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Comparison Of Contrast Enhanced Color Doppler Targeted Biopsy To Conventional Systematic Biopsy In Carcinoma Prostate

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Abstract: *In carcinoma prostate the low sensitivity of systematic biopsy makes target biopsies by identifying the location of the lesions more desirable. Micro-bubble contrast agents has been introduced as an innovative technology that can improve prostate cancer detection. They enhance the small vessels in the prostate, by providing an enhanced acoustic reflectivity.On the basis of this hypothesis, we undertook this study to evaluate the usefulness of Contrast Enhanced Sonography(CEUS) for prostate cancer detection in patients with PSA >4 ng/ml and compare this with conventional system.The objective of the study is to compare the detection of prostate cancer with contrast enhanced ultrasound with conventional systematic biopsy and impact on gleason score.A Prospective study was carried out between January 2013 and February 2014.All study subjects underwent standard transrectal sonographic examination of the prostate, repeat examination during contrast material infusion and targeted biopsy followed by sextant biopsy of the prostate during a single visit. Pathologic evaluation of the biopsy cores was the reference standard for calculation of sensitivity and specificity. The cancer detection rates of the 2 techniques and the Gleason scores between two techniques were compared. In this study, 25 patients were biopsied.Of the 25 patients evaluated ,21 patients showed positivity for prostate cancer by contrast enhanced TRUS biopsy(84%).But systematic biopsy demonstrated cancer in 17 patients only out of the 25(68%).Contrast enhanced sonography could improve the sensitivity as well as accuracy for cancer detection.The use of CEUS also may be useful in patients with indeterminate serum PSA .Targeted biopsy has a definite impact on gleason scores, detecting high grade cancers with limited number of cores thus helping in planning the treatment in carcinoma prostate.*

Keywords*: Contrast USG, Carcinoma Prostate.*

I. Introduction

One of the most common cancer diagnosed in men is carcinoma prostate, and it is the second most common cause of cancer death in the Western world.Because of improvements in diagnostic testing, it's incidence has been increasing. Gray scale ultrasound guided biopsy has been the standard procedure for prostate cancer detection in men with elevated serum.PSA or an abnormal digital rectal examination. Due to the low sensitivity of systematic biopsy , it would be desirable to target biopsies by identifying the location of the lesions.

Micro-bubble contrast agents has been introduced as an innovative technology that can improve prostate cancer detection .Various studies have demonstrated that contrast enhanced ultrasound (CEUS) of prostatic blood flow enhance prostate cancer visualization and helps in targeted biopsy.Comparisons between systematic biopsy and CEUS guided targeted biopsy have shown that targeted approach detects more cancer with lower number of biopsy cores.CEUS has been shown to detect cancers with higher Gleason scores, which improves cancer grading.

Microbubble contrast agent images the microvasculature in the prostate. They enhance the small vessels in the prostate, by providing an enhanced acoustic reflectivity. On the basis of this hypothesis, we undertook this study to evaluate the usefulness of CE sonography for prostate cancer detection in patients with PSA >4 ng/ml and compare this with conventional system.

II. Aim And Objective

To evaluate the detection of prostate cancer with contrast enhanced ultrasound compared with conventional systematic biopsy and impact on Gleason score.

III. Materials And Methods

A Prospective study was carried out between January 2013 and February 2014.The institutional ethical review board approved the study

3.1.**Inclusion Criteria** 1.Patients with serum PSA > 4 ng/ml 2.Normal or abnormal DRE 3.2.**Exclusion Criteria 1.**Active UTI 2.Prostatitis 3.Un Co-Operative Patients 4.Allergy to ultrasound contrast agents 5.Contra-indications to ultrasound contrast agents like -Recent acute myocardial infarction (< 7 days), Right-to-left shunts, Class III / IV cardiac failure, Severe pulmonary hypertension.

IV. Method Of Study

Informed consent was obtained from all the patients. All patients were begun on a course of a fluoroquinolone antibiotic the night before biopsy. A cleansing enema was administered on the morning of biopsy. Patients were instructed not to ingest aspirin or nonsteroidal anti-inflammatory agents for at least 5 days before biopsy.

All study subjects underwent standard transrectal sonographic examination of the prostate, repeat examination during contrast material infusion and targeted biopsy followed by sextant biopsy of the prostate during a single visit. Contrast enhanced sonography was done using sonovue , as the ultrasound contrast agent. The lyophilized powder is shaken with 5 mL of distilled water for 20 sec. By using a 20-gauge cannula, 1.5 ml contrast agent bolus was injected into the left antecubital vein manually. 1 ml solution contains 8ug/ml. 5 mL of normal saline is injected each time, after injecting the ultrasound contrast agent.

With a mechanical index of 0.6–1.2, contrast enhanced ultrasound was done, after the intravenous injection of the contrast agent. 20 seconds of inter sweep delay were given, to prevent the unnecessary destruction of micro bubbles. From the prostatic base to the apex, contrast ultrasound was done. Each scan requires 5 to 10 sec depending upon the prostate volume. The entire imaging sequence was performed at baseline and was repeated during infusion of contrast material. These images were obtained finally stored in digital format for further interpretation.

During intravenous injection of the US contrast agent SonoVue, a targeted core of biopsy was taken from the contrast enhanced areas alone. Contrast enhanced imaging was always performed before systematic biopsies to avoid biopsy induced hyperemia on the contrast enhanced imaging study. Biopsies from contrast enhanced areas were performed into a maximum of 2 hypervascular areas in the peripheral zone only. No targeted biopsies were performed in the transitional zone .

Subsequently ,the same patient underwent systematic biopsy from 8 sites after imaging protocol.The prostate gland is divided into eight sites. They are apex; base; medial; and lateral portions of the mid gland , on the right and left sides. Biopsies were obtained transrectally using an 18 gauge biopsy needle .The time required for the ultrasound examination and biopsy was about 30–40 minutes for each patient. Eight biopsy samples from each patient , were sent in eight bottles separately, according to biopsy site for each patient. Biopsies were obtained without regard to prostate US appearance. In each biopsy specimen , the histopathological study was done to detect the presence of cancer foci and gleason grading was assigned to each core of biopsy.

The biopsies from contrast enhanced areas are sent separately and examined histopathologically for the presence of cancer foci and corresponding Gleason grading assigned.

In this study, 25 patients were biopsied. So, totally 25 x 8=200 sites were biopsied totally in these 25 patients. Also ,total biopsy cores from contrast enhanced sites from these 25 patients was 35.The number of patients were less but the number biopsy sites were very large , so this study is not limited by the number of samples. Pathologic evaluation of the biopsy cores was the reference standard for calculation of sensitivity and specificity. The cancer detection rates of the 2 techniques and the Gleason scores between two techniques were compared.Using Paired T test, specificity , sensitivity for prostate cancer detection were analyzed. Chi square test and ROC curve analysis were used.In all the above statistical analysis, a *p* value of < 0.05 was considered significant.

V. Observation And Results

Our study consists of 25 patients who are suspected to have carcinoma prostate either due to elevated serum PSA or abnormal digital rectal examination or both.

5.1.Age Distribution Of Cases

The subjects ranged in age from 41 – 80 years with a mean age of 62.2 years.

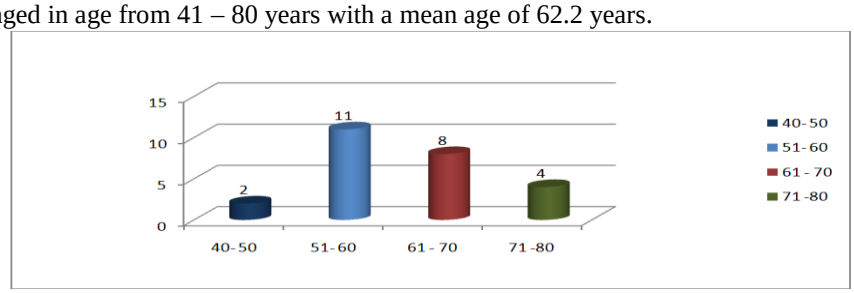


Figure 1. Age distribution of study group

5.2. Patient Characteristics

Sixteen patients were included due to presence of both abnormal DRE and elevated serum PSA >4 ng/ml.Two patients had only abnormal DRE with a normal serum.PSA.Seven patients had an elevated serum PSA alone with a normal DRE.

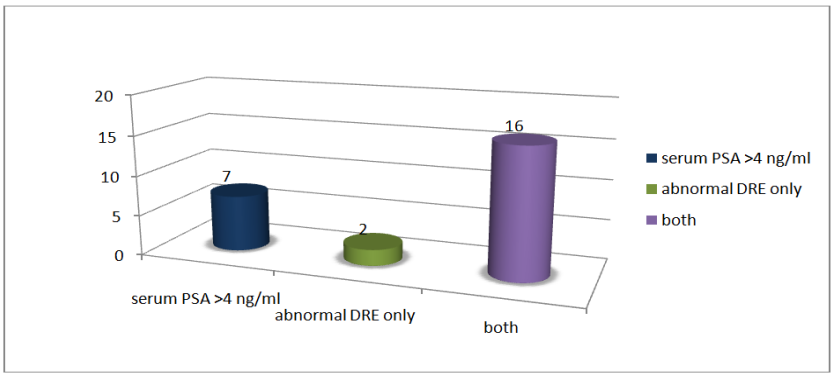


Figure 2. Patient characteristics of study group

A total of 35 cores were taken from contrast enhanced areas out of which 26 cores were positive for malignancy.Out of 200 cores taken by systematic biopsy,30 cores were positive.Seperate Gleason score was given to each of the cores from contrast enhanced areas as well as systematic biopsy cores.

5.3. Gleason Score Distribution In Contrast Enhanced Biopsy Vs Systematic Biopsy

Biopsy from contrast enhanced areas showed a gleason score of 8 in 2 patients,seven in 9 patients,six in 3 patients ,five in 6 patients and three in one patient.out of 25 cases,21 cases were positive for malignancy by contrast enhanced biopsy and 4 patients were negative for cancer. Out of this 4 patients,3 were BPH and 1 showed high grade PIN.

In the systematic biopsy group,a gleason score of 6 and 7 were present in 4 patients each, a score of 5 in 7 patients and 3 in 2 patient.

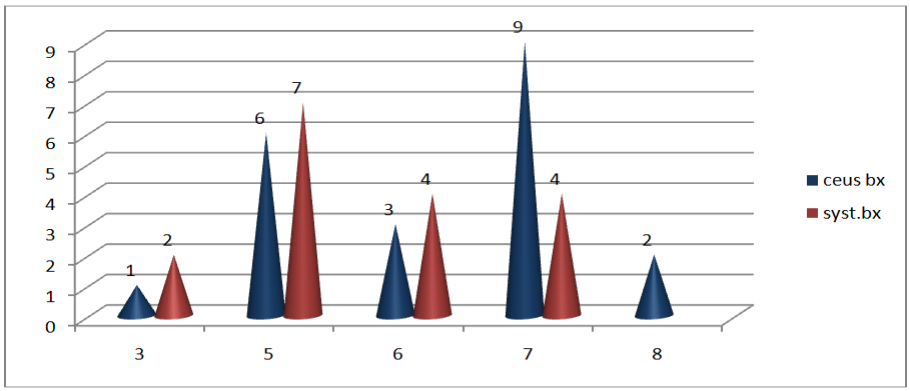


Figure 3. Gleason score distribution in contrast enhanced biopsy vs systematic biopsy

Higher grade cancer (Gleason score 7 or greater) was more common in patients with a positive targeted biopsy.

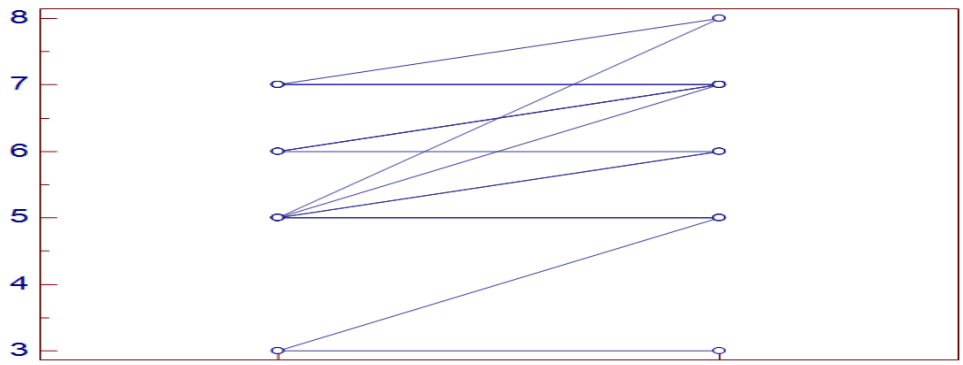


Figure 4. Dot and Line diagram

Analysis by Paired samples t-test of gleason scores between systematic and contrast enhanced biopsy showed a 95% CI of 0.22 to 1.11 with a significant p value (p=0.0032).

5.4. Distribution Of Positive Cores In Contrast Enhanced Biopsy And Systematic Biopsy :

Prostate cancer were detected in the base (*n* = 6), mid gland (n=15), Apex (n=5) in contrast enhanced biopsy.In systematic biopsy,cancers were detected in base(n =5), mid gland (n =21),apex (n=4).

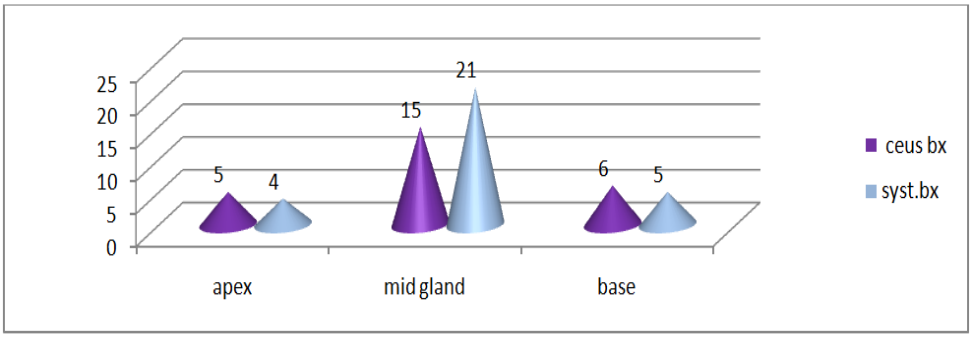


Figure 5. Distribution of positive cores in contrast enhanced biopsy and systematic biopsy :

5.5.Ultrasonographic Findings Vs Biopsy Results

Findings At Usg	Negative Cores	Positive Cores
Baseline Trus		
Negative	**156**	**18**
Positive	**14**	**12**
Contrast Enhanced Trus		
Negative	**5**	-
Positive	**4**	**26**

Table 1. Ultrasonographic Findings Vs Biopsy Results

Rating of biopsy sites as benign or malignant on the basis of USG and core biopsy results is shown in table .Sensitivity and specificity for detection of prostate cancer was calculated. Pathology of biopsy cores is used as reference standard. For baseline TRUS ,sensitivity was 40 % (12 /30) with specificity of 91.7%(156/170).For contrast enhanced ultrasound ,sensitivity was 100 % (26/26) but specificity was 56%(5/9).Chi square and Exact Measures of Association showed a significant P value <0.0000001.

5.6.Results Of Gray-Scale And Contrast-Enhanced Ultrasonography By Biopsy Site In 25 Patients

Of the 25 patients evaluated ,21 patients showed positivity for prostate cancer by contrast enhanced TRUS biopsy.(84%).But systematic biopsy demonstrated cancer in 17 patients only out of the 25.(68%).

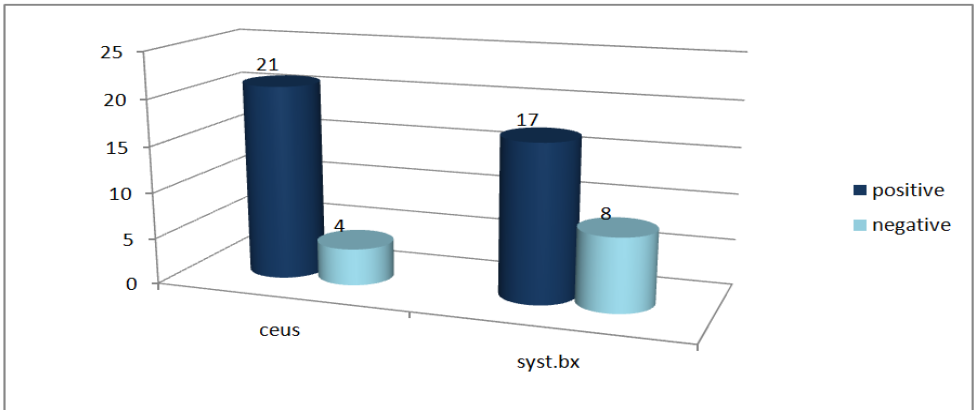


Figure 6. Results of Gray-Scale and Contrast-Enhanced ultrasonography by Biopsy Site in 25 Patients

5.7. Sensitivity Of Contrast Enhanced Trus With Respect To Serum Psa:

Our study included patients with serum PSA values ranging from 0.6 – 24.06 ng/ml with a mean of 12.73 .ROC analysis of sensitivity of contrast enhanced TRUS in relation to serum PSA showed area under curve of 0.863095 and 95% Confidence interval of 0.666917 to 0.966550 with a significant P value of <0.0001.

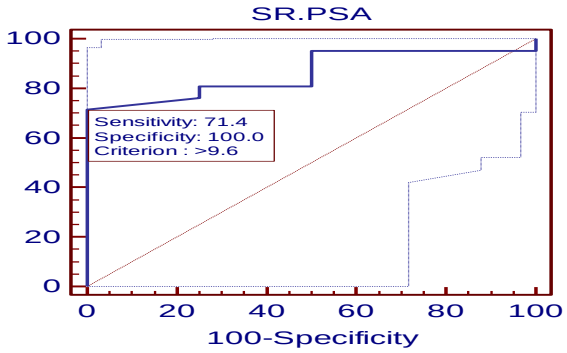
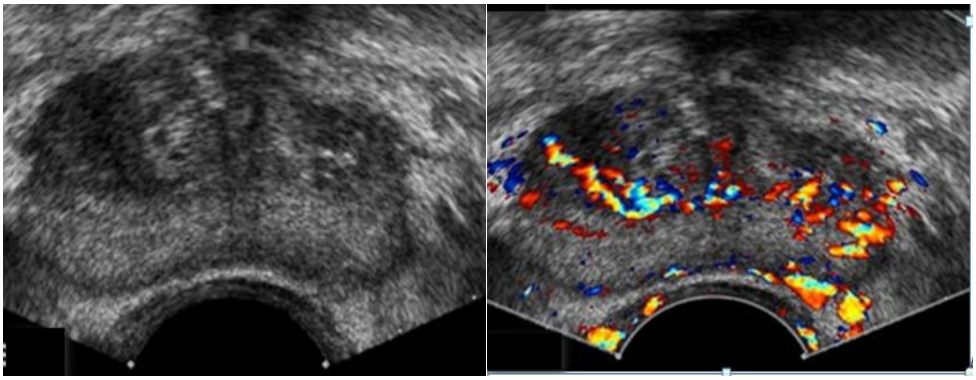


Figure 7. Sensitivity of contrast enhanced TRUS with respect to serum PSA



Gray Scale USG **Contrast Enhanced USG**

Figure 8.A. Conventional gray scale transverse image does not show any suspicious lesion. in the left base of the prostate B. Only contrast-enhanced colour Doppler transverse image shows increased flow associated with the cancer

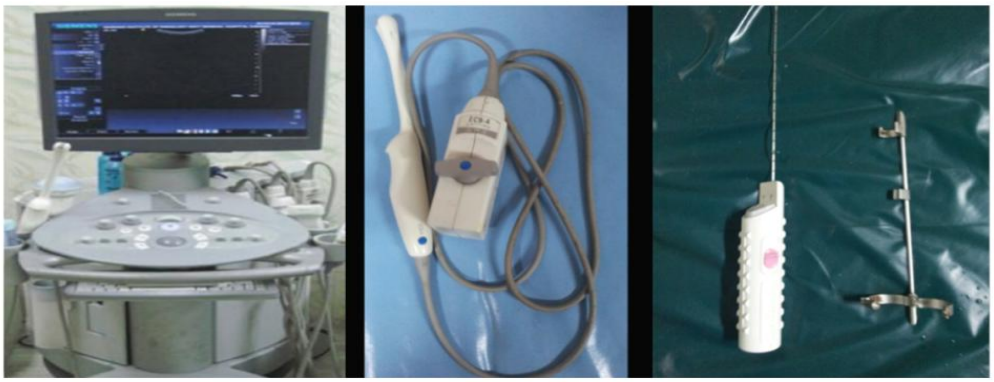


Figure 9. Ultrasound Machine, Probe And Biopsy Gun And Kit

VI. Discussion

6.1. Contrast-Enhanced Ultrasonography And Targeted Biopsy

Contrast-enhanced ultrasonogaphy (CEUS) uses gas-filled microbubbles that are administered intravenously as a bolus or an infusion pump to reach a steady state. Denaturated albumin, surfactants, or phospholipids are used as stabilizing shells. The gases consist of air or perfluoro gas.The microbubbles can withstand hydrostatic pressure within the vascular system and acoustic pressure from the ultrasound wave because of their stabilizing shell.So ,while passing through the pulmonary capillaries, they remain without being excreted. The encapsulated gas bubbles are smaller than erythrocytes ,varying in size from 1 to 4 micro metre ,enabling them to move through the micro circulation without difficulty. Microbubble contrast agents remain intravascular as blood-pool markers of the microcirculation. The intense reflected signal from the microbubbles in microvessels from malignant lesions can be visualized. Increased mechanical index of the ultrasound beam causes destruction of microbubbles resulting in total depletion of contrast microbubbles in the

targeted imaging area. Imaging the inflow of new contrast microbubbles after the destruction may reveal aberrant flow patterns correlated to prostate cancer.

There are a variety of microbubbles contrast agent and they differ in their shell makeup, gas core makeup, and whether or not they are targeted.

Microbubble shell: The shell material determines the ease with which the microbubble is taken up by the immune system. A hydrophilic material, though taken up more easily, reduces the microbubble residence time in the circulation and decreases the time available for contrast imaging.The microbubble mechanical elasticity is also affected by shell material . The microbubble shells are composed of albumin, galactose, lipid, or polymers.

Microbubble gas core: The gas core determines the echogenicity and is the most important part of the ultrasound contrast microbubble .The gas bubbles compress, oscillate, and reflect a characteristic echo when caught in an ultrasonic frequency field. This generates the strong and unique sonogram in contrast-enhanced ultrasound. Gas cores can be composed of air, or heavy gases like perfluorocarbon, or nitrogen. Heavy gases are less water-soluble so they are less likely to leak out from the microbubble leading to microbubble dissolution.As a result, microbubbles with heavy gas cores last longer in circulation.

Specific agents

SonoVue, made by Bracco, consists of sulphur hexafluoride microbubbles. It is mainly used to characterize liver lesions that cannot be properly identified using conventional ultrasound.

Optison, made by GE Healthcare, has an albumin shell and octafluoropropane gas core.

Levovist, made by Schering, has a lipid/galactose shell and an air core.

Perflexane lipid microspheres is an injectable suspension developed by Alliance Pharmaceutical . Beside its use to assess cardiac function and perfusion it is also used as an enhancer of the images of prostate, liver, kidney and other organs.

Perflutren lipid microspheres are composed of octafluoropropane encapsulated in an outer lipid shell.

Targeted microbubbles

Targeted microbubbles are outfitted with ligands that bind specific receptors expressed by cell types of interest, such as inflamed cells or cancer cells. Current microbubbles in development are composed of a lipid monolayer shell with a perflurocarbon gas core. The lipid shell is also covered with a polyethylene glycol (PEG) layer. PEG prevents microbubble aggregation and makes the microbubble more non-reactive. It temporarily "hides" the microbubble from the immune system uptake, increasing the amount of circulation time, and hence, imaging time. In addition to the PEG layer, the shell is modified with molecules that allow for the attachment of ligands that bind certain receptors. These ligands are attached to the microbubbles using carbodiimide, maleimide, or biotin-streptavidin coupling. Biotin-streptavidin is the most popular coupling strategy because biotin's affinity for streptavidin is very strong and it is easy to label the ligands with biotin. Currently, these ligands are monoclonal antibodies produced from animal cell cultures that bind specifically to receptors and molecules expressed by the target cell type.

Safety

Ultrasound contrast agents usually causes minor adverse events which are rare and transient. The most frequently reported side-effects of microbubble contrast agents are headache, altered taste, local pain at the injection side, a warm facial sensation, and a general flush. No cases of an allergic reaction have been reported to date. Because the gaseous content of the microbubble agents is eliminated by the lungs, it is of importance to evaluate whether impaired pulmonary function could be a contraindication for the use of microbubbles. CEUS appeared to be as safe and well tolerated in patients with Chronic Obstructive Pulmonary Disease as in a healthy control group. Recently, some cardiac events occurred in patients previously known with severe heart problems after use of Sonovue. Although relationship with the injection of the contrast agent cannot be proven, the use of this microbubble agent has since been restricted to noncardiac imaging and patients without serious cardiac morbidity.

6.2.Detection Of Prostate Cancer

CEUS of the prostate detects lesions that cannot be seen on grayscale ultrasound or found with systemic biopsies, because it allows the assessment of angiogenesis, increased vascularity, and abnormal blood flow associated with prostate cancer . CEUS was found to improve the sensitivity of detecting malignant tissue in a group of prostate cancer patients. Better visualization of prostate cancer with CEUS will increase diagnostic accuracy because biopsies can be taken targeted instead of randomly. CEUS-guided targeted biopsies may detect a larger number of prostate cancers with fewer needle biopsy cores, compared to grayscale ultrasoundguided biopsies. Sextant biopsies were scored prospectively as benign or malignant with grayscale

imaging and again for CEUS. Specificity was similar for both conventional grayscale ultrasound and CEUS. Doppler ultrasound can be used to enhance the contrast signal. Adding microbubble contrast agents to three-dimensional power Doppler imaging offers an increased detection of prostate cancer. Sensitivity increased from 38 to 85% using microbubble contrast agents during needle-guided prostate biopsies. Specificity did not change.

6.3. SUPPORT OF PROSTATE CANCER TREATMENT

Like conventional grayscale imaging, CEUS can support treatment of prostate cancer . CEUS has a higher sensitivity in detecting prostate cancer, resulting in a higher certainty to predict where and how extensive the tumor is. This information can be used to determine the right treatment modality or to support treatment. One could decide to spare one neurovascular bundle on the side without aberrant vascularity on CEUS, assuming there is no malignant tissue on that site. In case of suspicion of capsular invasion based on CEUS, one could decide to resect or freeze the prostate more radically.

CEUS could support prostate cancer brachytherapy planning by detecting intraprostatic lesions. These data can be used to optimize dose distributions and improve oncological outcomes. During active surveillance of prostate cancer, CEUS can be used to image and follow up the possible tumor foci. In case of an increase in contrast enhancement during follow up, associated with more clinical significant prostate cancer, treatment can be adjusted to the new situation.

6.4.Follow Up Of Prostate Cancer Treatment

CEUS can be used for several purposes in the follow up of prostate cancer , using the absence of blood signals as an indicator of treatment outcomes. Both high-intensity focused ultrasound (HIFU) and cryosurgery are treatments causing ablation of tissue by very high or very low temperatures, respectively. After successful prostate cancer treatment with HIFU or cryosurgery, blood flow should be absent in the treated area caused by direct thermal and indirect physiological effects of treatment. At this moment follow up after HIFU or cryotherapy is based on digital rectal examination, prostate specific antigen (PSA), and in some cases prostate biopsies to determine biochemical and pathological disease-free survival. CEUS can be used as an extra surveillance, to detect treatment failures or recurrence of prostate cancer. Sedelaar et al. consider CEUS as a promising method to determine the size of the defect after HIFU therapy for prostate carcinoma. The absence of blood flow after treatment reflected the affected tissue. As for HIFU, CEUS can be used to determine the size of the affected tissue after treatment with cryotherapy. CEUS after optimal treatment will show a total absence of contrast enhancement in the prostate, meaning an absence of blood perfusion. Treatment failures or cancer recurrence will be shown by areas of contrast enhancement, corresponding to remaining vital tissue. This way CEUS can be used as a verification of the used therapy. CEUS can be used to monitor the hormonal treatment of prostate cancer. Eckersley et al. found by means of CEUS that the vascular enhancement of the carcinoma declined with therapy, similar to PSA. The demonstrated reduction in vascularity produced by antiandrogen hormone therapy can be used to monitor therapy. The same should be possible with follow up after radiotherapy treatment. Radiotherapy kills cancer cells, theoretically resulting in a reduction of vascularization of prostate cancer foci, found by means of CEUS.

6.5. Limitations Of Our Study

The sample size of 25 subjects yielded 21 patients with cancer on contrast enhanced TRUS . To confirm that clinically important cancers are identified, it would be best to have a larger sample size with 5–10-year follow-up of negative US findings. Furthermore, a single imager in a single center examined all of the subjects in the current study. To be of value to the wider medical community, the results must be reproduced in a larger trial at multiple centers.

The requirement for sextant biopsy in all subjects ensured that there were many biopsy sites without US abnormalities. Furthermore, since biopsy was directed to any focus of abnormality seen either at baseline or after contrast material administration, a similar bias was introduced for both pre- and postcontrast imaging. The study design is further limited by the use of biopsy cores for pathologic correlation. Although each biopsy site was correlated with imaging findings, we cannot be certain that the biopsy needle passed through each visible sonographic abnormality. A sampling error of a few millimeters can result in a false-negative biopsy finding that is interpreted as a false-positive finding with enhanced transrectal sonography. Similarly, we cannot be certain that all of the cancers were identified, since none of the patients with negative biopsy results underwent pathologic examination of the remaining prostate tissue. Thus, we may have underestimated the false-negative rate of enhanced transrectal sonography. Nonetheless, our study design does provide a prospective evaluation of enhanced transrectal sonography relative to the current standard for the diagnosis of prostate cancer.

One final limitation is the issue of inner gland tumors. Sextant biopsy cores were obtained from only the outer gland. Inner gland cancers will be more difficult to detect because they are often superimposed on changes of benign prostatic hyperplasia.

Future studies of enhanced transrectal sonography should be conducted to investigate new techniques to maximize the difference in signal between benign and malignant tissues. Greater enhancement may be obtained with bolus administration of contrast material. New imaging techniques may reduce bubble destruction. Newer bubble agents that resonate at higher imaging frequencies may provide better signal, since the prostate is generally evaluated at 6–7 MHz.

VII. Conclusion

The results of this study shows that

- Contrast enhanced sonography could improve the sensitivity as well as accuracy for cancer detection, in analysis ,by biopsy site in patient population with prostate cancer
- The use contrast agents in TRUS will help in targeted biopsy of the enhancing lesions thereby decreasing the number of biopsy cores and associated complication.
- The use of CEUS also may be useful in patients with indeterminate serum PSA .
- Targeted biopsy has a definite impact on gleason scores,detecting high grade cancers with limited number of cores thus helping in planning the treatment in carcinoma prostate.

Conflict of interest:
None

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