PCA3 Molecular Urine Assay Correlates With Prostate Cancer Tumor Volume: Implication in Selecting Candidates for Active Surveillance

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Purpose: Prostate cancer gene 3 (PCA3) has shown promise as a molecular marker in prostate cancer detection. We assessed the association of urinary PCA3 score with prostatectomy tumor volume and other clinical and pathological features.

Materials and Methods: Urine specimens were collected after digital rectal examination from 59 men scheduled for prostate biopsy and 83 men scheduled for radical prostatectomy. Prostatectomy findings were evaluable for 96 men. PCA3 and prostate specific antigen mRNAs were quantified with Gen-Probe DTS® 400 System. The PCA3 score was defined as the ratio of PCA3 mRNA/prostate specific antigen mRNA $\times 10^3$.

Results: The PCA3 score in men with negative biopsies (30) and positive biopsies (29) were significantly different (median 21.1 and 31.0, respectively, $p = 0.029$). The PCA3 score was significantly correlated with total tumor volume in prostatectomy specimens ($r = 0.269$, $p = 0.008$), and was also associated with prostatectomy Gleason score (6 vs 7 or greater, $p = 0.005$) but not with other clinical and pathological features. The PCA3 score was significantly different when comparing low volume/low grade cancer (dominant tumor volume less than 0.5 cc, Gleason score 6) and significant cancer ($p = 0.007$). On multivariate analysis PCA3 was the best predictor of total tumor volume in prostatectomy ($p = 0.001$). Receiver operating characteristic curve analysis showed that the PCA3 score could discriminate low volume cancer (total tumor volume less than 0.5 cc) well with area under the curve of 0.757.

Conclusions: The PCA3 score appears to stratify men based on prostatectomy tumor volume and Gleason score, and may have clinical applicability in selecting men who have low volume/low grade cancer.

Key Words: genes, neoplasm; prostatic neoplasms; prostate-specific antigen

Prostate specific antigen has been used worldwide for the early detection of prostate cancer.[1] However, the increased use and continued reliance on PSA based screening has led to an increase in the diagnosis of low volume/low grade cancer that in some cases will not progress clinically during a lifetime.[2,3] This issue is an important consideration when selecting men with a diagnosis of prostate cancer for active surveillance.[4] Recently several studies have shown that the correlation between PSA and tumor volume has decreased.[5,6] Consequently a new prostate cancer marker with greater accuracy than serum PSA is urgently needed.[6,7]

Prostate cancer gene 3 (PCA3), first described by Bussemakers et al in 1999, is a noncoding, prostate specific mRNA that is highly over expressed in prostate cancer tissue compared with benign prostatic tissue.[8] The possible usefulness of the urinary PCA3 assay as a prostate cancer marker was suggested by de Kok et al in 2002.[9] Hessels et al reported that PCA3 was over expressed in 95% of the prostate cancer cells tested, with a median 66-fold up-regulation compared with adjacent nonneoplastic prostatic cells.[10] They also demonstrated the potential clinical usefulness of a PCA3 based urine test that measured relative over expression of PCA3 mRNA compared with PSA mRNA, and generated a PCA3 score. On the basis of these results Groskopf et al developed an investigational PCA3 urinary assay for general clinical use.[11] Recent studies have shown that the PCA3 urine assay has promise in improving the diagnostic accuracy of prostate cancer detection, especially in the PSA gray zone.[11–13]

On the basis of the molecular-biological features of PCA3 we hypothesized that the PCA3 score would associate with prostate cancer volume.[8–10] We report the association of the PCA3 score with the clinical parameters in men undergoing prostatectomy and the pathological features including tumor volume in radical prostatectomy specimens.

PATIENTS AND METHODS

Before study initiation this protocol was approved by the institutional review board and all subjects provided written informed consent. Our study population was composed of 2 groups. The first group, the biopsy group, consisted of 59

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consecutive men who were scheduled for a prostate biopsy at our institution with serum PSA 2.5 ng/ml or greater and/or abnormal DRE. The second group, the prostatectomy group, consisted of 83 men who had already been diagnosed with prostate cancer. In both groups eligibility was restricted to men with a serum PSA less than 50 ng/ml and age 40 to 70 years old. No man in either group was taking medications that could affect serum PSA such as finasteride. In the biopsy group biopsies were performed with a 10 to 13-core extended scheme as previously described.[14] In the prostatectomy group biopsies were performed with at least a sextant scheme. The actual scheme used depended on the source of patient referral. All men undergoing prostatectomy had clinical T1c-T3 N0M0 disease.

For this study urine samples (at least 20 ml first catch) were collected from June 2005 to May 2006 after an attentive DRE consisting of exactly 3 strokes per prostatic lobe, performed by an attending urologist. Firm pressure was applied from the base to the apex and from lateral to midline for each lobe. The urine samples were held at 2C to 8C and processed within 4 hours by mixing with an equal volume of detergent based stabilization buffer and then stored at −70C until analyzed.[11,12]

The PCA3 and PSA mRNAs were isolated, amplified and quantified, and the quantitative ratio of the PCA3 score was determined with the DTS 400 System as described previously.[11,12] Briefly, in separate procedures PCA3 and PSA mRNAs were isolated from the processed urine samples by capture onto magnetic microparticles and amplified by transcription mediated amplification, and the products were detected with chemiluminescent DNA probes using the hybridization protection assay. The PCA3 score was defined as the ratio of PCA3 mRNA-to-PSA mRNA $\times 10^3$.[11,12] PSA mRNA expression has been considered to be relatively constant in prostate cells including cancer cells[15] and, therefore, used to normalize for noncancerous prostate cells.[10]

The histopathological features of the outside biopsy specimens, including Gleason score and tumor length in biopsy cores, were reevaluated at our institution. The prostatectomy specimens were analyzed by a single pathologist (PT) according to our institutional protocol.[14,16] Briefly the glands were fixed in neutralized 10% formalin for 48 hours. The apical portion of the prostate (distal 0.5 to 1.0 cm) was separated from the rest of the prostate and radially sectioned. The remaining prostate was then sectioned at 4 mm intervals. The margin at the base of the prostate was evaluated with perpendicular sections. The tumors were graded according to the Gleason grading system. The assigned histological grade for the specimen was that of the dominant tumor focus, except for dominant transition zone tumors of lower histological grade than peripheral zone foci, in which case the assigned grade was that of the peripheral zone focus with the highest Gleason score. Each tumor focus was outlined on the histological sections. For each specimen, the volume of each individual tumor focus was determined using the formula 0.4 × length × width × cross section thickness. We already reported that this streamlined 3-dimensional volume estimation method is accurate and that it correlates significantly with other measurement methods.[17] The 5 largest tumor foci were analyzed as previously described.[5,17] The tumor foci were ranked by size, with focus 1 being the largest and focus 5 being the smallest. Total tumor volume was then determined by adding the volume of each cancer focus.

Low volume/low grade cancer was defined as organ confined cancer with a dominant tumor volume less than 0.5 cc and the absence of any Gleason grade 4 or 5 disease.[14,18] Significant cancer was defined as all cancers not meeting the criteria for low volume/low grade disease.

The patients underwent clinical evaluation, including DRE, TRUS and serum PSA determination. Prostate volume was measured by TRUS using the formula for elliptical volume ($\pi / 6 \times$ height × width × length). The proportion of positive cores in biopsy (% positive cores) was calculated using the following formula: number of positive cores divided by number of total biopsy cores.

The relationship of the PCA3 score with clinical and pathological features was evaluated using Mann-Whitney's U test. After evaluating the relationship between pairs of covariates using Pearson's correlation coefficient test, the ability to predict tumor volume with a combination of various variables was analyzed using a forward stepwise multiple logistic regression analysis. ROC curve analysis was generated by plotting sensitivity vs 1-specificity. Likewise, AUC and asymptotic significance were calculated for each variable. All statistical calculations were performed using a commercially available computer software package (SPSS® version 12.0) with $p < 0.05$ considered statistically significant.

RESULTS

In the biopsy group 29 were positive for prostate cancer and 30 were negative. Of the 29 men with a positive biopsy result 13 underwent prostatectomy. For purpose of the analysis we added these 13 patients to the prostatectomy group. Consequently, a total of 96 patients underwent prostatectomy and were evaluable. All of the 142 urine specimens (59 in the biopsy group and 83 in the prostatectomy group) yielded sufficient mRNA for analysis, corresponding to an informative rate for the PCA3 score of 100%.

Table 1 lists clinical and pathological characteristics of the patients who underwent radical prostatectomy. Using Pearson's correlation coefficient test the correlation between total tumor volume and PCA3 score was significant ($p = 0.008$) but was widely distributed (correlation coefficient $r = 0.269$). The PCA3 score did not correlate with the serum PSA level ($r = 0.027$, $p = 0.797$).

Figure 1, *A* demonstrates the relationship of the PCA3 score to total tumor volume, and figure 1, *B* shows the relationship of the PCA3 score in low volume/low grade cancer compared to significant cancer. The median/average PCA3 score in men with low volume (total tumor volume less than 0.5 cc in 27), intermediate (0.5 to less than 2.0 cc in 35) and high volume cancer (2.0 cc or greater in 34) was 21.5/29.3, 41.8/54.4 and 48.1/69.9, respectively. There is a direct correlation between tumor volume and the PCA3 score, ie as the tumor volume increased so did the PCA3 score, and the converse is also true, ie the lower the PCA3 score the greater was the probability that tumor volume would be smaller. Low volume/low grade cancer was present in 11% (11/96) of men with 89% (85/96) having significant cancer. The median/average PCA3 score in men with low volume/low grade cancer and significant cancer was 17.8/24.5 and 42.4/56.5, respectively. The PCA3 score was significantly different when comparing low volume/low grade cancer to significant cancer.

The relationship between the PCA3 score and the biopsy and prostatectomy Gleason scores is illustrated in figure 1,

TABLE 1. *Clinical and pathological characteristics of patients treated with radical prostatectomy for clinically localized prostate cancer*

Clinical features		
Median/av age (range)	60/60	(45–70)
No. ethnicity (%):		
White	75	(78.1)
Black	15	(15.6)
Hispanic	6	(6.3)
Median/av ng/ml PSA (range)	4.8/5.7	(1.0–27.0)
Median/av cc TRUS prostate vol (range)	31.5/34.7	(16.0–74.1)
No. clinical stage (%):		
T1c	68	(70.8)
T2 or greater	28	(29.2)
Median/av PCA3 score (range)	36.2/52.8	(2.0–306.6)
Prostate biopsy features		
No. Gleason score (%):		
6	39	(40.6)
7 (3+4)	39	(40.6)
7 (4+3)	12	(12.5)
8	6	(6.3)
Median/av % pos cores (range)	30/34	(8–100)
Median/av max tumor mm (range)	3.0/4.1	(0.5–13.5)
Prostatectomy features		
No. Gleason score (%):		
6	15	(15.6)
7 (3+4)	62	(64.6)
7 (4+3)	12	(12.5)
8	3	(3.1)
9	4	(4.2)
No. pathological stage (%):		
T2	79	(82.3)
T3a	10	(10.4)
T3b	7	(7.3)
Median/av cc total tumor vol (range)	1.24/1.76 (0.003–8.64)	
Median/av cc dominant tumor vol (range)	0.89/1.36 (0.002–8.64)	

C and *D*. Biopsy Gleason score did not equal the prostatectomy Gleason score in 41% (39/96) (upgrading in 31 men and downgrading in 8 men). In biopsy specimens, there was no significant difference between the PCA3 score in men with low grade (Gleason score 6 or less in 39) and high grade cancer (Gleason score 7 or greater in 57). However, in prostatectomy specimens the PCA3 score in low grade cancer (15) was significantly lower than that in high grade cancer (81).

The results shown in table 2 illustrate the absence of a relationship between PCA3 score and clinical and pathological characteristics. A limitation of our study is the relatively small sample size, especially with respect to the number of patients with low volume/low grade cancer. However, our findings suggest that the PCA3 score is significantly associated with prostatectomy tumor volume and Gleason score.

On multiple regression analysis TRUS prostate volume, biopsy Gleason score, percent positive cores in biopsy, log PSA, and log PCA3 score were significant independent predictors of low volume cancer (total tumor volume less than 0.5 cc) in prostatectomy specimens. Among these variables log PCA3 score was the best significant predictor (table 3). The prediction of dominant tumor volume less than 0.5 cc was significantly associated with TRUS prostate volume, maximum tumor length in biopsy cores, log PSA and log PCA3 score.

ROC curve analysis revealed that the PCA3 score demonstrated the best performance (AUC = 0.757) in predicting low volume cancer defined by total tumor volume (fig. 2, *A*). The AUC for the PCA3 score to predict a dominant tumor volume less than 0.5 cc decreased to 0.673 (fig. 2, *B*). A PCA3 score of 25 corresponded to the point on the ROC curves with the greatest diagnostic accuracy. The sensitivity, specificity, PPV, NPV, diagnostic accuracy and odds ratio of predicting total or dominant tumor volume using a PCA3 score cutoff of 25 in combination with Gleason score are shown in table 4.

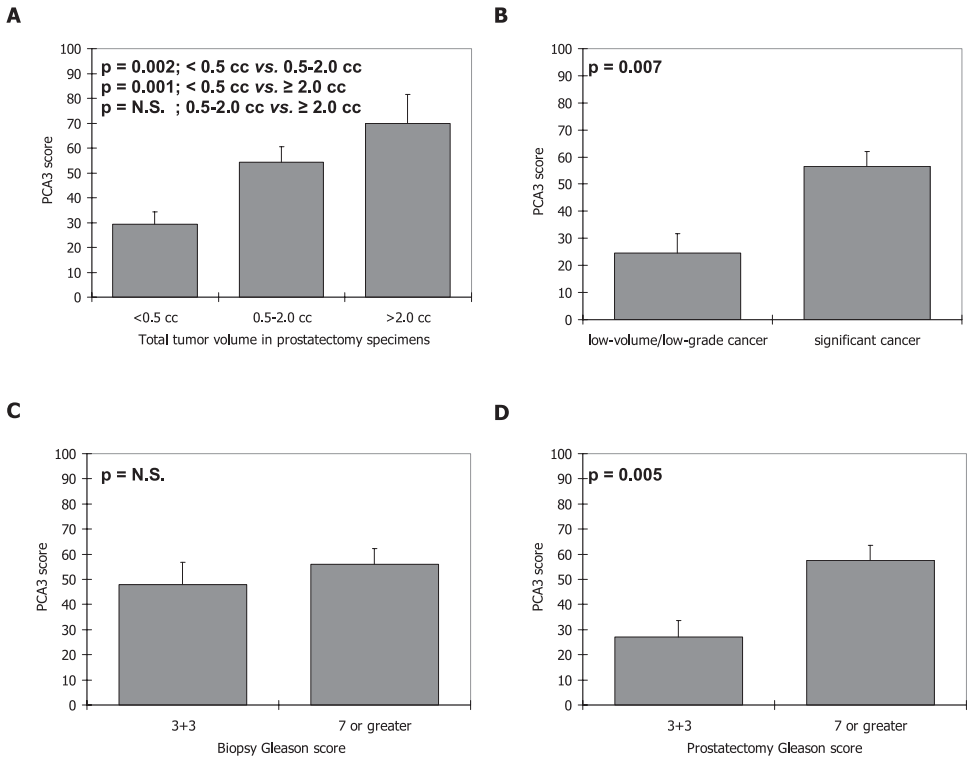


FIG. 1. Changes in PCA3 score by (*A*) total tumor volume, (*B*) low volume/low grade cancer vs significant cancer, (*C*) biopsy Gleason score and (*D*) prostatectomy Gleason score. Average PCA3 scores are shown. Error bars represent standard error of average.

TABLE 2. *Relationship between PCA3 score and clinical and pathological variables*

	No. Pts	Median/Av PCA3 Score	p Value*	
Age:			0.334	
Younger than 50	45	29.5/52.1		
50 or Older	51	42.4/53.5		
Ethnicity:				
White	75	35.4/48.6	0.093	(white vs black)
Black	15	55.0/79.9	0.576	(white vs Hispanic)
Hispanic	6	26.3/37.7	0.186	(black vs Hispanic)
Serum PSA (ng/ml):†				
Less than 2.5	9	69.8/61.0	0.325	(less than 2.5 vs 2.5–4.0)
2.5–4.0	24	29.0/44.4	0.580	(less than 2.5 vs 4.0 or greater)
4.0 or Greater	61	36.9/55.1	0.308	(2.5–4.0 vs 4.0 or greater)
TRUS prostate vol (cc):‡				
Less than 25	21	55.0/66.0	0.609	(less than 25 vs 25–50)
25–50	52	38.4/53.7	0.124	(less than 25 vs 50 or greater)
50 or Greater	12	23.4/30.6	0.087	(25–50 vs 50 or greater)
% Pos cores in biopsy:			0.170	
Less than 33.4	62	35.0/46.7		
33.4 or Greater	34	47.6/63.9		
Max tumor length (mm) in biopsy cores:			0.384	
Less than 7	77	35.5/49.6		
7 or Greater	19	39.9/65.9		
Clinical stage:			0.266	
T1c	68	39.2/49.2		
T2 or greater	28	48.0/61.6		
Pathological stage:			0.852	
T2	79	36.9/52.1		
T3	17	29.8/56.3		

* Mann-Whitney U test was used for each category.
† Measured in 94 patients.
‡ Measured in 85 patients.

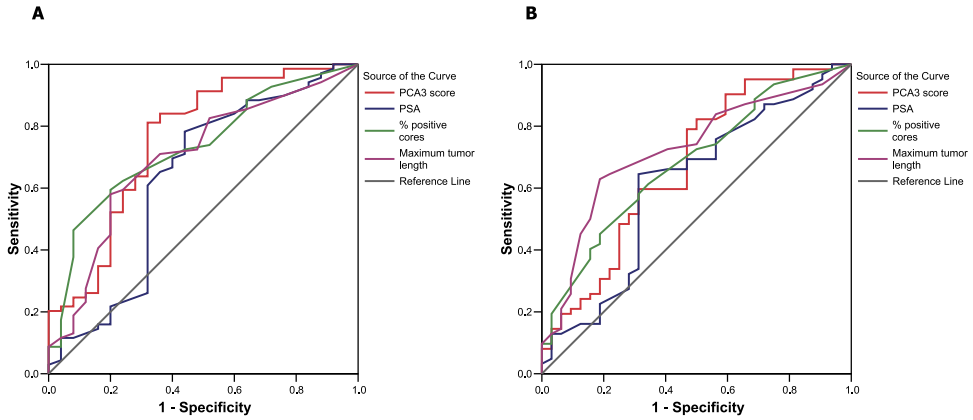
DISCUSSION

In this study we observed an association of PCA3 score with tumor volume as well as prostatectomy Gleason score. Although TRUS prostate volume, biopsy GS, serum PSA and percent positive biopsy cores were also associated with total tumor volume, the PCA3 score was the best predictor of total tumor volume, exceeding the other variables known to associate with tumor volume.[5,14] A comparison of the significant continuous variables identified in this study reveals that the PCA3 score had the biggest AUC to predict low volume cancer, suggesting that the PCA3 score may be useful in selecting men for nonaggressive therapies such as active surveillance. To our knowledge this is the first report to show a significant correlation of PCA3 score with prostate cancer volume in prostatectomy specimens. These findings are particularly important in light of the decreasingly observed association of serum PSA and prostate cancer volume in the modern era.

Several investigators have shown that the novel tumor marker PCA3 can improve specificity in prostate cancer diagnosis compared to serum PSA.[9–13] Marks et al reported the superiority of the urinary PCA3 score to serum PSA in determining the need for a repeat prostate biopsy in men with previous negative biopsies.[12] Using a PCA3 score threshold of 35 they achieved a specificity of 72% with an odds ratio of 3.6. In the current study we focused on the

TABLE 3. *Stepwise multiple logistic regression analysis for prediction of tumor volume less than 0.5 cc using preoperative variables*

	Univariable Analysis		Multivariable Analysis			
	Score	p Value	Regression Coefficient	Odds Ratio	95% CI	p Value
Total tumor vol:						
Age	0.000	0.998	—	—	—	—
Ethnicity (white or black or Hispanic)	4.083	0.130	—	—	—	—
Clinical stage (T1c or T2)	0.124	0.725	—	—	—	—
TRUS prostate vol (cc)	6.384	0.012	0.072	1.075	1.012–1.142	0.018
Biopsy GS (6 or greater)	9.548	0.002	−1.520	0.219	0.048–0.988	0.048
Max tumor length	5.614	0.018	—	—	—	—
% Pos cores	8.895	0.003	−0.061	0.941	0.895–0.990	0.018
Log PSA (ng/ml)	5.590	0.018	−5.584	0.004	0.000–0.173	0.004
Log PCA3 score	17.752	<0.001	−3.818	0.022	0.002–0.218	0.001
Dominant tumor vol:						
Age	0.126	0.722	—		—	—
Ethnicity (white or black or Hispanic)	0.747	0.688	—		—	—
Clinical stage (T1c or T2)	1.467	0.226	—		—	—
TRUS prostate vol (cc)	10.165	0.001	0.087	1.091	1.034–1.151	0.001
Biopsy GS (6 or greater)	7.576	0.006	—		—	—
Max tumor length	8.431	0.004	−0.275	0.760	0.599–0.964	0.024
% Pos cores	7.664	0.006	—		—	—
Log PSA (ng/ml)	4.099	0.043	−4.254	0.014	0.001–0.296	0.006
Log PCA3 score	11.736	0.001	−2.013	0.134	0.026–0.689	0.016



Total Tumor Volume < 0.5 cc

Variables	AUC	Asymptotic significance
PCA3 score	0.757	**<0.001**
PSA	0.632	0.051
% positive cores	0.733	**0.001**
Maximum tumor length	0.700	**0.003**

Dominant Tumor Volume < 0.5 cc

Variables	AUC	Asymptotic Significance
PCA3 score	0.673	**0.006**
PSA	0.612	0.076
% positive cores	0.682	**0.004**
Maximum tumor length	0.723	**<0.001**

Abbreviations: AUC, area under the curve; PSA, prostate specific antigen.

FIG. 2. ROC curve analyses and AUC in 96 prostatectomy patients by (*A*) total tumor volume less than 0.5 cc and (*B*) dominant tumor volume less than 0.5 cc.

correlation of the PCA3 score with pathological features, particularly prostatectomy tumor volume. We chose a PCA3 threshold of 25 to discriminate between total tumor volumes less than 0.5 cc and 0.5 or greater cc based on ROC curve analysis in men already diagnosed with prostate cancer. At this cutoff we observed a diagnostic accuracy of 76% (73/96), with a high odds ratio of 7.3 to predict total tumor volume less than 0.5 cc. Of the men with a PCA3 score of 25 or greater 94% (62/66) had significant cancer (dominant tumor volume 0.5 cc or greater and or Gleason score 7 or greater). It is noteworthy that the PCA3 scores in men with a negative biopsy and in men with low volume/low grade cancer were not significantly different ($p = 0.462$). However, both were significantly different from the PCA3 score in men with significant disease ($p = 0.002$ and 0.007, respectively). These findings suggest that the urinary PCA3 score measurement is potentially an effective tool for determining which men are candidates for active surveillance.

As one would predict based on the biological characteristics of PCA3 (ie over expression in cancer cells),[8,9] the PCA3 score associates better with total tumor volume compared to dominant tumor volume because of a summation effect of PCA3 being over expressed by cancer cells at each tumor focus.

We also observed the absence of a correlation between PCA3 score and serum PSA, although both were significantly correlated with tumor volume. In addition, men who had a tumor volume of 2 cc or more and a serum PSA of 4.0 ng/ml or less had relatively high PCA3 scores. Median and average PCA3 scores were 49.0 and 77.0, respectively (range 21.1 to 162.6 in 7 patients). The PCA3 score may potentially reflect different features or biological characteristics of prostate cancer compared to serum PSA, and is consistent with the independent mechanism of cancer detection, suggesting that it may have usefulness in assessing cancer aggressiveness alone or, more likely, in combination with other known parameters such as in a nomogram.

Previously one of the limitations of the PCA3 score measurement had been the low informative rate. Earlier versions of the test lacked analytical sensitivity in that up to 21% of specimens contained insufficient genetic material for a valid test.[19] In our study recent modifications in the PCA3 assay allowed us to measure the PCA3 score in all samples. Marks et al also reported that 226 of 233 specimens yielded sufficient RNA for analysis, corresponding to an informative specimen rate of 97%.[12] The greater informative rate is likely a result of the current streamlined specimen processing procedure that uses whole, unspun urine instead of urine sediments as reported by Hessels[10] and van Gils[13] et al, as well as improvements in mRNA capture and amplification technology.[11,12]

TABLE 4. *Performance characteristics of PCA3 score 25 as a cutoff to predict tumor volumes less than 0.5 cc in combination with Gleason score*

	Dominant Tumor Vol Less Than 0.5 cc	Total Tumor Vol Less Than 0.5 cc	Dominant Tumor Vol Less Than 0.5 cc With GS 6	Total Tumor Vol Less Than 0.5 cc With GS 6
No.	34	27	11	10
% Sensitivity (No./total No.)	50.0 (17/34)	63.0 (17/27)	63.6 (7/11)	70.0 (7/10)
% Specificity (No./total No.)	79.0 (49/62)	81.2 (56/69)	72.9 (62/85)	73.3 (63/86)
% PPV (No./total No.)	56.7 (17/30)	56.7 (17/30)	23.3 (7/30)	23.3 (7/30)
% NPV (No./total No.)	74.2 (49/66)	84.3 (56/66)	93.9 (62/66)	95.5 (63/66)
% Diagnostic Accuracy (No./total No.)	68.8 (66/96)	76.0 (73/96)	71.9 (69/96)	72.9 (70/96)
Odds ratio	3.8	7.3	4.7	6.4

CONCLUSIONS

The PCA3 score is significantly associated with tumor volume and Gleason score in prostatectomy specimens, suggesting that the urinary PCA3 score may be a novel molecular marker not only for prostate cancer detection, but also for the classification of men diagnosed with prostate cancer. The PCA3 score appears to stratify men based on tumor volume and may have clinical applicability in selecting men who have low volume/low grade cancer as active surveillance candidates.

Abbreviations and Acronyms

DRE	=	digital rectal examination
GS	=	Gleason score
NPV	=	negative predictive value
PCA3	=	prostate cancer gene 3
PPV	=	positive predictive value
PSA	=	prostate specific antigen
TRUS	=	transrectal ultrasound

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EDITORIAL COMMENTS

The PCA3 gene, unlike PSA, is over expressed by prostate cancer cells (60 to 100- fold), giving the gene a relative specificity for this malignancy. A method to quantify the gene in urine has been developed, and the test is commercially available at this time (reference 11 in article). A digital rectal examination performed just before collection of the urine is required to milk prostate cells into the specimen. In clinical studies of the new test urinary PCA3 levels reflected early prostate cancer more accurately than serum PSA levels ($p < 0.01$) (reference 12 in article).

The present report by Nakanishi et al expands on previous PCA3 studies in an important way. Levels of the gene appear to correlate with cancer severity, ie tumor volume and Gleason grade in radical prostatectomy (but not biopsy) specimens, and further, to differentiate significant from insignificant cancers (less than 0.5 cc, Gleason 6 or less). These results must be regarded as preliminary, but if they are validated in definitive trials, PCA3 testing could become an important tool to help us decide not only who should undergo biopsy, but also who should undergo treatment. As compelling as the PCA3 story now appears, in the future test panels of molecular markers including PCA3 and the TMPRSS2-ETS gene fusion products will likely prove to yield more clinical information than single tests.[1]

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Probably the most commonly cited statistic in the prostate cancer literature is the estimated 218,890 new cases and 27,050 deaths in the United States in 2007.[1] Despite these grim statistics, a significant number of men diagnosed with and treated for prostate carcinoma have clinically indolent disease. Studies examining surgical specimens have estimated that between 6% and 27% of patients undergoing radical prostatectomy have tumors similar to those identified incidentally at autopsy,[2] and mathematical models analyzing PSA screening data have estimated the risk of over detection from 27% to 56%.[3] Given that a large number of men are being diagnosed with clinically unimportant tumors, markers to identify these patients would be of clear use.

Is PCA3 that marker? Nakanishi et al clearly raise the possibility. Low PCA3 strongly correlated with smaller tumor size and lower pathological Gleason score, both clinically relevant predictors of tumor aggressiveness. However, while these associations are extremely interesting and encouraging, it remains to be proven that patients with low PCA3 levels can be safely observed. It is likely that some of the pathologically indolent tumors in this study would have progressed if untreated. Analysis of banked serum from watchful waiting trials will be critically important before any marker, including PCA3, can be accepted as a marker of indolent prostate carcinoma.

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2. Humphrey, PA, Keetch DW, Smith DS, Shepherd DL and Catalona WJ: Prospective characterization of pathological features of prostatic carcinomas detected via serum prostate specific antigen based screening. J Urol 1996; **155:** 816.
3. Draisma G, Boer R, Otto SJ, van der Cruijsen IW, Damhuis RA, Schroder FH et al: Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. J Natl Cancer Inst 2003; **95:** 868, 2003.