PCA3 Urinary Test *Versus* ^{1}H-MRSI and DCEMR in the Detection of Prostate Cancer Foci in Patients with Biochemical Alterations

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Abstract. *Aim: To compare the prostate antigen 3 (PCA3) test with [1]H-magnetic resonance spectroscopic imaging (^{1}H-MRSI) and dynamic contrast-enhanced magnetic resonance imaging (DCEMR) combined examination in the detection of prostate tumor foci in patients with persistently elevated prostate-specific antigen (PSA) levels and prior negative random transrectal ultrasound (TRUS)-guided biopsy. Patients and Methods: Forty-three patients with a first random biopsy negative for prostate adenocarcinoma, persistent elevated PSA and negative digital rectal examination were recruited. All the patients were submitted to MRSI examination (MRSI-DCEMR) and were submitted to an attentive prostate massage in order to perform PCA3 assay. Afterwards, 10-core laterally-directed random TRUS-guided prostate biopsy was performed. Results: The overall sensitivity and specificity of a PCA3 score ≥35 for positive biopsy were 76.9% and 66.6%, respectively, with a positive predictive value (PPV) of 80% and a negative predictive value (NPV) of 62.5%; as for MRSI sensitivity and specificity were, respectively, 92.8% and 86.6% with a PPV of 92.8% and a NPV of 86.6%. Receiver operating characteristic (ROC) analysis rates were 0.755 for PCA3 and 0.864 for MRSI. Conclusion: Combined MRSI/DCEMR can better improve the cancer detection rate in patients with prior negative TRUS-guided biopsy and altered PSA serum levels than PCA3. Optimization of MRSI will allow more precise diagnosis of local invasion and improved bioptical procedures.*

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The widespread use of prostate-specific antigen (PSA) as a biomarker of prostate cancer (PCa) has led to the detection of some carcinomas that would have otherwise remained undetected during life; the deficiencies of serum PSA as a PCa-specific diagnostic test are well recognized (1), thus creating a new diagnostic dilemma: only a fraction of men with increased serum PSA have detectable prostate cancer. Clinicians are both missing PCa in patients with a non-elevated PSA, and performing a large number of unnecessary biopsies to detect a smaller proportion of questionably clinically significant tumors. Additionally, PSA does not have the capability to predict lethal prostate cancer with precision (2). Men with at least one negative biopsy often have persistently increased serum PSA, primarily attributable to an enlarged gland and benign prostatic hyperplasia (BPH). However, a significant proportion of men with slightly increased serum PSA (2.5-4.0 ng/ml) either have, or will develop, clinically significant PCa (3). Although biopsy remains the gold standard for PCa detection, more accurate tests, with better specificity, are needed to decide whether or not to biopsy the prostate. Additionally early detection of PCa has proved difficult and current detection methods are inadequate. Thus, the development of novel biomarkers for PCa detection remains an important and exciting challenge.

Noninvasive urine-based tests are particularly attractive candidates for large-scale screening protocols since urine is readily available and can be used to detect either exfoliated cancer cells or secreted products and biomarkers urine samples have emerged for detecting and predicting aggressiveness of prostate cancer. First described by Bussemakers *et al.* (4), PCA3 (prostate cancer antigen 3, also known as differential display code 3 or DD3) is a prostate-specific noncoding mRNA, with no resultant protein that is significantly overexpressed in PCa tissue compared to non-neoplastic prostatic cells (5) and detectable in the urine and prostatic fluid of men with PCa. In recent clinical trials, the potential diagnostic value of the PCA3 urine test was soon

established (6, 7). The PCA3 urine test is probably the best adjunct to serum PSA for predicting biopsy outcome, and has proven its clinical relevance by surpassing the predictive abilities of traditional serum biomarkers. PCA3 is overexpressed 60-100 times in 95% of PCa and PCa metastatic specimens than in benign prostate tissue (8).

Recently some studies (9, 10) have revealed the high diagnostic accuracy of combined proton [1]H-magnetic resonance spectroscopic imaging (^{1}H-MRSI) and dynamic contrast-enhanced imaging magnetic resonance (DCEMR) in the management of prostate cancer. In the prostate, the substances analyzed by MRSI are citrate, creatine and choline and then their ratio is calculated (11). However, the final diagnosis confirmed by histology and samples are obtained by transrectal ultrasound (TRUS)-guided biopsy. Bioptic samples are taken on a random scheme because the nodule is not detectable at TRUS and a negative set of biopsies does not rule out the presence of cancer; in fact more than 30% neoplastic foci are misdiagnosed and 23% of them are of high grade (12, 13). The present aim was to evaluate the ability of MRSI combined with MRSI and DCEMR *versus* PCA3 urinary test to improve PCa biopsy detection in cases of PSA increase and previous negative prostate biopsy. The association between the PCA3 results and MRSI examination with the clinical-pathological features known to be associated with PCa aggressiveness were investigated.

Patients and Methods

Study design and population. This was a prospective single-center study on patients with prior negative random TRUS-guided prostate biopsy and persistent elevated PSA levels. The study was performed after approval of the protocol from the institutional board committee of Sapienza Rome University and informed consent for inclusion was obtained from all the patients. Forty-three consecutive patients referred to the Department of Urology, Policlinico Umberto I for prostate biopsy from September 2009 to February 2010 were recruited into the study. The age of the patients ranged from 48 and 69 years (mean 60.3) and the inclusion criteria were: a first random TRUS-guided prostate biopsy negative for prostate adenocarcinoma or high-grade prostate intraepithelial neoplasm; persistent elevated PSA levels (total PSA ≥4 ng/ml and <10 ng/ml) and negative digital rectal examination (DRE). Exclusion criteria for the study were previous hormonal, surgical or radiation therapies for prostate diseases, urine not collected after DRE and prior to prostate biopsy, inadequate prostate biopsy with fewer than 10 cores and cases in which MRSI with a complete MRSI and DCEMR study was not possible or men who declined to consent for participation in the study.

All the patients were submitted to an attentive prostate massage (3 compressions for each prostatic lobe) and whole urine specimens were collected in order to perform PCA3 assay and then the patients were submitted to MRSI examination with MRSI and DCEMR, prior to TRUS-guided prostate biopsy. All the biopsies were homogeneously performed in the Department of Urology, Policlinico Umberto I, by the same physician (MC), as part of the patient's urological work-up after urine specimen collection, according to a standard biopsy

protocol: 10-core laterally directed random TRUS-guided prostate biopsy (two cores from the basal portion lateral and paramedial, two from the midgland lateral and paramedial, and one from the apex, on each side of the gland for each patient, plus 3 additional biopsies from other areas suspicious for PCa at MRSI) (14). In cases of histological diagnosis of PCa, the patients were advised of all treatment options and, if accepted and without contraindication for surgery, the patients were submitted to radical retropubic prostatectomy (RRP). Both prostate biopsies and radical prostatectomy specimens were evaluated at the Department of Pathology, Policlinico Umberto I, and where PCa was present, the Gleason Score (GS) was determined. If a tumor was reported in a set of biopsies, a radical prostatectomy was performed by an expert urologist and correlated with the MRSI findings by step section at histopathology.

PCA3 urine assay. The PCA3 clinical test requires urine to be collected after an attentive DRE to increase the number of prostate cells shed into the urine (5). The attentive DRE included firm pressure on the prostate from base to apex and from lateral to median lobe, with three strokes per lobe and enough pressure to slightly depress the prostate surface following the manufacturer's instructions for the Gen-Probe Progensa PCA3 Assay. Following DRE, the patients collected their initial void of 20-30 ml of urine and then *PCA3* and *PSA* mRNAs were isolated from a total of 2.5 ml of urine and were submitted to transcription-mediated amplification. The *PCA3* and *PSA* mRNAs level were quantified using the PCA3 assay (15). The PCA3 scores were reported as a quantitative *PCA3/PSA* mRNA ratio ×1000 to normalize the *PCA3* to the prostate RNA level in the urine sample. Cases with insufficient *PSA* mRNA were considered inconclusive and excluded. A PCA3 score of ≥35 was considered positive (per laboratory standard).

MRSI and MRSI-DCEMR. All the examinations were performed on a 3T magnet (Magnetom Vario, Siemens Medical Solutions, Erlanger, Federal Republic of Germany; gradient strength, 45 mT/m; slew rate, 346 T/m/s; rise time, 400 micro/s; featuring total imaging matrix-TIM1 technology), equipped with surface phased array (Body Matrix, Siemens Medical Solutions, Erlanger, Federal Republic of Germany) and endorectal coil (e-Coil, Medrad, combined with Endo-Interface, Siemens Medical Solutions, Erlanger, Federal Republic of Germany) for the *in vivo* MRSI measurements. The endorectal radio frequency (RF) coil was positioned close to the prostate through the rectum of each patient and filled with 50 to 60 ml of room air on the basis of patient tolerance, which not only shapes the coil, but also reduces the dielectric RF load of the coil, at minimal susceptibility difference with the tissue. The correct position and the efficiency of the coil for magnetic field generation *in vivo* was estimated from sagittal spin echo sequences at different RF power levels. The ballooned coil also supports the prostate and minimizes motion. Morphological imaging of the prostatic gland was carried out by acquiring turbo spin-echo (TSE) T2-weighted sequences in the axial, sagittal and coronal planes, with the use of optimized parameters for a better spatial resolution (repetition time (TR), 5.190 ms; echo time (TE), 95 ms; flip angle, 150°; average, 3; field of view (FOV) read, 256 mm; FOV phase, 100; thickness, 3 mm; section gap, 0; matrix, 512×512; phase resolution, 100%; band width, 130; scan time, 3.40 min). At ^{1}H-MRSI, a point-resolved spectroscopic sequence was obtained with the use of three-dimensional (3D) chemical shift imaging sequence with spectral/spatial pulses optimized for
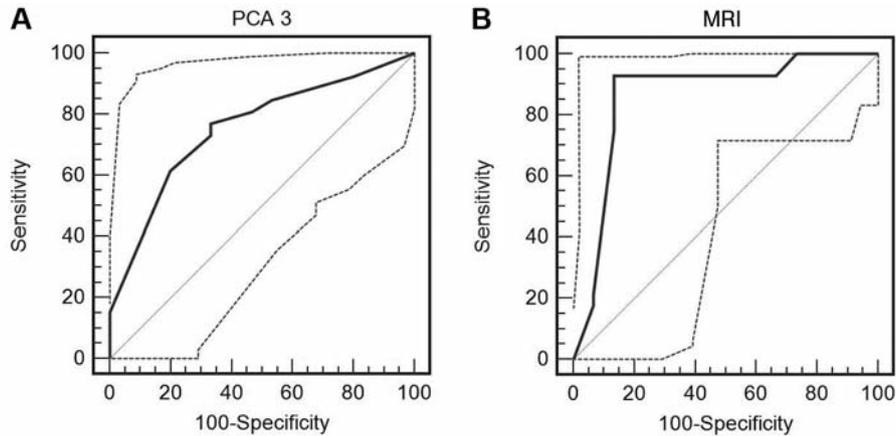
Figure 1. *ROC curves. The area under the ROC curve was 0.755 for PCA3 (A) and 0.864 for MRSI (B), with a 95% confidence interval of 0.596 to 0.875 and 0.725 to 0.949, respectively. ROC curve analysis was performed using biopsy as the diagnostic indicator in the comparison method. The ROC curve is a plot of sensitivity as a function of false-positive rate (1–specificity).*

quantitative detection of choline and citrate (FOV, 60×60×60 mm; Vol, 30×30×30 mm; TR, 550 ms; TE, 145 ms; flip angle, 65°; interpolation, 16; vector size, 512; time acquisition (TA), 7.32 min; δ frequency, −1.80 ppm; average, 5; voxel isotropic, 0.25 cm^3). The ^{1}H-MRSI pulse sequence used frequency-selective water and lipid suppression to selectively obtain the relevant metabolite signals from the prostate. The DCEMR images were acquired by using a gradient-echo (GRE) T1-weighted sequence during *i.v.* contrast agent administration (TR, 2.15 ms; TE, 0.85 ms; flip angle, 16°; average, 1; thickness, 2.5 mm; section gap, 0; time resolution, 16 sections/3 s; matrix, 256×328; scan time, 3.15 min) immediately following completion of an *i.v.* bolus injection of 1.0 mmol/ml of gadobutrol (Gadovist, Bayer Schering Pharma AG, Leverkusen, Germany). Contrast was administered with a power injector (Spectris; Medrad, Warendale, PA, USA) at 3.0 ml/s and was followed by a 15 ml saline flush. During contrast agent administration, subtraction images were generated by an automated algorithm that uses the first 3s of the sequence as baseline for the following measurements. This technique was used to improve the selection of regions of interest (ROI), in subsequent signal intensity time (SI-T) concentration curves analysis. The 3D volume was acquired with the same positioning angle and center as the transverse T2-weighted sequence, covering the entire prostate gland. Relative gadolinium chelate concentration curves were calculated (16). For practical purposes, prostate adenocarcinoma can be distinguished from healthy peripheral zone tissue on the basis of the (choline + creatine)/citrate ratio (17). Normal peripheral zone tissue is characterized by voxels with a (choline + creatine)/citrate ratio of <0.8; suspicious of cancer is defined as a voxel with (choline + creatine)/citrate ratio >0.8 (18). In this study, for MRSI, the (choline + creatine)/citrate ratio threshold for cancer suspicion was defined as 0.80, as previously reported in the literature (19).

Statistical analysis. Statistical data analysis was conducted with statistical software MedCalc Software for Windows, version 9.3 (Mariakerke, Belgium). A *p*-value <0.05 was considered to indicate a significant difference. All the variables were also included in logistic multivariate models. Classification tables (2×2) were used to calculate the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy of each feature. Receiver operating characteristic (ROC) curves comparison for each analysis phase was also carried out.

Results

Fourty-one out of the 43 urinary sediment specimens were analyzed successfully (*i.e.* adequate concentrations of *PCA3* and *PSA* mRNA to calculate the PCA3 ratio), 95.3%. The PSA levels ranged from ≥4 ng/ml to <10 ng/ml; mean 6.37 ng/ml. The performance of the PCA3 test was evaluated in terms of sensitivity and specificity by comparing the PCA3 score to the biopsy results. The sensitivity and specificity of PCA3 and MRSI were explored using ROC analysis. The ROC curve analysis was performed using biopsy as the diagnostic indicator in the comparison method. The ROC curve is a plot of sensitivity as a function of false-positive rate (1–specificity).

The overall sensitivity and specificity of a PCA3 score ≥35 for positive biopsy in this cohort were 76.9% and 66.6 %, respectively, (Figure 1A), with a PPV of 80 % and an NPV of 62.5%. For the MRSI sensitivity and specificity were, respectively, 92.8% and 86.6% (Figure 1B) with a PPV of 92.8 % and a NPV of 86.6%. The area under the ROC curve was 0.755 for PCA3 and 0.864 for MRSI, with a 95% confidence interval of 0.596 to 0.875 and 0.725 to 0.949, respectively. Out of the 41 individuals that yielded informative specimens, 28 biopsies were positive for PCa (Figure 2), and the remaining 13 were biopsy-negative; 5 out of the 13 biopsy-negative males had BPH and/or inflammation. Out of the 28 patients with PCa on biopsy, 13
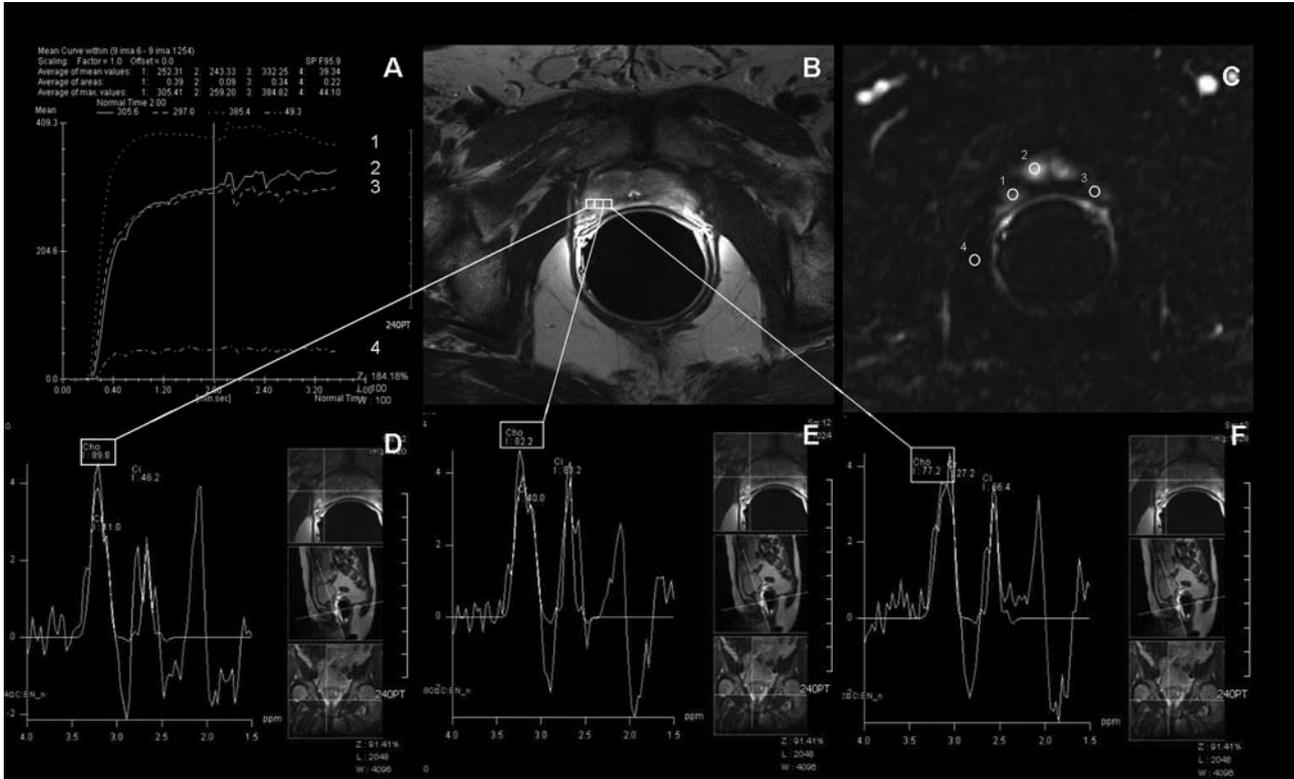
Figure 2. *Representative prostate cancer-positive profile of a 58-year-old patient (PSA 4.6 ng/ml; ratio 18%), negative first TRUS-guided biopsy and negative PCA3 test. A: Signal-intensity time curve, note the malignant pattern curve 1. B: Bilateral mild hypointense area in the peripheral zone at level of apex. C: Dynamic subtracted image representing region enhancement and ROI. ROI 1 on enhancing zone, ROI 3 on opposed site of peripheral zone, ROI 2 on benign prostate hyperplasia and ROI 4 on the pelvic muscle as baseline. D-F: MRSI analysis (3D-CSI sequence, voxel size 2.5 cm³) with reference images on the right side, high choline level detected in 3 consecutive voxels. Histological prostate cancer, Gleason score 6 (3+3). ROI, Region of interest.*

(46.4 %) had an unfavorable prognosis based on a Gleason score of 7 or greater (Figure 3). RRP was performed in 10 cases and the remaining patients affected by PCa (18) were submitted to radiation therapy and/or hormonal therapy. Among the men undergoing radical prostatectomy after positive biopsy, the concordance of MRSI and macrosection was 93.2%.

Discussion

Using the early assay method, approximately 15%-20% of PCA3 samples were deemed 'non-evaluable' because the urine did not contain a sufficient quantity of *PSA* mRNA to allow detection of background genetic material. In this study, 95% of the urinary sediments contained enough prostate epithelial nuclear material to be evaluated and the *PCA3* to *PSA* mRNA urinary levels exhibited 66-82% sensitivity and 76-89% specificity for PCa. Both values compared quite favorably with PSA accuracy. In an earlier study of 443 men undergoing prostate biopsy, 66% sensitivity and 89% specificity were achieved with PCA3, and in a subgroup of

94 patients with a PSA level of <4.0 ng/ml, the sensitivity was 74% and specificity 91% (5).

The Gleason scoring system is regarded as the most powerful prognostic factor in PCa (20). Men who have organ-confined disease and a GS ≥7 tumor have significant worse outcome after radical prostatectomy and radiotherapy than men with a GS <6 tumor (21). In one study, a higher PCA3 score in men with a biopsy Gleason score of 7 or greater compared to those with a Gleason score less than 7 was reported (22). These results suggested a possible specific use for PCA3 in identifying aggressive prostate carcinomas. However the ROC analysis suggested that PCA3 alone could not be used to identify men with progressive disease. Differentiation between GS ≤6 and ≥7 is important for treatment decision, but in this study, no exact correlation between PCA3 and clinical-pathological features was identified.

MRSI of the prostate is not routinely used in the initial diagnosis of prostate adenocarcinoma, but rather for staging (23). Recently several studies have demonstrated the significant role of MRSI as an effective technique to localize
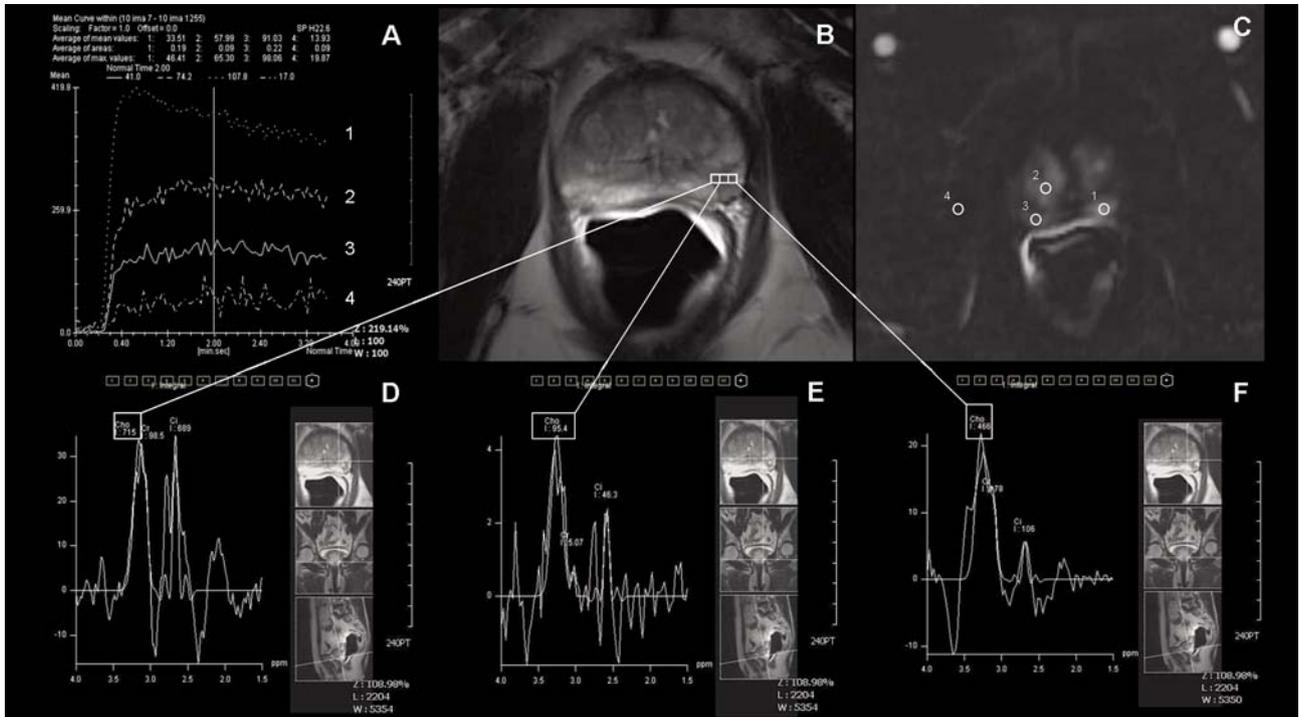
Figure 3. *Representative profile of a high Gleason score (GS) prostate cancer case (63 years old). PSA 7.2 ng/ml; ratio 13%, negative PCA3 test. A: Signal-intensity time curve, note the malignant pattern in curve 1 (see high peak enhancement and wash-out). B: Focal hypointense area in the left portion of the peripheral zone at level of middle gland. C: dynamic subtracted image representing region enhancement and ROI. ROI 1 on enhancing zone, ROI 3 on opposed site of the periheral zone, ROI 2 on benign prostate hyperplasia and ROI 4 on pelvic muscle as baseline. D-F:MRSI analysis (3D-CSI sequence, voxel size 2.5 cm^3) with reference images on the left side, high choline level detected at level of morphological enhanced focus. Histological intracapsular prostate cancer, GS 7 (4+3). ROI, region of interest.*

prostate cancer also in men with repeat negative biopsies and increased PSA (24, 25). MRSI demonstrates zonal anatomy with excellent contrast resolution and can reveal tumors in areas not routinely sampled on biopsy and not palpable on DRE. MRSI which allow the assessment of local extent (including extracapsular extension and seminal vesicle invasion) could provide a visual road-map for treatment planning (26). Furthermore the combination of MRSI and MRSI yields superior diagnostic results than either modality alone (11). MRSI spectroscopic analysis provides metabolic information regarding prostatic tissue by displaying the relative concentrations of chemical compounds within contiguous small volumes of interest (voxels) (10, 27, 28).

While PCA3 appears to improve PCa detection, it has inherent limitations. There is no international standard for the urinary assay (although not approved by the FDA for prostate cancer detection, a commercial PCA3 DD3 test is available in Europe and the Gen-Probe Progensa PCA3® assay received marketing clearance in 2006) and all methods rely upon urine obtained immediately after an attentive DRE, unlike PSA for which there are several assays, although reported values vary based upon the assay method.

While urinary specimen informative rates are generally high, a small proportion of men have to provide repeat urine samples after an inadequate DRE, to express a sufficient number of prostate cells. Furthermore, it is unclear if a suboptimal DRE or a small peripheral tumor producing a minimal number of shed cells into the urine can result in a falsely negative PCA3 score, and while no relationship has been found between PCA3 score and prostate volume, a recent report suggested that *PCA3* mRNA can be detected in high-grade prostatic intraepithelial neoplasia and benign tissue proximal to neoplastic glands, suggesting precursor molecular changes (29). Recently, Deras *et al*. (30) compared the accuracy of PCA3 with other prostate cancer detection methods in 570 men about to undergo prostate biopsy. The accuracy of PCA3 was significantly greater than serum PSA, and its sensitivity and specificity were similar to all serum PSA levels. Prostate volume did not influence the results. Further studies are needed to assess the value and exact position of PCA3 testing in the clinical management of PCa. The current diagnosis of prostate adenocarcinoma is mainly based on the use of PSA determination and TRUS-guided biopsies.

The addition of ^{1}H MRSI to MRSI can improve prostate cancer detection and assessment of tumor volume; it also contributes indirectly to improved local staging, especially using 3T magnet (31) and more information using MRSI combining metabolic (spectroscopy) and lesion environment data (perfusion) along with angiogenesis in addition to anatomical high resolution images can be obtained; allowing the correct localization and characterization of the neoplastic and pre-neoplastic focus in the prostate gland. With greater understanding of the relationship between spectroscopic data, tumor biology and carcinogenesis, it may become possible to use MRSI/^{1}H MRSI to achieve more precise stratification of patients in clinical trials, to monitor the progress of patients who select watchful waiting or minimally aggressive cancer therapies and to guide and assess emerging local prostate cancer therapies.

Conclusion

The combination of MRSI and DCEMR at 3T shows the potential to guide biopsy to cancer foci in patients with previously negative TRUS biopsy and shows higher sensitivity and specificity than PCA3 assay.

In the not too distant future, with improvement of the PCA3 test and MRSI, and using these methods together, the diagnosis of PCa might be achieved without performing invasive prostate biopsy. In any case, the PCA3 provides genetic information, MRSI gives vascular, anatomical and metabolic details and clinical information is obtained by DRE.

Conflict of Interests Statement

The Authors have no conflicts of interest to declare.

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