PCA3: A Molecular Urine Assay for Predicting Prostate Biopsy Outcome

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Purpose: A urinary assay for PCA3, an mRNA that is highly over expressed in prostate cancer cells, has shown usefulness as a diagnostic test for this common malignancy. We further characterized PCA3 performance in different groups of men and determined whether the PCA3 score could synergize with other clinical information to predict biopsy outcome.

Materials and Methods: Prospectively urine was collected following standardized digital rectal examination in 570 men immediately before prostate biopsy. Urinary PCA3 mRNA levels were quantified and then normalized to the amount of prostate derived RNA to generate a PCA3 score.

Results: The percent of biopsy positive men identified increased directly with the PCA3 score. PCA3 assay performance was equivalent in the first vs previous negative biopsy groups with an area under the ROC curve of 0.70 and 0.68, respectively. Unlike serum prostate specific antigen the PCA3 score did not increase with prostate volume. PCA3 assay sensitivity and specificity were equivalent at serum prostate specific antigen less than 4, 4 to 10 and more than 10 ng/ml. A logistic regression algorithm using PCA3, serum prostate specific antigen, prostate volume and digital rectal examination result increased the AUC from 0.69 for PCA3 alone to 0.75 ($p = 0.0002$).

Conclusions: PCA3 is independent of prostate volume, serum prostate specific antigen level and the number of prior biopsies. The quantitative PCA3 score correlated with the probability of positive biopsy. Logistic regression results suggest that the PCA3 score could be incorporated into a nomogram for improved prediction of biopsy outcome. The results of this study provide further evidence that PCA3 is a useful adjunct to current methods for prostate cancer diagnosis.

Key Words: prostate; prostatic neoplasms; prostate cancer antigen 3, human; tumor markers, biological; diagnosis

The current standard for the diagnosis of CaP consists of a serum test for PSA and DRE. Serum PSA levels above 2.5 to 4 ng/ml and/or abnormalities felt during DRE may indicate the presence of CaP, although the positive predictive value of these methods is only 24% to 37%.[1,2] Noncancerous conditions such as prostatitis and BPH can cause an increase in serum PSA, resulting in a high false-positive rate relative to prostate biopsy.[3–5] In addition, the widespread use of the serum PSA test has led to an increase in the number of biopsies performed each year, of which many are negative for cancer.[6]

Several derivatives of the serum PSA assay have been used to improve accuracy for predicting the biopsy outcome. For example, the ratio of uncomplexed PSA (free PSA) to total PSA is often used to improve the specificity of cancer detection in patients with serum PSA 4 to 10 ng/ml.[7] The rate of change of serum PSA with time (PSA velocity) is a method of using longitudinal information in the diagnosis of CaP.[8] Another kallikrein, human kallikrein 2, has also been shown to improve cancer discrimination and it may be associated with extracapsular extension and biochemical recurrence.[9] While these surrogate markers have been shown to improve CaP diagnosis, there is an unmet need for more cancer specific tests.

PCA3 is a new molecular marker that has shown promise for improving the diagnosis of CaP.[10–13] PCA3 is a prostate specific noncoding mRNA that is over expressed 60 to 100-fold in more than 90% of prostate tumors compared to that in benign prostatic tissue.[10,14,15] The over expression of PCA3 mRNA can be quantified and expressed relative to a prostate specific gene that is not altered in expression in cancer cells.[10,16] We previously described a quantitative molecular urine assay that uses PSA gene expression to nor-

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malize PCA3 mRNA levels to generate a PCA3 score.[17] In several studies the urine test for PCA3 was found to be superior to the serum PSA test for predicting the outcome in men undergoing first or repeat biopsy.[11,13,16]

In this research study we assessed the performance of the PCA3 assay in men scheduled for prostate biopsy. The PCA3 score was investigated with respect to prostate volume, serum PSA and biopsy result. The ability of the PCA3 score to synergize with other clinical information to predict biopsy outcome was also examined.

MATERIALS AND METHODS

Specimen Collection and Processing

The study was prospective, involving 570 consecutive men scheduled for prostate biopsy due to serum PSA 2.5 ng/ml or greater, abnormal DRE, a family history of CaP or other risk factors. Subjects were enrolled at 4 North American sites, including Université Laval, Quebec City, Quebec, Canada; Urological Sciences Research Foundation, Los Angeles, California; University of Washington, Seattle, Washington; and Johns Hopkins Medical Institutions, Baltimore, Maryland. A consent form approved by the institutional review board at each site was signed by all participants.

Urine specimens were collected between April 2004 and May 2006. Serum PSA testing was performed at each site an average of 4 months before urine specimen collection. Of the patients 77% underwent PSA testing using the assay of a single manufacturer (Roche, Nutley, New Jersey). An assay of other manufacturers was used at some test sites. Approximately 18% of specimens were tested using the Hybritech® test and approximately 5% were tested using the Tosoh (Tosoh, South San Francisco, California) test. Biopsies consisting of at least 10 cores were performed by urologists within 6 months of urine specimen collection and prostate volume measured by transrectal ultrasound was recorded during biopsy. The pathologists at each institution who examined the biopsy cores were blinded to PCA3 score results.

Urine samples (20 to 30 ml first catch) were collected after DRE by a urologist. DRE was performed by applying firm pressure (enough to depress the prostate surface 0.5 to 1 cm) from base to apex and from the lateral to the median line for each lobe using 3 strokes per lobe. Urine samples were processed within 4 hours by mixing with an equal volume of detergent based stabilization buffer, which lyses the cells and stabilizes the RNA. Processed specimens were stored at –70C until tested.

PCA3 Assay Procedure

PCA3 and PSA mRNA levels were quantified as previously described using the PROGENSA™ PCA3 assay.[17] Briefly, target mRNA was isolated from whole urine samples by capture onto magnetic microparticles coated with sequence specific oligonucleotides. Captured mRNA was amplified by transcription mediated amplification and detected with chemiluminescent DNA probes. PCA3 and PSA mRNA copy levels were calculated based on transcript calibrators. PSA mRNA levels were used to normalize PCA3 to the total amount of prostate RNA present in the sample and ensure that the RNA yield was sufficient for analysis. The PCA3 score was calculated using the equation, [PCA3 mRNA]/[PSA mRNA] $\times$ 1,000. As previously described, the interassay coefficient of variation for the PCA3 score is 15% to 24%.[17]

Statistical Analysis

Linear regression analysis was performed to identify a relationship between serum PSA or the PCA3 score and prostate gland volume. The variables were log transformed before analysis. The slope of the regression line was calculated by least squares estimation. To determine the presence of any significant trend statistical inference on the slope was done using the t test.

The effect of the biopsy result on the PCA3 score was investigated using ANOVA. Four comparisons were made, including inflammation vs no pathological findings, ASAP/PIN vs no pathological findings, cancer vs no pathological findings and cancer vs ASAP/PIN. The resulting p values were adjusted using the Tukey-Kramer test to protect against the inflated familywise error due to multiple comparisons.

Logistic Regression Models

LR was used for the multivariate prediction model. The binary response variable was the presence or absence of CaP on biopsy. The independent variables were PCA3 score, serum PSA, suspicious DRE, patient age and prostate gland volume. PCA3 score and serum PSA were log transformed to decrease deviance. LR models were evaluated by a 4-fold cross-validation method. The complete data set of 553 subjects with no missing values for the 5 independent variables was randomly divided into 4 groups of equal size and equal prevalence using a block randomization scheme. Three of the 4 groups were then used as a training set to develop an LR model. The trained model was subsequently tested on the remaining group. Permutations were made 4 times to obtain prediction results for all 4 groups. Prediction results of the cross-validated LR model were assessed using ROC analysis. To obtain the best LR model in terms of the area under the ROC curve all combinations of 5 independent variables were considered and compared using a nonparametric method.[18]

RESULTS

Table 1 lists the characteristics of the 570 men in the study. Overall 206 biopsy specimens (36%) were found to show CaP. Figure 1 shows the relationship between PCA3 score and biopsy result. The probability of a cancer positive prostate biopsy increased continuously as the PCA3 score increased. Men with a PCA3 score of less than 5 showed a positive biopsy rate of 14%, whereas 69% with a PCA3 score of greater than 100 were biopsy positive. The data demonstrated a direct correlation between the quantitative PCA3 score and the probability of a positive prostate biopsy.

Using ROC analysis we compared the performance of the PCA3 score between men undergoing first biopsy and men who had at least 1 previous negative biopsy (fig. 2). The AUC in the 277 men undergoing the first biopsy was 0.703, whereas the AUC in the 280 men undergoing repeat biopsy was 0.684. These data demonstrated that PCA3 diagnostic accuracy does not depend on whether the individual has a first biopsy or a repeat biopsy.

TABLE 1. *Patient characteristics*

No. pts	570	
No. biopsy pos (%)	206	(36)
Mean/median age (range)	64/64	(32–89)
Mean/median (cc) prostate vol (range)	45/40	(5–225)
Serum PSA (ng/ml):		
Mean/median (range)	7.8/5.6	(0.3–484)
No. less than 2.5 (%)	54	(9.5)
No. 2.5–4.0 (%)	77	(13.5)
No. 4.0–10 (%)	346	(60.7)
No. greater than 10 (%)	86	(15.1)
Not available	7	(1.2)
No. DRE result (%):		
Normal	455	(79.8)
Abnormal	86	(15.1)
Not available	29	(5.1)
No. Gleason score (%):		
6 or Less	111	(54.9)
7	60	(29.1)
8–10	25	(12.1)
Not available	10	(4.9)
No. ethnicity (%):		
White	470	(82.5)
Black	30	(5.3)
Hispanic	13	(2.3)
Asian	2	(0.4)
Other or not available	55	(9.6)

We next examined the distribution of PCA3 score relative to several histological diagnoses. The diagnoses of no pathological condition, including BPH, and inflammation resulted in the lowest PCA3 scores with a median value of 15 and 13 (mean 30 and 24, respectively) (fig. 3). A slight increase in the PCA3 score was observed for the histological diagnoses ASAP, PIN or ASAP and PIN together. The median PCA3 Score was 27 for ASAP, 24 for PIN and 23 for ASAP plus PIN (mean 33, 35 and 26, respectively). The diagnosis of CaP resulted in the highest median PCA3 score of 38 (mean 63). Results were similar when comparing men undergoing first biopsy vs those undergoing repeat biopsy (data not shown). Using ANOVA the cancer group was significantly different than the no pathological findings group (raw and adjusted $p < 0.0001$). The cancer group was also significantly different than the ASAP, PIN and ASAP plus PIN groups combined (raw and adjusted $p < 0.0001$). PCA3 scores for Gleason score 6 vs 7 or greater were not significantly different. Median PCA3 scores for Gleason scores 6 and 7+ were 38 and 41 (mean 61 and 69, respectively). These data indicate that the PCA3 score distinguishes between cancer and other histological diagnoses of the prostate.

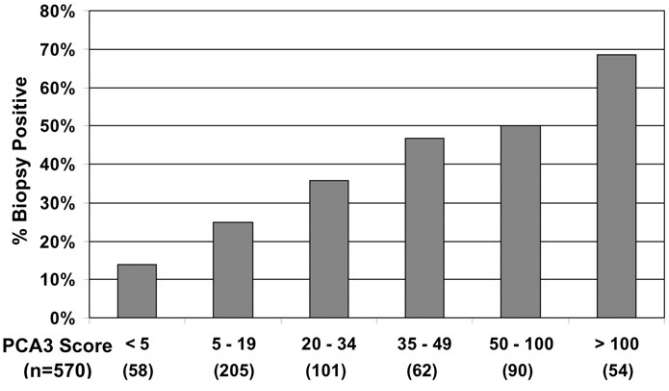

FIG. 1. Percent of men with positive biopsy by PCA3 score. Values in parentheses indicate sample size.

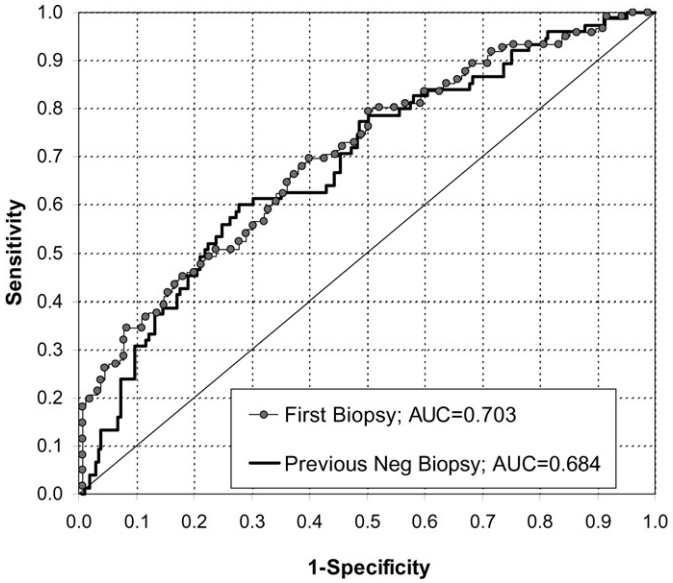

FIG. 2. ROC curve analysis in 277 men undergoing first biopsy and in 280 with previous negative biopsy. ROC curve shows plot of sensitivity as function of false-positive rate (1 − specificity). PCA3 score was analyzed with prostate biopsy used as comparison method.

Of the 570 men in the study population PV data were obtained on 552. As expected, men with increased PV had significantly higher serum PSA ($p < 0.0001$, fig. 4, *A*). Men with a PV of less than 30 cc had a median PSA of 4.8 ng/ml, men with a PV of between 30 and 50 cc had a median PSA of 5.4 ng/ml and men with a PV of greater than 50 cc had a median PSA of 7.0 ng/ml. The same trend was seen in biopsy negative and positive men (data not shown). In contrast, PCA3 score did not correlate with PV ($p = 0.54$). The median PCA3 score in men with a PV of less than 30, 30 to 50 and greater than 50 cc was 25, 21 and 23, respectively (fig. 4, *B*). These data indicate that, unlike serum PSA, the PCA3 score is not significantly affected by PV.

To examine the performance of the PCA3 assay between groups of men for which serum PSA has varying usefulness

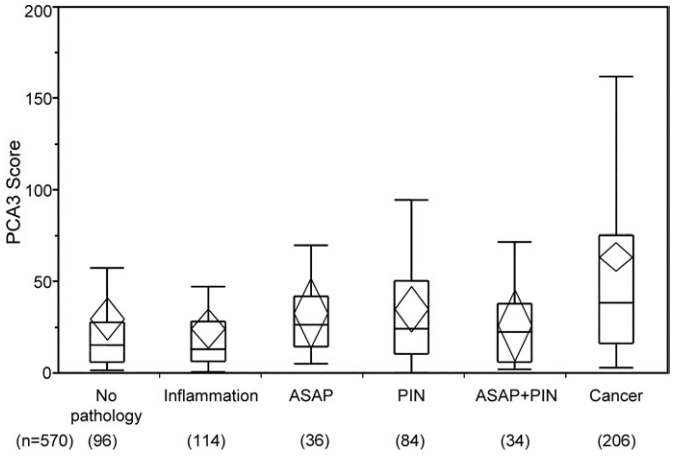

FIG. 3. PCA3 score by biopsy result. Boxes indicate 25th and 75th quartiles. Vertical lines indicate 1.5 × IQR. Horizontal line in box represents median. Sides of diamonds represent mean. Top and bottom of diamonds represent 95% CI. Values in parentheses indicate sample size.

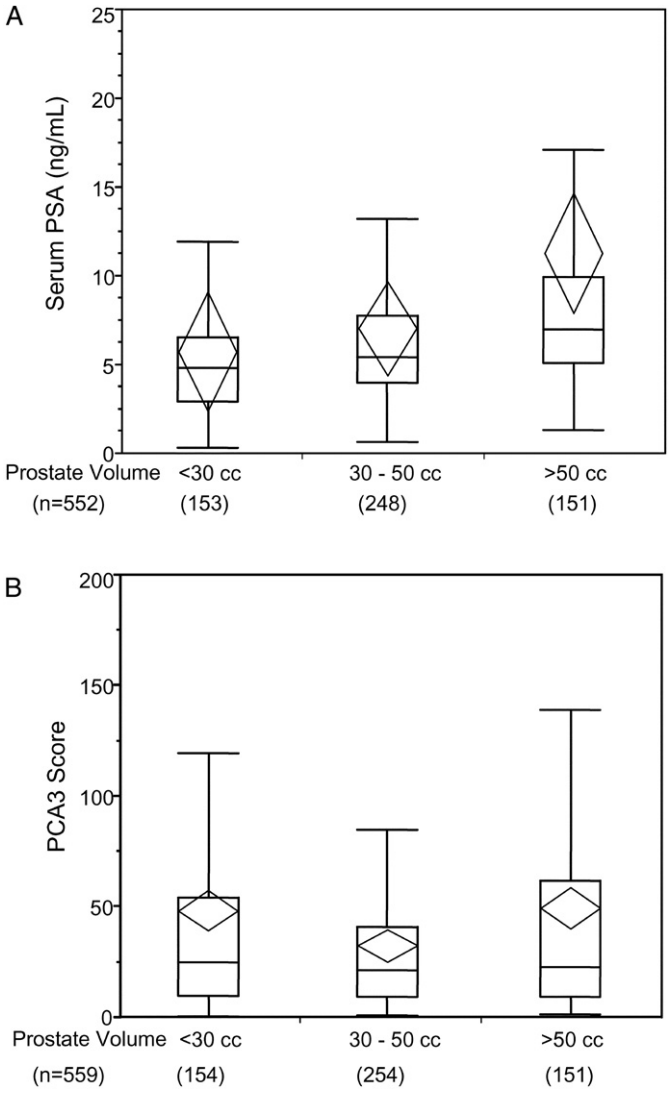

FIG. 4. *A*, serum PSA by PV. *B*, PCA3 score by PV. Boxes indicate 25th and 75th quartiles. Vertical lines indicate 1.5 × IQR. Horizontal lines in box represents median. Sides of diamonds represent mean. Top and bottom of diamonds represent 95% CI. Values in parentheses indicate sample size.

we compared PCA3 assay sensitivity and specificity in men with different serum PSA levels (less than 4, 4 to 10 and more than 10 ng/ml). In the overall subject population a PCA3 score cutoff of 35 yielded 54% sensitivity and 74% specificity (table 2). Table 3 lists sensitivity and specificity values at a range of cutoffs. For the different serum PSA ranges PCA3 sensitivity was between 50% and 61%, and specificity was between 71% and 80% (table 2). The 95% CIs at each level overlapped well, indicating that the diagnostic accuracy of the PCA3 assay is similar at all serum PSA levels.

TABLE 2. *PCA3 assay performance at different serum PSA levels*

Serum PSA (ng/ml)	No. Pts (% biopsy pos)	PCA3 Assay (95% CI)*	
		% Sensitivity	% Specificity
Less than 4	131 (26)	50 (36–63)	77 (73–82)
Greater than 4–10	346 (38)	53 (46–59)	71 (67–75)
Greater than 10	86 (48)	61 (50–69)	80 (70–88)
Overall	563 (37)	54 (49–59)	74 (71–77)

* Sensitivity and specificity at a PCA3 score cutoff of 35.

TABLE 3. *PCA3 assay sensitivity and specificity at various cutoffs*

PCA3 Cutoff	% Sensitivity	% Specificity
5	96	14
10	85	32
15	77	47
20	71	56
25	63	61
30	56	70
35	54	74
40	49	78
45	44	82
50	40	83
55	38	86
60	33	89
65	32	91
70	26	92
75	25	94
80	22	94
85	22	94
90	20	95
95	18	95
100	18	95

To evaluate the potential of the PCA3 score to synergize with other diagnostic indicators of CaP and improve predictive accuracy we combined several variables using LR. After 4-fold cross-validation the incorporation of 4 of 5 variables, that is PV, DRE result, log[serum PSA] and log[PCA3 score], resulted in the greatest diagnostic accuracy. The AUC was 0.752 compared to 0.686 for the PCA3 score (fig. 5). The increase in the AUC in the LR model was strongly significant ($p = 0.0002$). In comparison, the LR model with the PCA3 score removed (PV, DRE and log[serum PSA]) resulted in an AUC of 0.672. The serum PSA test showed less diagnostic accuracy in this data set with an AUC of 0.547, although it should be noted that serum PSA performance in the subset of men undergoing first biopsy was slightly improved with an AUC of 0.618 (data not shown). Together these results suggest that the PCA3 score could be combined

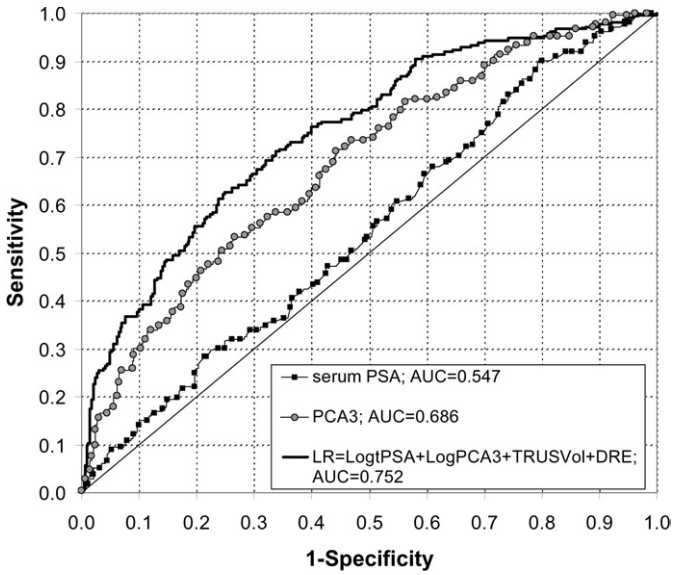

FIG. 5. ROC curve analysis of serum PSA, PCA3 score and LR algorithm in 553 men. LR incorporated terms log(PCA3 score), log(PSA), prostate volume on transrectal ultrasound (*TRUSVol*) and DRE result.

with other factors for an improved prediction of biopsy outcome.

DISCUSSION

Although the currently used diagnostic indicators of CaP are useful in aiding the diagnosis of CaP, there is much room for improvement. An increase in the serum concentration of PSA is not a specific indication of CaP since it could be a symptom of other prostatic conditions, such as BPH.[3,4] Furthermore, men with increased serum PSA and negative biopsy represent a dilemma to physicians because serum PSA has lost usefulness in those men. The use of the free PSA test has provided increased specificity (20%) in men in the 4 to 10 ng/ml PSA range.[7] Despite this improvement many unnecessary biopsies could be avoided using a more specific test.

The PCA3 molecular urine assay provides a method for the direct detection of CaP cells that is completely independent of blood based tests for surrogate markers. In this study PCA3 diagnostic accuracy was significantly greater than that of serum PSA. Unlike the serum PSA test, which can be confounded by an enlarged prostate, the PCA3 score was not influenced by PV.[19] Furthermore, PCA3 sensitivity and specificity were similar at all serum PSA levels. PCA3 testing may have the most immediate usefulness in patients with increased PSA and a previous negative biopsy.[13] The data presented indicate that PCA3 has equivalent diagnostic accuracy when applied before a first biopsy. Together these results suggest that PCA3 may be a more specific diagnostic test and a useful adjunct to the serum PSA test.

Importantly we found that, as the PCA3 score increased, the frequency of prostate cancer detected by biopsy also increased.[13,16] Therefore, PCA3 may be useful for helping to stratify patients according to the risk of a positive biopsy. Previous studies have shown that a combination of factors can enhance diagnostic accuracy compared to each single factor alone[2,20] and LR analysis showed that PCA3 has the potential to add value using this type of approach.

The main limitation of the current study was that it focused on a high risk pre-screened population. More than 75% of the patients had PSA more than 4 ng/ml and more than half were undergoing repeat biopsy. As such, the usefulness of PCA3 in a screening setting cannot be extrapolated from the data presented, although it should be noted that PCA3 diagnostic accuracy was still greater than serum PSA in men undergoing first biopsy (AUC 0.703 vs 0.618). Future studies will incorporate other information, including free PSA and PSA isoforms,[9] into a risk calculator or nomogram with the goal of providing physicians with useful information to guide decisions to biopsy the prostate.

CONCLUSIONS

In this study the urinary PCA3 assay improved diagnostic accuracy for CaP detection. The quantitative PCA3 score was found to be directly related to the probability of positive biopsy. The test performed similarly regardless of serum PSA level and the PCA3 score was not influenced by PV or by whether the patient had undergone previous negative biopsy. In addition to other prostate markers and patient data, the PCA3 test can provide valuable information to guide physicians in the diagnosis of CaP.

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Abbreviations and Acronyms

ASAP	=	atypical small acinar proliferation
BPH	=	benign prostatic hyperplasia
CaP	=	prostate cancer
DRE	=	digital rectal examination
LR	=	logistic regression
PCA3	=	prostate cancer gene 3
PIN	=	prostatic intraepithelial neoplasia
PSA	=	prostate specific antigen
PV	=	prostate volume

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