

uPM3, A NEW MOLECULAR URINE TEST FOR THE DETECTION OF PROSTATE CANCER

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ABSTRACT

Objectives. To evaluate, in a multicenter study, the diagnostic performance of a new molecular test uPM3 for detecting prostate cancer cells in urine because of the need for better methods to identify patients at risk of prostate cancer.

Methods. The uPM3 test is a nucleic acid amplification assay detecting simultaneously in the urine the relative expression of prostate-specific antigen (PSA) mRNA as a marker of prostate cells and PCA3RNA, which is selectively expressed in most types of prostate cancer. The test is performed using the isothermic nucleic acid-based amplification method, and the two targets are simultaneously detected in real-time fluorescence using specific beacons as probes in a thermostated spectrofluorometer. The test was performed on the first voided urine obtained after careful digital rectal examination of the prostate in men undergoing transrectal ultrasound-guided prostate biopsy.

Results. Of 517 patients undergoing biopsy at five centers, 443 (86%) had an assessable sample. Of those, 21%, 55%, and 24% had a total PSA level of less than 4 ng/mL, between 4 and 10 ng/mL, and greater than 10 ng/mL. The corresponding percentage of biopsies positive for cancer in these three groups was 20%, 35%, and 44%. The overall uPM3 sensitivity and specificity was 66% and 89%, respectively. In men with a PSA level less than 4 ng/mL, the sensitivity was 74% and specificity 91%. In those with a PSA level of 4 to 10 ng/mL, the sensitivity was 58% and specificity 91%. In those with a PSA level greater than 10 ng/mL, the sensitivity and specificity was 79% and 80%, respectively. The positive predictive value of uPM3 was 75% compared with 38% for total PSA, and the negative predictive value was 84% compared with 89% and 80% for a PSA cutoff of 2.5 and 4.0 ng/mL, respectively. The overall accuracy was 81% compared with 43% and 47% for total PSA at a cutoff of 2.5 and 4.0 ng/mL, respectively.

Conclusions. These results suggest that the uPM3 molecular urine test may be an important adjunct to current methods for the detection of early prostate cancer. *UROLOGY* 64: 311–316, 2004. © 2004 Elsevier Inc.

Prostate cancer is the second leading cause of cancer-related mortality in the Western male population. Serum prostate-specific antigen (PSA) testing and digital rectal examination (DRE) of the

prostate have been widely accepted as effective methods of early detection of cancer.¹ However, in 2002, the U.S. Preventive Services Task Force did not recommend routine screening,² in good part because of the potential harm resulting from the lack of specificity with current screening methods. In 2 of 3 men with a serum PSA level of 4 ng/mL or greater, biopsies will be negative. However, 20% of those men will have cancer detected if rebiopsied.³ Furthermore, the rate of cancer in men with a PSA level of 2.5 to 4 ng/mL undergoing systematic biopsies reaches 20% to 23%.^{4,5} In screened populations, it has been estimated that approximately one half of the tumors missed in men with a PSA level of 0 to 4 ng/mL had aggressive characteristics and were organ confined.⁶ However, lowering the

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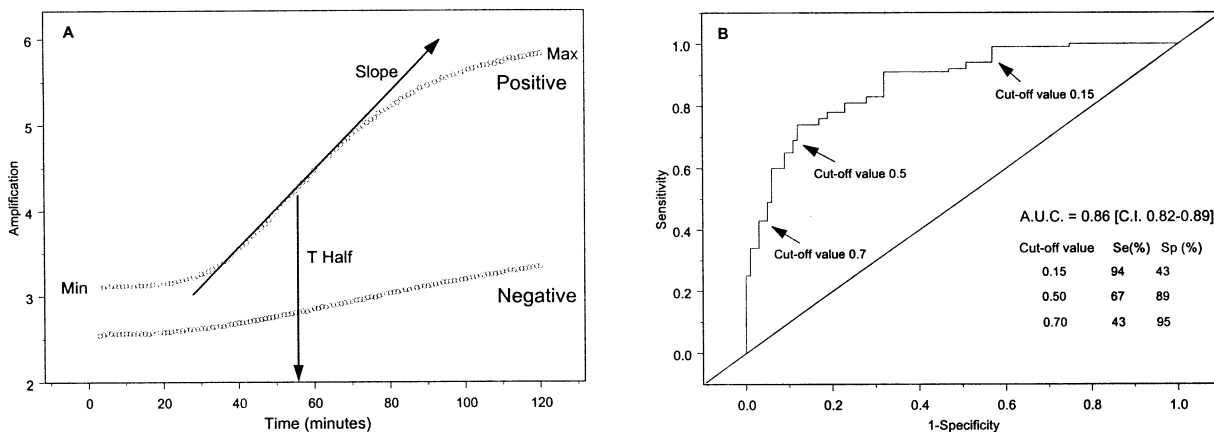


FIGURE 1. (A) Examples of amplification curves of positive and negative sample showing four parameters measured. (B) Receiver operator characteristic curve of sensitivity and specificity of prediction probability obtained by analysis of amplification curves compared with biopsy results. Cutoff of 0.5 selected for other analysis. A.U.C. = area under curve; C.I. = 95% CI; Se = sensitivity; Sp = specificity.

threshold for biopsy to 2.5 ng/mL would almost triple the number of men at risk of biopsy and would significantly raise the number of unnecessary biopsies.⁷ These dilemmas clearly emphasize the need for more sensitive and specific screening strategies.

To date, only modest improvements in specificity have been observed using tests to measure various forms of free and complexed PSA.⁸⁻¹⁰ A promising novel approach is based on the molecular detection of prostate cancer cells in urine obtained after prostatic massage by measuring cancer-specific markers such as GSTP1, telomerase, or PCA3^{DD3} RNA by reverse transcriptase-polymerase chain reaction.¹¹⁻¹³ PCA3^{DD3} is one of the most prostate cancer-specific genes described so far, with overexpression in 95% of cancers tested and a median 66-fold upregulation compared with adjacent non-neoplastic prostatic tissues.^{14,15} The quantitative reverse transcriptase-polymerase chain reaction analysis of PCA3^{DD3} gene in urine samples obtained after prostatic massage showed 67% sensitivity and 80% specificity for prostate cancer detection in a recent single-institution study.¹³

We report the results of a multicenter study evaluating the clinical performance of the uPM3 test, a nucleic acid-based amplification assay to measure PSA and PCA3^{DD3} RNA in first voided urine specimens after attentive DRE in 443 patients undergoing transrectal ultrasound-guided prostate biopsy. The study was performed under routine clinical practice conditions and showed a diagnostic accuracy of 81%.

MATERIAL AND METHODS

Urine samples were obtained from 517 men undergoing transrectal ultrasound-guided prostate biopsy at five medical centers. The respective ethical review board of the participating institutions approved the study, and all patients provided written informed consent.

Before transrectal ultrasound-guided biopsy, subjects underwent an attentive DRE, performed by nine different physicians with instruction of doing a thorough prostate palpation for 15 to 30 seconds. After the DRE, the first 20 to 30 mL of voided urine was collected and mixed with an equal volume of phosphate buffer (pH 7.0) and then stored and shipped at 2° to 8°C. The biopsy procedure was not standardized across the five centers, but 6 to 10 cores were taken, depending on the practice of each investigator.

The uPM3 assay was performed in one central laboratory at which the urine samples were processed within 3 days of collection. Spun cells were lysed in a guanidine thiocyanate buffer, and nucleic acid extracts were prepared by the Boom method¹⁶ and eluted in 200 μ L DNase/RNase-free water.

Both PSA and PCA3^{DD3} RNA were amplified in the same tube using the nucleic acid sequence-based amplification technology¹⁷ and real-time fluorescence using specific beacon probes to detect the amplification products.¹⁸ The test was performed in duplicates containing 5 μ L each of the total nucleic acid extract. The reaction tubes were incubated at 41°C and then read kinetically during a 2-hour period in a thermostated spectrofluorometer (NucliSens Easy Q, Bio-Merieux, Durham, NC) with the filters set for measurement of fluorescein for PCA3^{DD3} and rhodamine fluorescence for PSA. Figure 1A shows the amplification kinetic curves for a positive and negative PCA3 amplification. The curves were analyzed using a logistic curve fitting routine, including the following four parameters: Max, the upper horizontal asymptote; Min, the lower horizontal asymptote; T Half, the time at the inflexion point; and Slope, a time-scale parameter. Two other parameter estimates were determined: the difference between the asymptotes (Max minus Min) and the ratio of the asymptotes (Max over Min). The six parameter estimates of the PCA3^{DD3} RNA results were used to construct classification trees to predict the outcome of patients (ie, cancer and no

TABLE I. Positive biopsies versus serum tPSA and DRE categories

tPSA Range (ng/mL)	Subjects (%)	Positive Biopsies (%)
<4	21 (94/443)	20 (19/94)
DRE suspicious	61 (57/94)	21 (12/57)
DRE negative	39 (37/94)	19 (7/37)
4–10	55 (243/443)	35 (86/243)
DRE suspicious	23 (55/243)	51 (28/55)
DRE negative	77 (188/243)	31 (58/188)
>10	24 (106/443)	44 (47/106)
DRE suspicious	26 (28/106)	68 (19/28)
DRE negative	74 (78/106)	36 (28/78)
Total	100 (443/443)	34 (152/443)

KEY: tPSA = total prostate-specific antigen; DRE = digital rectal examination.

cancer on biopsies) according to the serum total PSA (tPSA) range. To confirm the presence of exfoliated prostate cells in the sample, the PSA RNA curve was required to have a Max/Min ratio of at least 1.3 for the sample to be evaluated.

The S-PLUS 2000 software (Insightful, Seattle, Wash) was used to estimate the six parameters of each patient and to construct the classification trees that resulted in a probability of cancer of 0.0 to 1.0. The sensitivity and specificity were computed by comparing the probability outcome predicted by the classification trees and the actual patient biopsy outcomes to construct a receiver operating characteristic curve (Fig. 1B).

Continuous variables, including age and tPSA, were compared between patients with prostate cancer and those with benign conditions using the nonparametric Mann-Whitney U test ($P < 0.05$ considered statistically significant). JMP software (SAS Institute, Cary, NC) was used for the computation.

RESULTS

Of the 517 subjects recruited, 443 (86%) provided samples with sufficient PSA RNA signal for analysis of PCA3^{DD3} RNA. The cohort was predominantly white (419 [95%] of 443), with a median age of 64 years (range 40 to 87). Of the 443 men, 152 (34%) had cancer on biopsy. The median tPSA level was 7.5 ng/mL (range 1 to 144) for those with cancer versus 6 ng/mL (range 0.1 to 83) for those without cancer found on biopsy ($P < 0.0001$). Suspicious DRE findings were noted in 48 (32%) of 152 patients with cancer and 55 (19%) of 291 without cancer. The Gleason scores were preponderantly (72%) in the range of 6 to 7 (median 6).

The distribution of subjects according to tPSA level, DRE findings, and percentage of positive biopsies is presented in Table I. The uPM3 test result was a predictive probability obtained by analysis of the amplification curves using a four-parameter logistic curve fitting routine (Fig. 1A) and classification tree. Figure 1B shows the receiver operating characteristic curve computed using these results

TABLE II. Sensitivity and specificity of uPM3 by tPSA range

tPSA Range (ng/mL)	n	Sensitivity (%)	Specificity (%)
<4	94	74 (51–88)	91 (82–95)
4–10	243	58 (48–68)	91 (86–95)
>10	106	79 (65–88)	80 (68–88)
Overall	443	66 (59–74)	89 (85–92)

KEY: tPSA = total prostate-specific antigen.
Data in parentheses are 95% confidence intervals.

compared with the actual biopsy outcome. The area under the curve was 0.86 (95% confidence interval [CI] 0.82 to 0.89).

Tables II and III give the clinical performance of the uPM3 urine test according to the serum tPSA values using a cutoff of 0.5 predictive probability. The overall sensitivity was 66%, with a specificity of 89%. The sensitivity and specificity was 74% and 91% for a PSA level less than 4 ng/mL, 58% and 91% for a PSA level of 4 to 10 ng/mL, and 79% and 80% for a PSA level greater than 10 ng/mL, respectively. No statistically significant difference was found in the sensitivity or specificity of the uPM3 assay in patients with or without suspicious DRE findings. The positive predictive value (PPV) for the uPM3 test was 75% versus ~40% for tPSA. The negative predictive value (NPV) was equivalent between the two tests, but, because of the greater PPV for uPM3, the accuracy of the uPM3 test was nearly twofold greater than tPSA determination (81% versus 43% and 47% for tPSA cutoffs of 2.5 and 4 ng/mL, respectively).

Of the total population of 443 assessable men, 91 underwent subsequent biopsy after one or more previous negative biopsies. The performance of the uPM3 test in this subset was similar to that in the overall cohort, with a sensitivity of 74% (95% CI 57% to 86%) and a specificity of 87% (95% CI 76% to 93%) corresponding to an NPV of 87% (95% CI 76% to 93%) and a PPV of 74% (95% CI 57% to 86%).

COMMENT

The widespread acceptance of serum PSA testing for the early detection of prostate cancer has been hampered by the low specificity of the assay and the resulting cost and anxiety generated by unnecessary biopsies. The problem is further complicated by the results of recent studies showing that almost 1 in 4 men with a PSA level of 2.5 to 4 ng/mL have prostate cancer on biopsy,^{4,5} of which more than one half are aggressive cancers that are

TABLE III. uPM3 performance versus serum tPSA level

Variable	PPV (%)	NPV (%)	Accuracy (%)
tPSA \geq 2.5 ng/mL	37 (33–42)	89 (77–95)	43 (39–48)
tPSA \geq 4 ng/mL	38 (33–43)	80 (71–87)	47 (42–52)
uPM3	75 (67–82)	84 (79–87)	81 (77–84)

KEY: tPSA = total prostate-specific antigen.
Data in parentheses are 95% confidence intervals.

organ confined and thus within the appropriate window of opportunity for cure.⁶ Large-scale randomized prostate cancer screening trials have shown that approximately 20% of men have a PSA level between 2 and 4 ng/mL, stressing the important burden that would result from lowering the PSA threshold for prostate biopsy.⁷ The results of the present study have shown that the uPM3 urine test may represent a statistically significant improvement, with an overall accuracy twofold greater than that of the tPSA assay. A salient feature of this assay was its high specificity, which averaged 89% in the 443 patients tested. The specificity was slightly lower in men with a PSA level greater than 10 ng/mL, but such men have the greatest probability of cancer on repeat biopsy. The NPV was similar to that of the tPSA assay, but a positive uPM3 test was associated with a positive biopsy 75% of the time, twice the PPV of tPSA measurement. Because the test is the equivalent of molecular cytology of the prostate, it was interesting to note that individuals with a positive uPM3 test may be at greater risk of cancer than those with prostate biopsy features considered to be high risk such as high-grade prostatic intraepithelial neoplasia and atypia, which have a PPV for cancer on repeat biopsies varying from 10% to 50% depending on the study.^{19–21} Only 1 of 14 with high-grade prostatic intraepithelial neoplasia and 0 of 6 with atypia in the present study had a positive uPM3 test.

The performance of the uPM3 test in this multi-center setting was very similar to that of previously reported single-institution research assays.^{11–13} In previous reports, the assays were performed after prostatic massage that was described as extensive in at least one study.¹³ In the present trial, nine different physicians performed what was requested to be an attentive DRE but was not standardized. It is possible that a true prostatic massage would have increased the cell yield and thus the sensitivity of cancer detection. The test was usable in only 443 (86%) of the 517 patients tested because of the lack of detection of prostate cells. It is also possible that additional technical improvements in the assay may increase its sensitivity in the future. Others have

shown, in a different type of assay, an improvement of 36% to 100% in the detection of cancer in urine after prostatic massage by changing the kit used for RNA isolation.^{11,22} All biopsies performed in our study were ultrasound guided, but the number of cores taken varied among centers from 6 to 8 or 10. Uncertainties remain as to the optimal biopsy protocol, because the only randomized trial showed no improvement in cancer detection by increasing the number of cores from 6 to 12.²³ Nevertheless, the frequency of positive biopsies in our study was very similar to that reported in contemporary series. The performance of the uPM3 test was similar in men with suspicious DRE findings or negative DRE findings.

One of the most promising characteristics of the uPM3 test was its high accuracy in the 94 men with a PSA level less than 4 ng/mL. In this range, the uPM3 test had 74% sensitivity and 91% specificity. This type of noninvasive test may be particularly attractive to identify those at high risk of cancer among the large population of men with a PSA level between 2.5 and 4 ng/mL. Another significant challenge in current practice is to determine who should undergo repeat biopsy and how often. The uPM3 test also performed very well in the 91 subjects undergoing subsequent biopsies after one or more previous negative biopsies. In this subset, the uPM3 test had 74% sensitivity and 87% specificity, corresponding to an NPV of 87% and a PPV of 74%. On the basis of the limited data from the present study, the PPV of the test may be better than commonly used risk factors such as prostatic intraepithelial neoplasia or atypia. No information was available on the extent of cancer in the present study. Future investigations should address the potential prognostic value of the uPM3 test to determine cancer aggressiveness.

The high specificity of the uPM3 test was likely a result of the very high discriminating power of the gene expression in prostatic cancer cells. In real-time quantitative reverse transcriptase-polymerase chain reaction studies performed on radical prostatectomy specimens, the median expression of PCA3^{DD3} in tumor tissues was 5849 normalized

mRNA copies versus 10 for the telomerase reverse-transcriptase (hTERT) gene.¹⁵ The same group recently showed a median 11-fold upregulation in 13 prostatic tissue samples containing less than 10% of tumor cells, suggesting that nucleic acid amplification testing using the PCA3^{DD3} gene is capable of detecting very few malignant cells in a background of predominantly nonmalignant prostatic cells.¹³ Because of the nature of this type of assay in detecting rare events, it was not possible to determine the robustness of the test in individual patients. The results of the present study will need to be validated in other clinical trials that should also aim at elucidating the relationship of the test result with the extent of cancer, as well as the possible causes of false-positive and false-negative results.

CONCLUSIONS

The nucleic acid amplification test uPM3 to detect PSA and PCA3^{DD3} RNA in voided urine after DRE performed well in routine clinical settings and was highly specific. With an NPV comparable to that of the PSA assay, the overall accuracy of the uPM3 test was 81% compared with approximately 40% for tPSA. The uPM3 test may provide some answers to the current dilemmas of early prostate cancer detection and may be particularly useful in the monitoring of men with lower PSA values and those with previously negative biopsies.

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EDITORIAL COMMENT

In this report, Fradet *et al.*, present the results of a multicenter study on the diagnostic value of the first gene-based test for prostate cancer.

The uPM3 test measures the expression of the PCA3^{DD3} gene, which was identified by Bussemakers and colleagues.¹ The first exploratory study by Hessels *et al.*² indicated that gene-based testing for the diagnosis of prostate cancer was feasible and that the use of the relatively novel substrate (ie, urinary sediment after extended DRE), was very promising. Fradet *et al.* now provide additional evidence that the uPM3 test can predict the presence of prostate cancer with extremely high accuracy. This will have profound additional value within the PSA range of 4 to 10 ng/mL, because of very high

NPV resulting from the high specificity of the test (89%). I, therefore, conclude that we are at the verge of the clinical introduction of a gene-based test for prostate cancer.

Several issues remain to be resolved, the most important of which is how predictive the test is for clinically significant prostate cancer. Follow-up of the pathologic data of the studies by Fradet *et al.* and Hessel *et al.*² should provide insight into whether the cases identified are indeed clinically significant.

Although the use of this novel substrate seems to be very promising, caution should be used. It is to be expected that urologists worldwide will perform the DRE differently, which will inevitably result in different cell yields. Standardization of the attentive DRE, therefore, deserves attention. Additionally, because a significant amount of cells end up in the urine, it is tempting to speculate that PCA3 mRNA could be released in the urine without a DRE. This possibility should also be addressed in the coming years.

Considering the unique characteristics of the PCA3 gene, I believe that the first gene-based test for prostate cancer comes within reach. Extension with a panel of other prostate cancer-specific and/or progression markers would further extend the

potential value of such tests. Thus, it is a promising new test with interesting development potential.

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