# PCA3 Molecular Urine Assay for Prostate Cancer in Men Undergoing Repeat Biopsy

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**OBJECTIVES** Men with elevated serum prostate-specific antigen (PSA) levels and negative prostate biopsy findings present a dilemma because of the lack of an accurate diagnostic test. We evaluated the potential utility of the investigational prostate cancer gene 3 (PCA3) urine assay to predict the repeat biopsy outcome.

**METHODS** Urine was collected after digital rectal examination (three strokes per lobe) from 233 men with serum PSA levels persistently 2.5 ng/mL or greater and at least one previous negative biopsy. The specimens were collected from April 2004 to January 2006. The PCA3 scores were determined using a highly sensitive quantitative assay with transcription-mediated amplification. The ability of the PCA3 score to predict the biopsy outcome was assessed and compared with the serum PSA levels.

**RESULTS** The RNA yield was adequate for analysis in the urine samples from 226 of 233 men (ie, the informative specimen rate was 97%). Repeat biopsy revealed prostate cancer in 60 (27%) of the of 226 remaining subjects. Receiver operating characteristic curve analysis yielded an area under the curve of 0.68 for the PCA3 score. In contrast, the area under the curve for serum PSA was 0.52. Using a PCA3 score cutoff of 35, the assay sensitivity was 58% and specificity 72%, with an odds ratio of 3.6. At PCA3 scores of less than 5, only 12% of men had prostate cancer on repeat biopsy; at PCA3 scores greater than 100, the risk of positive biopsy was 50%.

**CONCLUSIONS** In men undergoing repeat prostate biopsy to rule out cancer, the urinary PCA3 score was superior to serum PSA determination for predicting the biopsy outcome. The high specificity and informative rate suggest that the PCA3 assay could have an important role in prostate cancer diagnosis. UROLOGY 69: 532–535, 2007. © 2007 Elsevier Inc.

A specific test for prostate cancer (CaP) is not currently available. Serum prostate-specific antigen (PSA) levels have been widely used for diagnostic purposes for more than 25 years, but false-positive and false-negative results are commonplace.[1] When prostate biopsy is performed irrespective of the serum PSA level, CaP can be found regardless of the PSA result. Thompson and colleagues[2] have concluded that no specific PSA level can accurately separate men with CaP from men with only benign prostatic hyperplasia (BPH). In addition, a large population of men with false-positive serum PSA values has now emerged (ie, the PSA level is elevated for non-CaP reasons such as BPH). These men are at risk of developing clinically significant CaP as they age, but methods such as the PSA velocity and free PSA may not allow for effective treatment of these patients. Consequently, many men with negative biopsy findings undergo repeat biopsies to rule out CaP. A call for CaP-specific markers has been issued.[3]

Prostate cancer gene 3 (PCA3), a gene closely and specifically associated with CaP, could be such a marker. First described by Bussemakers and colleagues[4] in 1999, PCA3 encodes a prostate-specific mRNA that is highly overexpressed in CaP tissue compared with benign prostatic tissue.[5] The possible use of urinary PCA3 as a CaP marker was suggested by de Kok *et al.*[6] in 2002. The potential value of urinary PCA3 mRNA testing has been demonstrated in first-generation, semiquantitative research assays.[5,7,8]

On the basis of this preliminary evidence, Groskopf and colleagues[9] developed an investigational PCA3 urinary assay with the potential for general use in clinical

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settings. This PCA3 assay has been shown to be quantitative, sensitive, and relatively quick and easy to use compared with the earlier versions.[9] We explored the potential utility of the PCA3 assay in an especially problematic group of men, those with elevated serum PSA levels but negative prostate biopsy findings.

## MATERIAL AND METHODS

The urine samples were obtained from three North American sites: Laval University (Quebec City, Quebec, Canada), Urological Sciences Research Foundation (Los Angeles, Calif), and the University of Washington School of Medicine (Seattle, Wash). The respective institutional review boards approved the study protocol, and all study subjects provided written informed consent. The specimens were collected from April 2004 to January 2006.

The study population consisted of 233 consecutive men with serum PSA levels of 2.5 ng/mL or greater who had a history of at least one negative biopsy documented by the study site investigator and who had been scheduled for a follow-up biopsy. The average patient age ± SD was 64 ± 7 years (median 64, range 45 to 83), average serum PSA level was 7.4 ± 4.3 ng/mL (median 6.1, range 2.5 to 31.1), and average prostate volume as determined by transrectal ultrasonography was 49 ± 29 $cm^3$ (median 43, range 13 to 225). The population was 95% white, 4% black, 1% Hispanic, and less than 1% Asian (1 subject). Biopsies were performed according to the procedure described by Marks *et al.*[10] and usually consisted of 12 cores from the peripheral zone.

Urine samples (20 to 30-mL first catch) were collected after digital rectal examination, consisting of exactly three strokes per lobe, performed by an attending urologist. Firm pressure (enough to depress the prostate surface approximately 0.5 to 1.0 cm) was applied from the base to the apex and from the lateral to the median line for each lobe. The urine samples were held at 2° to 8°C and processed within 4 hours by mixing with an equal volume of detergent-based stabilization buffer and then stored at −70°C until testing.[9] Ongoing stability studies have shown no evidence of degradation of PCA3 or PSA mRNA under these storage conditions (data not shown).

The specimens were batch tested in December 2005 and January 2006. For each processed urine specimen, the quantitative ratio of PCA3 to PSA mRNA, or the PCA3 score, was determined as described previously.[9] In brief, in separate assays, 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.[11] Calibrators containing PCA3 or PSA RNA transcripts were included in each assay run and were used to convert signal to mRNA copies. PSA mRNA levels were used to normalize PCA3 to the total amount of prostate RNA present in the sample and to ensure that the RNA yield was sufficient for analysis. The PCA3 score was calculated as PCA3 mRNA/PSA mRNA × 1000. The precision of the PCA3 assay, defined as the inter-run coefficient of variation for the PCA3 score (multiple reagent lots, instruments, operators), was ~20%.[9]

## RESULTS

For the subject group studied, 226 of 233 specimens yielded sufficient RNA for analysis, corresponding to an informative specimen rate of 97%. For the 226 subjects with informative specimens, 60 repeat biopsies were positive for CaP and 166 were negative. All CaP cases found were Gleason grade 6 (65%) or 7 (35%).

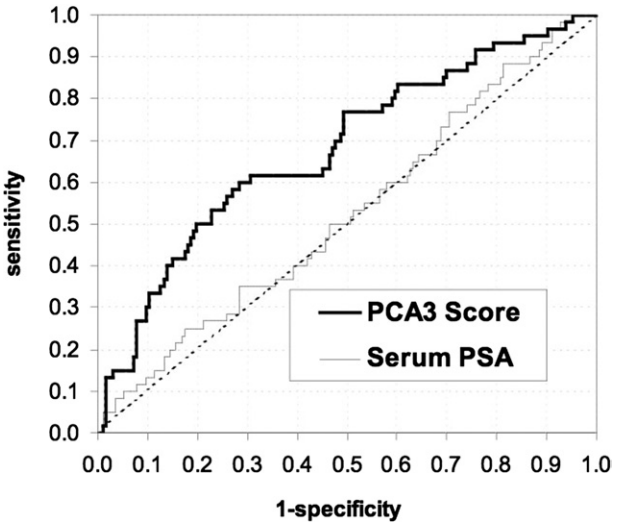


**Figure 1.** ROC analysis using PCA3 score or serum PSA level as diagnostic indicator and prostate biopsy as reference method.

**Table 1.** Sensitivity and specificity of PCA3 assay

| PCA3 Score Cutoff | Sensitivity (%) | Specificity (%) | Odds Ratio |
|---|---|---|---|
| 10 | 87 | 28 | 2.5 |
| 35 | 58 | 72 | 3.6 |
| 50 | 47 | 81 | 3.7 |

To assess the ability of the PCA3 assay to predict the prostate biopsy outcome, receiver operating characteristic (ROC) curve analysis was performed using the biopsy result as the reference method (Fig. 1). For comparison, the performance of the serum PSA assay on this subject population was also evaluated. For the PCA3 score, the area under the ROC curve was 0.678 (95% confidence interval 0.597 to 0.759). The serum PSA assay yielded an area under the curve of 0.524 (95% confidence interval 0.438 to 0.610), indicating that the serum PSA level has little diagnostic value for this subject population. The difference between the areas under the curve for PCA3 and serum PSA was statistically significant ($P = 0.008$). The PCA3 scores for Gleason grade 6 versus Gleason grade 7 were not significantly different ($P = 0.24$).

The ROC curve was used to determine the sensitivity and specificity of the PCA3 assay at different PCA3 score cutoffs (Table 1). A PCA3 score of 35 corresponded to the point on the ROC curve with the greatest diagnostic accuracy. Using 35 as the cutoff, the PCA3 assay sensitivity was 58% and specificity 72%, with an odds ratio of 3.6.

The correlation between the PCA3 score and the probability of positive biopsy findings was also deter-

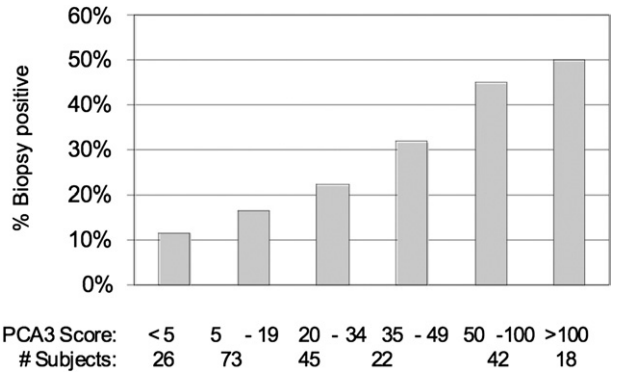


**Figure 2.** Probabilities of positive biopsy findings at different PCA3 score ranges. Number of subjects in each range shown at bottom.

mined (Fig. 2). The risk of positive biopsy findings increased with an increasing PCA3 score. At PCA3 scores of less than 5, only 12% of the subjects had positive biopsy findings. In contrast, men with a PCA3 score greater than 100 had a 50% probability of positive biopsy findings.

## COMMENT

CaP will have been diagnosed in approximately 230,000 U.S. men during 2006.[12] The great majority of these men will have undergone prostate biopsy because of an elevated serum PSA level. The positive predictive value of a serum PSA level of 4.0 ng/mL or more, the biopsy trigger currently recommended by the American Urological Association,[13] is approximately 24% for men in their seventh decade.[14] Thus, about 1 million U.S. men will have undergone a prostate biopsy in 2006 to detect CaP in one fourth of them. Furthermore, men with negative biopsy findings but elevated PSA levels still have the possibility of having CaP, because 25% of CaP cases remain undiagnosed after a single set of biopsy cores.[15] Many will undergo additional biopsies subsequently. The dollar cost, risk of morbidity, and emotional turmoil of repeat prostate biopsies are considerable, and a more accurate CaP test would be an important clinical advance for this vexing problem.

The method of PCA3 determination, as described in the present study and in detail previously,[9] is a substantial change from earlier versions and provides improved analytical sensitivity. Because BPH (and normal) prostate cells express low levels of PCA3 mRNA, the assay results are reported as copies of PCA3 per copies of mRNA of PSA, with the latter a measure of prostate RNA present in the specimen. Previous versions of the test lacked analytical sensitivity in that up to 21% of specimens contained insufficient genetic material for a valid test (ie, the test was "noninformative").[8] In the present study, the informative rate was 97%. The greater informative rate was likely a result of the streamlined specimen processing procedure that used whole, unspun urine (instead of urine sediments), as well as improvements in mRNA capture and amplification technology.[11]

The present data are evidence that urinary PCA3 determination can add specificity to a diagnostic algorithm for CaP in men with negative biopsy findings but elevated serum PSA levels. For the 226 subjects evaluated in this study, the serum PSA levels provided little value in separating those whose repeat biopsy demonstrated CaP and those whose repeat biopsy showed only BPH (Fig. 1). The area under the PSA-ROC curve was 0.524, indicating little better than a "coin toss" probability of predicting the presence of CaP. However, for the urinary PCA3 score in the same subjects, the area under the PCA3-ROC curve was 0.678. The difference between the two methods was statistically significant ($P < 0.01$). For men with elevated serum PSA levels who are undergoing repeat prostate biopsy, the PCA3 assay appears to represent an incremental improvement in the ability to predict the prostate biopsy outcome.

These data suggest that the best PCA3 score cutpoint to denote a positive result would be approximately 35, which provided relatively high specificity (72%), preserved the sensitivity (58%), and yielded an odds ratio of 3.6. Using lower PCA3 scores as the cutpoint resulted in greater sensitivity at the expense of specificity, although at a sensitivity of 87%, the PCA3 specificity of 28% was still greater than that of the serum PSA assay (18% at a cutoff of 4 ng/mL).

As shown in Figure 2, the diagnostic value of PCA3 determination is a continuum, with low PCA3 scores (less than 20) indicating a low probability of CaP and high scores (greater than 50) indicating a much greater likelihood of CaP. With additional experience, the PCA3 levels could be reported as a percentage of the probability of CaP, allowing personalized decisions regarding repeat biopsy on the basis of an individual risk-benefit assessment. In populations other than that used in our study, the normative values might be different.

Additional studies are ongoing to validate the predictive accuracy of the PCA3 score and to determine whether the PCA3 assay can synergize with other diagnostic methods such as the free/total serum PSA. In addition to providing information to guide biopsy decisions, the PCA3 score could potentially be used to monitor men with chronically elevated serum PSA levels for the development of clinically significant CaP. The results from this research study have indicated that the PCA3 assay may be a new tool to assist clinicians in the treatment of patients in the "PSA dilemma" population.

## CONCLUSIONS

PCA3 gene overexpression is detectable in urine, providing the basis for a specific CaP test. Improved PCA3 assay methods—applied in this study in 233 men with serum PSA levels greater than 2.5 ng/mL and previous negative biopsy findings—allowed for the prediction of CaP on repeat biopsy with a specificity of 72%, sensitivity of

58%, and an odds ratio of 3.6, using a PCA3 score cutpoint of 35 (copies of PCA3 per copy of PSA mRNA). The risk of positive biopsy findings correlated with the quantitative PCA3 score. In men with elevated serum PSA levels and previous negative prostate biopsy findings, the determination of the urinary PCA3 levels appears to have value in the prediction of repeat biopsy outcomes.

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## References

1. Freedland SJ, and Partin AW: Prostate-specific antigen: update 2006. Urology **67:** 458–460, 2006.
2. Thompson IM, Ankerst DP, Chi C, *et al:* Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA **294:** 66–70, 2005.
3. Stamey TA, Caldwell M, McNeal JE, *et al:* The prostate specific antigen era in the United States is over for prostate cancer: what happened in the last 20 years? J Urol **172:** 1297–1301, 2004.
4. Bussemakers MJ, van Bokhoven A, Verhaegh GW, *et al:* DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res **59:** 5975–5979, 1999.
5. Hessels D, Klein Gunnewiek JM, *et al:* DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol **44:** 8–16, 2003.
6. de Kok JB, Verhaegh GW, Roelofs RW, *et al:* DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res **62:** 2695–2698, 2002.
7. Fradet Y, Saad F, Aprikian A, *et al:* uPM3, a new molecular urine test for the detection of prostate cancer. Urology **64:** 311–316, 2004.
8. Tinzl M, Marberger M, Horvath S, *et al:* DD3PCA3 RNA analysis in urine—a new perspective for detecting prostate cancer. Eur Urol **46:** 182–187, 2004.
9. Groskopf J, Aubin SMJ, Deras IL, *et al:* APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. Clin Chem **52:** 1089–1095, 2006.
10. Marks LS, Epstein JI, and Partin AW: The role of prostate needle biopsy in evaluation of chemopreventive agents. Urology **57(suppl 4a):** 191–193, 2001.
11. Giachetti C, Linnen JM, Kolk DP, *et al:* Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol **40:** 2408–2419, 2002.
12. Jemal A, Siegel R, Ward E, *et al:* Cancer statistics, 2006. CA Cancer J Clin **56:** 106–130, 2006.
13. Thompson IM, Carroll P, Coley C, *et al:* Prostate-specific antigen (PSA) best practice policy. Oncology **14:** 267–286, 2000.
14. Vollmer RT: Predictive probability of serum prostate-specific antigen for prostate cancer: an approach using Bayes rule. Am J Clin Pathol **125:** 336–342, 2006.
15. Roehl KA, Antenor JA, and Catalona WJ: Serial biopsy results in prostate cancer screening study. J Urol **167:** 2435–2439, 2002.