UPM3: review of a new molecular diagnostic urine test for prostate cancer

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PSA elevation is the most common indication for urologic referral to rule out the presence of prostate cancer. Recently PSA screening and its usefulness in suggesting the presence of clinically significant prostate cancer has been put into doubt. PSA has limitations in detecting significant cancers even when elevated and on the other hand significant cancers are found in the presence of low PSA levels. In order to better predict patients at risk of harboring prostate cancer new diagnostic tests are required. A promising novel approach is based on the molecular detection of prostate cancer cells in urine. The uPM3 assay is based on the amplification of specific target RNA using the nucleic acid sequence-based amplification (NASBA) technology. In a large multi-center study of 517 cases the overall sensitivity was 66% with a specificity of 89%. The positive (PPV) predictive values for the uPM3 test were 75% compared to 38% for a serum PSA cutoff of 4. The negative predictive value (NPV) was equivalent between the tests, but due to the higher PPV for uPM3, the accuracy of uPM3 was nearly two-fold greater than PSA (81% versus 43% and 47% for PSA cutoffs of 2.5 ng/ml and 4 ng/ml, respectively). This test may become one of the first molecular diagnostic tools to aid in prostate cancer detection.

Key Words: diagnostic marker, prostate cancer, UPM3

Introduction

PSA elevation is the most common indication for urologic referral to rule out the presence of prostate cancer.[1,2] Recently PSA screening and its usefulness in suggesting the presence of clinically significant prostate cancer has been put into doubt.[3] PSA has limitations in detecting significant cancers even when elevated[3] and on the other hand significant cancers are found in the presence of low PSA levels.[4] The limitation in predictive accuracy of serum PSA has been addressed by efforts directed at increasing its specificity by using age-specific PSA ranges, PSA velocity and free over total PSA ratios.

Age-specific PSA ranges have been proposed based on the relationship of age with prostate size. Age-specific PSA ranges increase the sensitivity of PSA in younger men and increase its specificity in older men. The ranges are as follows: 40-49 years old: 0-2.5 ng/ml, 50-59: 0-3.5 ng/ml, 60-69: 0-4.9 ng/ml and 70-79: 0-5.9 ng/ml. A further attempt at improving the performance of PSA consisted of the rate of PSA change over time, termed PSA velocity. Rate of change exceeding 0.75 ng/ml per year has been shown to be 72% specific and 95% sensitive in predicting presence of prostate cancer on needle biopsy. However, at least three measurements need to be obtained within a 2

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year period, therefore the usefulness of PSA velocity is limited to men with adequate follow-up. Furthermore, assay related differences in individual PSA values as well as individual physiologic variation in PSA levels may confound the calculation of PSA velocity. Serum PSA is found in two parts, protein bound and free. The ratio of free PSA over total, has been shown to improve the specificity of the traditional PSA cut-off. The presence of cancer is associated with low free PSA levels. The most useful clinical application of free PSA is to detect cancer in men with normal (0-4 ng/ml) PSA levels and in men with borderline (4.1-10 ng/ml) PSA elevations. In men with normal PSA levels, free over total PSA ratio values between 0% and 25% have been shown to be 90% sensitive and 24% specific. In men with PSA values between 4.1 ng/ml and 10 ng/ml, a cut-off of 20% has been suggested and this practice was shown to result in sensitivity of 95% and specificity of 29%.[5-8]

Presence of suspicious rectal examination findings and/or elevation of serum PSA or of its enhanced forms indicates the need for prostate biopsy. Multi-core biopsies of the prostate are performed under trans-rectal ultrasound guidance. Suspicious (hypoechoic) appearance of the gland on ultrasound may suggest additional biopsies. In its absence a minimum of six biopsies are routinely obtained from the peripheral zone of the gland, where most cancers originate. Trans rectal ultrasound allows the determination of the volume of the prostate, which in turn allows to determine the expected benign contribution to circulating serum PSA. Since prostate cancer releases more PSA per unit of volume than does BPH, a PSA to volume ratio (PSA density) allows to grade the risk of cancer on biopsy. A higher risk based on this calculation may prompt additional biopsies. Unfortunately a significant percentage of men will have prostate cancer even in the presence of a low PSA density making biopsy a necessity in the face of elevated PSA.

Although PSA alone or in combination with other variables represent a good predictor of the likelihood of cancer on needle biopsy, needle biopsy alone represents the gold standard for presence of malignancy. Unfortunately, significant rates of false negative needle biopsy findings exist. In the presence of persistently elevated serum PSA, some centers perform as many as four consecutive needle biopsies appear to be required before presence of prostate cancer may be ruled out. Continued surveillance with PSA and rectal examinations and consideration of further biopsies may be required if suspicious findings persist.

UPM3

In order to better predict patients at risk of harboring prostate cancer new diagnostic tests are required. A promising novel approach is based on the molecular detection of prostate cancer cells in urine obtained after prostate massage by measuring cancer specific markers such as GSTP1, telomerase or $PCA3^{DD3}$ RNAs by reverse transcriptase polymerase chain reaction (RT-PCR).[9-11] $PCA3^{DD3}$ is one of the most prostate cancer-specific genes described to date, with over-expression in 95% of cancers tested and a median 66-fold up-regulation compared to adjacent non-neoplastic prostate tissues Figure 1.[12,13] The high specificity of the uPM3 test is likely due to the very high discriminating power of the PCA3 gene expression in prostatic cancer cells. Hessels et al showed a median 11-fold up-regulation of PCA3 in 13 prostate tissue samples containing less than 10% of tumor cells, suggesting that nucleic acid amplification testing using PCA3 gene is capable of detecting very few malignant cells in a predominantly non-malignant environment.[11]

The quantitative RT-PCR analysis of $PCA3^{DD3}$ gene in urine samples obtained after prostatic massage showed 67% sensitivity and 80% specificity for prostate cancer detection in a single institution study.[11] The uPM3 assay is based on the amplification of specific target RNA using the nucleic acid sequence-based amplification (NASBA) technology.[14,15] Both PSA and $PCA3^{DD3}$ RNA are assayed simultaneously on the same specimen. The products of amplification are measured in real-time fluorescence using specific

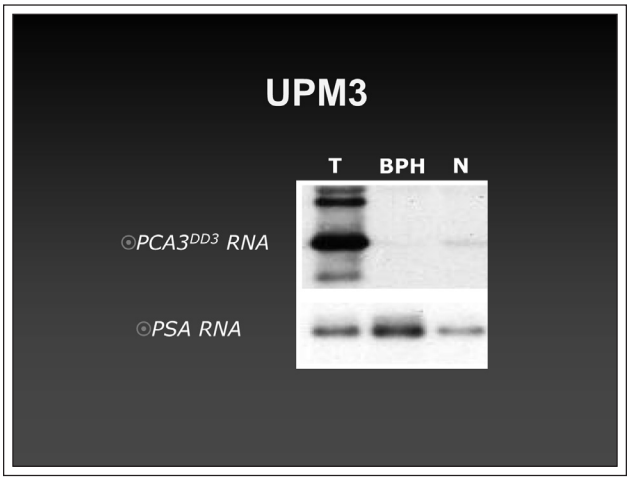

Figure 1. Expression of PSA RNA as a marker of prostate cells and PCA3 RNA which is selectively expressed in the majority of prostate cancers.

TABLE 1. **UPM3 sensitivity and specificity by total PSA range**

PSA range	% Sensitivity (95% CI)	% Specificity (95% CI)
< 4 ng/ml	74 (51-88)	91 (82-95)
4-10 ng/ml	58 (48-68)	91 (86-95)
>10 ng/ml	79 (65-88)	80 (68-88)
Overall	66 (59-74)	89 (85-92)

beacon probes.[16] PSA RNA is used to confirm that prostate cells are present in the sample. In a large multi-center study of 517 cases performed in the province of Quebec the overall sensitivity was 66% with a specificity of 89%. Sensitivity was 74% and specificity 91% for PSA <4, 58% sensitivity and 91% specificity for PSA 4 – 10, and 79% sensitivity and 80% specificity for PSA >10. Table 1 There was no apparent difference in sensitivity or specificity of the uPM3 assay in patients with suspicious DRE or negative DRE.

The positive (PPV) predictive values for the uPM3 test were 75% compared to 38% for a serum PSA cutoff of 4. The negative predictive value (NPV) was equivalent between the tests, but due to the higher PPV for uPM3, the accuracy of uPM3 was nearly two-fold greater than PSA (81% versus 43% and 47% for PSA cutoffs of 2.5 ng/ml and 4 ng/ml, respectively) Table 2.[17,18]

The performance of the uPM3 test in this multi-center setting was very similar to those of single institution research assays previously reported.[13-15] The authors state that although the uPM3 test in its current format underwent extensive experimentation to optimize its performance, it is possible that further technical improvements in the assay may increase its sensitivity in the future

One of the most promising characteristics of the uPM3 test was its high accuracy in men with PSA <4. In this range, uPM3 showed 74% sensitivity and 91% specificity. Given the current trend of lowering PSA threshold to 2.5 this type of non-invasive test may be particularly attractive to identify men at high risk of cancer among the large population of men with PSA between 2.5 ng/ml and 4 ng/ml. Another significant challenge in current practice is to determine whom to re-biopsy after a negative biopsy result. The study using the uPM3 performed very well in predicting the presence of cancer in subjects undergoing subsequent biopsies after one or more previous negative biopsies. In this subset, uPM3 had a 74% sensitivity and a 87% specificity corresponding to a negative predictive value of 87% and a positive predictive value of 74%. It is interesting to note that the positive predictive value of the test appears to be better than risk factors, such as PIN or ASAP, commonly used to justify repeat biopsies.[19,20]

TABLE 2. **Accuracy and predictive value of PSA compared to uPM3**

	PPV (%)	NPV (%)	Accuracy (%)
PSA above 2.5	37	89	43
PSA above 4	38	80	47
UPM3	75	84	81

Conclusion

The nucleic acid amplification test uPM3 to detect PSA and PCA3^{DD3} RNA in voided urine after DRE appears to be highly specific for prostate cancer. The negative predictive value is comparable to that of total PSA while the overall accuracy appears to be twice that of total PSA given it's high positive predictive value. This new molecular diagnostic test may help in addressing some of the complexities of early prostate cancer detection. Suggestions to lowering PSA cutoffs for screening may be partially offset by identifying patients at higher risk using the UPM3 test. Identifying patients at high risk for cancer with previously negative biopsies will also be very useful. This test may become one of the first molecular diagnostic tools to aid in prostate cancer detection. □

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