Nonenhanced B-mode scanning in combination with power Doppler identified only 2 hypoechoic areas that correlated with cancer. Due to preceding biopsy, there were cystic areas and calcifications, which did not affect contrast-enhanced US. Seventeen cases were classified as inconclusive by the three readers, among them one patient with cancer and all 7 cases of PIN in the targeted biopsy. Five of the 7 PIN cases were also diagnosed on the basis of randomized biopsy. Only the targeted biopsy from an area of heterogeneous enhancement, diagnosed one cancer.

In all 31 cases classified as negative for cancer on the basis of US, neither histology nor 12-month follow-up demonstrated prostate cancer.

In patients with asymmetrical BPH (n=16), isolated adenoma nodules could not be differentiated from hypervascular tumors because they also showed rapid inflow of the echo enhancer.

In one patient with a total of 6 negative biopsies and a PSA level of 12.6 ng/dl a tumor measuring 1.5 cm in diameter was identified in the transitional zone. Precise targeting of this area was possible and the biopsy specimen obtained in this way yielded the histological diagnosis of adenocarcinoma with a Gleason score of 3+4.

No patients had any complications from the US or biopsy protocol.

Retrospective analysis. A total of 30 prostate carcinomas were confirmed histologically. In the retrospective analysis, the localization of 22 of the 24 carcinomas confirmed by targeted biopsy correlated with the operative or histological findings. Localization of suspicious areas by assigning them to the apical, intermediate, lateral, or basal area of the prostate on the right or left side was practical and reliable (Figure 3).

Retrospective comparison of the artifacts (scores from 0=no artifacts to 4=not analyzable) on the ADF scans and power Doppler scans obtained immediately before biopsy showed that the new broadband technology significantly (p<0.05) reduced artifacts compared to power Doppler (1.4±0.4 versus 3.1±0.9).

All 65 patients with negative histology were also free of prostate cancer at 12-month follow-up.

Discussion

Ultrasonography has only a minor role in the diagnostic work-up of prostate cancer but is used as a guiding tool when systematic core biopsy is performed (2-4, 19). Endoluminal US combined with color or power Doppler exploration of the prostate has been investigated in many studies but published results have been contradictory. Since not all prostate carcinomas are hypoechogetic or hypervascular, US and Doppler US have a high false-negative rate (20) although Doppler US allows determination of the degree of tumor vascularization in some cases (21-26). The risk of finding focal cancer remains high on re-biopsy of patients with persistently increased PSA but the clinical significance is questionable (27).

In the present study analysis of the USCM inflow allowed significant differentiation of the vascularization of prostate cancer from that of normal prostate tissue. Targeted biopsy based on the results of the contrast-
enhanced US examination diagnosed 24 of the 30 carcinomas in our study population while standard repeat biopsy highlighted only eight and B-mode US only two. These results confirm the usefulness of targeted biopsy, especially as prior B-mode US and randomized biopsy missed not only small carcinomas with a diameter of 0.5 cm but also larger ones. Some authors have claimed that there is no need for immediate repeat sextant biopsy after negative initial sextant biopsy in men with PSA levels of 4.0 ng/ml or greater and have suggested repeat biopsies after 4 years, because the PPV (Positive Predictive Value) and detection rate did not increase significantly after repeat sextant biopsy (28). Djavan et al. reported that, in patients with negative previous biopsies and high PSA levels, the cancer detection rates on first, second, third and fourth repeat biopsy were 22% (231/1051), 10% (83/820), 5% (36/737) and 4% (4/94), respectively. They recommended that the third and fourth repeat biopsies should only be obtained in very selected patients in whom carcinoma was strongly suspected and/or who had poor prognostic factors on the first or second biopsy. These data further underline the need for a more reliable diagnostic tool such as targeted biopsy. Alternatively, stereotactic transperineal prostate re-biopsy with 3-dimensional mapping can be used to diagnose nonpalpable isoechoic occult prostate malignancy. However, this biopsy technique is very time consuming and requires anesthesia. In addition, there is a risk of complications such as bleeding or urinary retention in 10% of cases (29).

PIN could not be identified by contrast-enhanced US because heterogeneous enhancement was also seen in prostatitis and BPH. In 31 cases there was completely homogeneous enhancement of the entire prostate and none of these patients was diagnosed with prostate cancer in the subsequent biopsy or follow-up. Our experience has shown that evaluation of USCM dynamics is a fast and simple method for identifying prostate cancer on the basis of fast inflow. The images generated with this technique allow easy and reliable differentiation of prostate cancer compared with B-mode US. The method failed to detect 6 carcinomas, but in these cases the time-intensity curves also showed similar peak maxima for the tumor and surrounding prostate tissue. Centrally reduced perfusion or central necrosis in a tumor was also easily detected by contrast-enhanced US. It is noteworthy that even tumors as small as 5 mm are reliably depicted by US with its known high resolution and difference in perfusion from surrounding prostate tissue. These findings suggest that the tumor undergoes metabolic changes which in turn alter perfusion in relation to tumor growth (12, 13). Even small and slow blood flow was detected with the contrast-enhanced ADF US technique, making it superior to conventional power Doppler US and contrast-enhanced power Doppler (15, 16). The technique detected individual signals from echoeenhancer microbubbles which have an average size of 2.5 μm. Retrospective comparison of the two US techniques demonstrated markedly fewer artifacts on scans obtained with the ADF broadband technique compared with contrast-enhanced power Doppler (PD) scans (ADF: 1.4±0.4 versus PD 3.1±0.9, p<0.05). ADF is thus an adequate imaging technique for detecting slow blood flow, thereby improving the accuracy of gray-scale sonography or targeted prostate biopsy (30).

In conclusion, contrast-enhanced ADF broadband Doppler US allows reliable differentiation of prostate cancer and normal prostate tissue. ADF US scans are degraded by fewer artifacts than power Doppler images and thus help improve patient comfort by providing a good basis for performing targeted prostate biopsy instead of systematic biopsy.

References


