PCA3 Score Before Radical Prostatectomy Predicts Extracapsular Extension and Tumor Volume

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Purpose: *PCA3* is a prostate specific, nonprotein coding RNA that is over expressed in prostate cancer. Recent studies showed the diagnostic potential of a urine based *PCA3* for predicting biopsy outcome. We assessed the relationship between urine *PCA3* and pathological features in whole mount radical prostatectomy specimens.

Materials and Methods: Post-digital rectal examination urine specimens were obtained from 72 men with prostate cancer before radical prostatectomy. *PCA3* and *PSA* mRNA were measured. The ratio of *PCA3* to *PSA* mRNA was recorded as a *PCA3* score and correlated with data on each prostate specimen.

Results: Patients with extracapsular extension had a significantly higher median *PCA3* score than patients without extracapsular extension (48.8 vs 18.7, $p = 0.02$). *PCA3* score significantly correlated with total tumor volume ($r = 0.38$, $p < 0.01$). On multivariate analysis *PCA3* score was an independent predictor of extracapsular extension ($p = 0.01$) and total tumor volume less than 0.5 cc ($p = 0.04$). At a cutoff *PCA3* score of 47 extracapsular extension was predicted with 94% specificity and an 80% positive predictive value. When combined with serum PSA and biopsy Gleason score, the ROC AUC for predicting extracapsular extension was 0.90.

Conclusions: *PCA3* detected in the post-digital rectal examination urine of patients with prostate cancer correlated with pathological findings. Therefore, it could provide prognostic information. To our knowledge this is the first report of a molecular urine assay that predicts extracapsular extension.

Key Words: prostate; prostatic neoplasms; urine; tumor marker, biological; gene expression

Widespread use of PSA has led to earlier CaP detection but it has fallen short for identifying the extent of disease in an individual. Several nomograms have been developed to stratify the patient risk of nonorgan confined disease at prostatectomy using PSA, biopsy results and DRE findings[1–3] but they lack accuracy. Up to 50% of cases are upgraded at prostatectomy.[4,5] Therefore, recommending active surveillance in patients remains problematic, although many may not require treatment in their lifetime.[2,6] For these reasons biomarkers that can predict pathological stage, grade or tumor volume are desperately needed.

Investigations of novel serum biomarkers for CaP have yielded only slight improvements over PSA.[7–9] A recent focus is moving toward the molecular analysis of exfoliated prostatic epithelial cells from the gland by manipulation. Prostatic secretions can be difficult to obtain but adequate cellular material has been obtained in urine collected after an attentive DRE.[7,10] Of the many urine based molecular assays that are currently being evaluated the measurement of *PCA3* has shown the most promise in several studies.[11,12]

PCA3 is a prostate specific mRNA that is over expressed in most CaP and has high tumor cell specificity.[13–15] *PCA3* does not code for a protein but it can be amplified and quantified from whole urine after DRE.[16] While urine based *PCA3* assays show promise for predicting biopsy results,[11,12] limited data are available to address the possible prognostic value after a diagnosis is made.[17] We correlated the preoperative urine *PCA3* score with pathological prostatectomy features of prognostic significance, specifically stage, grade and tumor volume.

PATIENTS AND METHODS

Institutional review board approval was obtained before the commencement of this study and all patients provided written informed consent. Between September 2006 and November 2007, 72 men volunteered for the study. Patients on medications that affect PSA, eg 5α-reductase inhibitors or herbal medications, were not eligible for study. Urine specimens were obtained before biopsy in 33 men or at least 6 weeks after biopsy in 39 but before prostatectomy. Table 1 lists patient characteristics.

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For another article on a related topic see page 2206.

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TABLE 1. *Preoperative clinical data*

Median age (range)	58 (42–73)
Median ng/ml serum PSA (range)	4.7 (1.0–31.6)
Median cc total tumor vol(range)	0.87 (0.004–14.39)
Median *PCA3* score (range)	25.7 (4.0–269.0)
No. race (%):	
White	54 (75.0)
Black	18 (25.0)
No. clinical stage (%):	
cT1	52 (71.2)
cT2	21 (28.8)
No. GS biopsy grade (%):	
3+3	50 (69.4)
3+4	9 (12.5)
4+3	6 (8.3)
8–9	7 (9.7)

Urine specimens were obtained immediately after DRE, which involved 3 sweeps of the prostate on each lateral lobe. *PCA3* and PSA mRNA were then measured using a DTS® 400 System, as previously described.[11,12,16] *PCA3* mRNA was normalized to *PSA* mRNA in each sample and the ratio was multiplied by 10^3 to obtain the *PCA3* score. This score was correlated with comprehensive pathological data on radical prostatectomy whole mount specimens.

All prostatectomy specimens were processed and analyzed by the same genitourinary pathologists (BF and IS). Specimens were handed to the pathologist in the operating room at Walter Reed Army Medical Center, where they were inked and palpable tumors were incised to assess gross ECE. Specimens were then carried to the Armed Forces Institute of Pathology and processed using the institutional standard whole mount technique. Each prostate was formalin fixed, paraffin embedded and sectioned at 2.2 mm intervals before whole sections were mounted on slides.[18] Tumor histology was graded using the Gleason grading system. All tumors were measured in 3 dimensions and the product was multiplied by 0.4 to estimate volume. This estimation technique was shown to be accurate in previous studies.[19] Total tumor volume was determined by adding the volume of each tumor. Microscopic tumor foci were noted but considered to be of negligible volume in this study.

Spearman rank correlation analysis was used to determine associations among commonly used clinical variables, *PCA3* score and total tumor volume. The median test was performed to determine the association between *PCA3* score and patient clinicopathological characteristics. Stepwise LR was used to identify independent preoperative predictors (urine *PCA3* score, serum PSA, biopsy grade, clinical stage, race, etc) of total tumor volume, characterized as less vs greater than 0.5 cc or ECE. ROC analysis was used to assess prediction results of the univariate and multivariate LR models with $p < 0.05$ considered statistically significant. SAS®, version 9.1.3 was used for all data analysis.

RESULTS

In our patient cohort we observed upgrading in 21 of 72 cases (29.2%) and down grading in 11 (15.3%) of GS from biopsy to prostatectomy specimens. Table 2 lists overall prostatectomy characteristics. Current American Joint Committee on Cancer staging criteria were used to assign a pathological T stage to each specimen. By this commonly used staging system ECE defines pT3 disease.

Table 3 shows stepwise multivariate logistic regression analysis to evaluate the ability of preoperative clinical variables and *PCA3* score to predict ECE. Biopsy grade greater than GS 6, preoperative PSA and *PCA3* score independently predicted ECE. When ROC analysis was performed with these 3 factors combined, the AUC was 0.90. When *PCA3* score was evaluated independently, the AUC was 0.732 and the ROC was noticeably skewed toward greater specificity (see figure). Using a cutoff *PCA3* score of 47 in our series the resulting sensitivity, specificity and accuracy were 57%, 94% and 83%, respectively. Positive and negative predictive values using this cutoff were 80% and 84%, respectively. Of 47 patients with biopsy GS 6 and PSA less than 10 ng/ml ECE was seen in the prostatectomy specimens of 6 (13%). This finding was correctly predicted by the *PCA3* score in 4 of the 6 cases (67%) with a cutoff score of 47. Results in only 3 of 47 cases were falsely positive in this subset.

Spearman analysis showed that *PCA3* score significantly correlated with total tumor volume in 72 patients ($r = 0.38$, $p < 0.01$). Patients with a larger tumor volume of greater than 2.0 cc had a significantly higher *PCA3* score (median 47.6, range 7.5 to 269.0) than patients with a smaller tumor volume of 0.5 to 2.0 cc (median 17.5) and less than 0.5 cc (median 18.7) ($p = 0.01$). Clinical stage, preoperative PSA and *PCA3* score were independent predictors of a total tumor volume of less than 0.5 cc ($p = 0.04$, table 4).

DISCUSSION

The 44.4% rate of grade migration (32 of 72 cases) in this series highlights one of the major challenges of treating CaP today. There is an urgent need for markers to better predict pathological stage and the significance of disease in CaP. It would be valuable to have a marker that accurately predicts ECE and/or low volume disease in patients with otherwise low risk disease by biopsy GS and preoperative serum PSA. In these patients a predictive marker would affect decisions about lymph node dissection with prostatectomy and be invaluable for contemplating active surveillance. The data presented show that *PCA3* may be useful in this regard. It is important to confirm these results in larger scale multicenter studies.

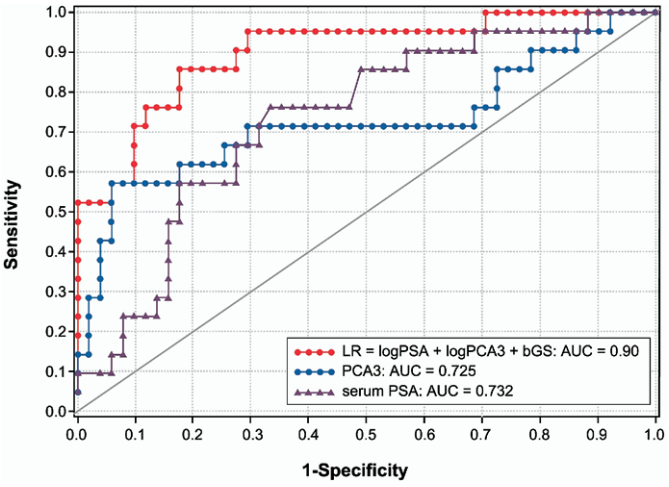
The figure shows the most remarkable result of this study. The AUC of the combination of serum PSA, biopsy GS and *PCA3* score shows that this new assay works synergistically with established prognostic tools. Individually the *PCA3* score does not have an overall advantage over serum PSA for predicting ECE but it adds specificity, which greatly

TABLE 2. *Pathological data*

	No. Pts (%)
Pathological T stage:	
pT2	51 (70.9)
pT3a	15 (20.8)
pT3b	6 (8.3)
GS prostatectomy grade:	
3+3	42 (58.3)
3+4	20 (27.8)
4+3	3 (4.1)
8–9	7 (9.7)
Total tumor vol(cc):	
Less than 0.5	28 (38.9)
0.5–2.0	25 (34.7)
Greater than 2.0	19 (26.4)

TABLE 3. *Stepwise multivariate LR analysis to predict ECE using preoperative variables in 72 patients*

Variable	Univariate		Stepwise Multivariate	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Age	0.985 (0.919–1.087)	0.68	—	—
Race (black vs white)	3.500 (1.136–10.779)	0.02	4.517 (0.720–28.352)	0.10
Clinical stage (cT2 vs cT1)	2.438 (0.828–7.176)	0.10	4.621 (0.855–24.980)	0.07
Biopsy grade (6 vs greater than 6)	15.710 (4.556–54.172)	<0.01	10.227 (2.145–48.762)	<0.01
Log PSA	5.096 (1.577–16.471)	<0.01	7.863 (1.406–43.987)	0.01
Log *PCA3* score	3.440 (1.519–7.795)	<0.01	4.155 (1.320–13.077)	0.01



ROC for predicting ECE by preoperative serum PSA, *PCA3* score, and combined PSA, *PCA3* score and Gleason score 6 or greater than 6 (red). *LR*, logistic regression. *bGS*, biopsy Gleason score.

improves the predictive ability when combined. Patients with a biopsy GS of less than 6, low PSA and a *PCA3* score of less than 47 would be excellent candidates for active surveillance in this cohort. The additional correlation with tumor volume makes *PCA3* score an excellent potential marker for following patients on active surveillance. If the *PCA3* score were to increase while on surveillance, patients would then require treatment.

Nakanishi et al recently reported that *PCA3* score correlates with tumor volume as well as prostatectomy grade but they did not find a correlation with pathological stage.[17] This inconsistency may be explained by differences among subject groups or in the pathological evaluation of specimens. We found an increased frequency of pT3 disease in our cohort than they found in their series (21 of 72 cases or 29.2% vs 17 of 96 or 17.7%), which could have been a result of increased detection from analyzing the prostate in whole mounted sections with smaller intervals (2.2 vs 4 mm). The other finding that varied was a correlation with prostatectomy grade. In this study the median *PCA3* score for prostatectomy GS 6 was less than the median score for GS greater than 6 but the difference was not significant (21.3 vs 30.8, p = 0.22). A larger study could better differentiate the data. It is interesting to note that recent evidence correlated preoperative tumor volume, as estimated from biopsy results, with prostatectomy grade.[4] Another aspect of this population that differs from populations in other series was the relatively high percent of black men (25%). There are interesting new findings supporting different genetic changes in this group and studies are under way to perform subset analyses to evaluate possible differences.[20]

Previous studies have indicated that in a pre-biopsy population a *PCA3* score cutoff of 35 provides an optimal balance of sensitivity and specificity for predicting CaP vs no CaP.[11,12] In this pre-prostatectomy cohort the mean tumor volume in patients with a *PCA3* score of greater than 35 was 1.1 cc (median 0.56). Of these 48 patients 23 (47.9%) had a tumor volume of less than 0.5 cc and 42 (87.5%) had a tumor volume of less than 2.0 cc. In contrast, 19 of 24 patients (79.2%) with a *PCA3* score of greater than 35 had a tumor volume of greater than 0.5 cc and 13 (66.7%) had a tumor volume of greater than 2.0 cc (mean 3.0, median 2.09). For a *PCA3* score of less vs greater than 35 the difference in the percent of patients with a tumor volume of less than 0.5 cc was significant (p = 0.02). Determining the optimal *PCA3* score cutoff point to predict prognosis, ie stratifying patients for active surveillance, is under study. It is also worth noting that for diagnostic or prognostic applications maximal predictive accuracy would likely be achieved by using *PCA3* score as a continuous (vs dichotomous) variable in combination with other clinical and/or pathological data.

The theory behind the correlation of *PCA3* detected in urine and tumor volume is based on the premise that cells from larger tumors are more likely to exfoliate into the prostatic ducts. These tumor cells are dislodged during DRE and expelled in first catch urine. One could also postulate that a tumor with a worse histological grade might shed cells more readily. It is difficult to understand how invasion through the prostatic capsule can be related to increased

TABLE 4. *Stepwise multivariate LR analysis to predict total tumor volume less than 0.5 cc stage using preoperative variables in 72 patients*

Variable	Univariate		Stepwise Multivariate	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Age	1.012 (0.948–1.080)	0.73	—	—
Race (black vs white)	1.000 (0.335–2.987)	1.00	—	—
Clinical stage (cT2 vs cT1)	3.778 (1.115–12.797)	0.03	12.253 (2.181–68.835)	<0.01
Biopsy grade (6 vs greater than 6)	6.333 (1.662–24.137)	<0.01	—	—
Log PSA	3.339 (1.323–8.425)	0.01	5.547 (1.748–17.596)	<0.01
Log *PCA3* score	2.064 (1.063–4.008)	0.03	2.222 (1.022–4.830)	0.04

gene expression or exfoliation of cells. There could be a common cellular mechanism or this finding could be related to the anatomical location of the tumor near the capsule, such that direct manipulation by DRE releases cells more efficiently. The correlation could also be explained by tumor volume affecting *PCA3* score and ECE.

CONCLUSIONS

PCA3 score in post-DRE urine of patients with CaP was a strong independent predictor of ECE that functioned synergistically with other clinical information. We also confirmed previous results showing a correlation between *PCA3* score and tumor volume. Therefore, the *PCA3* urine test has the potential to provide valuable prognostic information. Further studies of the value of this assay in various populations are currently under way.

Abbreviations and Acronyms

CaP	=	prostate cancer
ECE	=	extracapsular extension
DRE	=	digital rectal examination
GS	=	Gleason score
LR	=	logistic regression
PCA3	=	CaP gene 3
PSA	=	prostate specific antigen

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EDITORIAL COMMENTS

Despite changes in biopsy strategies there is significant upgrading at radical prostatectomy, as observed in this study. The fear of missed high grade cancer is the single most limiting factor for the adoption of watchful waiting for low risk cancer. The *PCA3* molecular urine test provides an additional independent predictor of pathological grade and stage. When combined with serum PSA and biopsy Gleason score, it increased diagnostic accuracy to 90% (ROC AUC 0.9) to predict ECE or clinically low volume cancer. These results confirm those recently published showing a low *PCA3* score for clinically nonsignificant cancer and a high score for Gleason 7 or greater disease (reference 17 in article). The current study also suggests that the *PCA3* score could be a factor in addition to those popularized by D'Amico et al to assess cancer risk (reference 1 in article). While initial studies suggested a cutoff value to assess the risk of positive biopsies, it appears that for diagnostic and prognos-

tic determination using the full range of *PCA3* scores as a continuous value would be much more useful.

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PCA3 gene expression separates benign prostate from CaP with an accuracy approaching 100% at the tissue level.[1] The gross over expression of *PCA3* by cancer cells is the basis for a urinary CaP test to quantify the amplified gene in urine samples (references 12 and 16 in article). In an era when the limitations of PSA testing are increasingly apparent, the specificity of the *PCA3* gene for CaP cancer has created considerable interest.

In this latest addition to the *PCA3* story these authors performed the test in 72 men with CaP before radical prostatectomy. The *PCA3* score correlated with final tumor volume ($r = 0.38$, $p < 0.01$) and it was also an independent predictor of ECE. Independently a *PCA3* score of greater than 47 predicted ECE with 94% specificity and 80% positive predictive value. Remarkably when the *PCA3* score was combined with preoperative PSA and biopsy Gleason score, the ROC AUC for predicting ECE was approximately 90%. Thus, *PCA3* expression appears to function synergistically with other clinical information.

However, the current data raise questions about PCA3. What is normal and what is abnormal? To date a *PCA3* score of 35 has been considered the optimal cutoff but these pathological data, which were obtained without regard to the *PCA3* level, suggest otherwise. The median *PCA3* score in men undergoing radical prostatectomy was 25, ie half of the patients had a score below that level. Do these low scores equate to falsely negative tests or do men with a low score have insignificant cancer, as suggested by Nakanishi et al (reference 17 in article)? Perhaps *PCA3* scores should not be viewed in terms of positive or negative, but rather as a gradation of risk. What regulates this peculiar noncoding stretch of mRNA? How should *PCA3* scores be integrated with other information, especially other molecular markers, to make treatment decisions? A lot remains to be learned about testing with this promising new marker, which at the tissue level is almost completely specific for CaP.

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REPLY BY AUTHORS

We highlight a promising prognostic use of the urine *PCA3* assay. Marks points out an important issue with respect to relatively lower *PCA3* scores in a significant fraction of our patients with prostate cancer. We also are intrigued by this observation, and at this time it is unclear whether it is due to our patient cohort, tumor biology or other technical issues. However, we did identify some uniqueness in our study population such as a higher percentage of black men and the fact that men on active duty are screened at age 40 years. Marks makes a salient observation when he suggests that "Perhaps *PCA3* scores should not be viewed in terms of positive or negative, but rather as a gradation of risk." This concept of *PCA3* score as a continous variable was also mentioned by Fradet. We look forward to future studies of *PCA3* in different cohorts and collaboration with other investigators to further elucidate the usefulness of this latest marker in prostate cancer.