

The use of *PCA3* in the diagnosis of prostate cancer

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Abstract | Although the routine use of serum PSA testing has undoubtedly increased prostate cancer detection, one of its main drawbacks has been its lack of specificity, which results in a high negative biopsy rate. Consequently, a large population of men with chronically elevated serum PSA and one or more negative biopsies has emerged. More accurate tests are needed that can help identify which patients are at high risk of developing prostate cancer, and for whom repeat prostate biopsies are mandatory. To improve the specificity of prostate cancer diagnosis, prostate-cancer-specific markers, such as prostate cancer gene 3 (*PCA3*), are needed. The strong association between *PCA3* mRNA overexpression and malignant transformation of prostate epithelium indicates its potential as a diagnostic biomarker. Quantification of *PCA3* mRNA levels in urine was found to help predict the outcome of prostate biopsies. The intensive and time-consuming reverse-transcriptase polymerase chain reaction *PCA3* urine test has been translated successfully into the fast and easy transcription-mediated amplification (TMA)-based *PCA3* test. This test is the first RNA-based molecular diagnostic assay in body fluids for prostate cancer that is available to urologists. This Review describes the translation of the molecular marker *PCA3* from the research laboratory to clinical practice.

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Introduction

Annually, 186,320 men in the US and 345,900 men in Europe are newly diagnosed with prostate cancer, and around 28,660 US and 87,400 European men die from this disease.^{1,2} Early detection of prostate cancer relies on serum PSA testing or digital rectal examination (DRE). Since its first clinical application, serum PSA has been the most valuable tool in the detection, staging and monitoring of this disease. Although the routine use of serum PSA testing has undoubtedly increased prostate cancer detection, one of its main drawbacks has been its lack of specificity that results in a high negative biopsy rate. This is especially true in patients with serum PSA values between 3–10 ng/ml, in whom the negative biopsy rate is approximately 60–75%.³ The specificity is low because an elevated serum PSA level is not a prostate-cancer-specific event; it can also be detected in men with benign prostatic hyperplasia (BPH) and prostatitis. Although prostate biopsy is considered the gold standard for prostate cancer diagnosis, this method has its limitations and associated morbidities. Methods to enhance PSA specificity have assisted clinicians in deciding which patients should undergo biopsy, but have not necessarily improved diagnostic accuracy or facilitated optimal therapeutic decision-making. More-accurate tests that can stratify patients according to their risk of

developing prostate cancer, and identify those who require repeat prostate biopsy, are needed. Implementation of prostate-cancer-specific markers in body fluids is needed in order to improve the specificity of prostate cancer diagnosis. A number of these biomarkers have been identified, but one of the biomarkers that has been critically evaluated and clinically investigated for its diagnostic potential is prostate cancer gene 3 (*PCA3*).^{4,5} This Review provides an overview of *PCA3*, from its identification and its systematic and critical evaluation, to a fully translated molecular assay in body fluids that is a valuable tool in predicting biopsy outcome.

Identification and characterization

In 1999, a new prostate-specific gene was identified using differential display analysis, a technique used to compare mRNA expression patterns of tumor and adjacent non-neoplastic tissue in radical prostatectomy specimens.⁶ Using northern blot analysis, *DD3* (differential display clone 3) was found to be highly overexpressed in prostate tumors compared to normal prostate tissue from the same patient. In accordance with current human genome nomenclature, it has been renamed *PCA3* to reflect its association with prostate cancer. Low *PCA3* expression was observed in normal prostate and benign prostatic hyperplasia (BPH) tissue. High overexpression of *PCA3* was observed in 95% of the primary prostate cancer specimens studied. Using reverse-transcriptase polymerase chain reaction (RT-PCR), *PCA3* was found to be prostate-specific as no expression could be detected in other normal human tissues, in tumors originating from

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Competing interests

D. Hessels has declared an association with the following company: NovioGendix. J. A. Schalken has declared an association with the following company: Gen-Probe Inc. See the article online for full details of the relationships.

Key points

- Unlike serum PSA, *PCA3* is specific for prostate cancer and is not affected by prostate volume or other non-cancerous prostate conditions
- The *PCA3* score correlates with the result of a subsequent biopsy; in men with a high *PCA3* score, the likelihood of a positive prostate biopsy is increased
- Preliminary data demonstrate a correlation between *PCA3* score and prostate cancer prognostic markers; therefore, *PCA3* might identify men who have clinically insignificant prostate cancer and are candidates for active surveillance
- The *PCA3* test is the first fully translated molecular assay in body fluids that is a valuable tool in predicting biopsy outcome
- Combining *PCA3* as a continuous variable with other clinical or pathological factors or biomarkers (for example, *TMPRSS2-ERG* gene fusions) will provide more-accurate prostate cancer diagnosis and prognosis

the breast, cervix, endometrium, ovary and testis, or in cell lines originating from bladder, kidney and ovarian cancers. *PCA3* expression was demonstrated in only two prostate cancer cell lines, LNCaP and 22Rv1.⁶

The gene encoding *PCA3* maps to chromosome 9q21–22 and consists of four exons. Molecular characterization of the *PCA3* transcription unit revealed that alternative polyadenylation at three different positions in exon 4 gives rise to three different-sized transcripts. In addition, alternative splicing occurs, in which exon 2 (present in only 5% of the transcripts) is skipped. The most frequently found transcript, which accounts for approximately 60% of all cDNA clones analyzed, contains exons 1, 3, 4a and 4b (Figure 1).⁶ Open reading frame analysis revealed that the *PCA3* exons are populated by an unusual number of stop codons. A gene that codes for proteins will typically possess one long open reading frame delimited by a stop codon. The multiplicity of stop codons across the three reading frames of *PCA3* and the lack of an extended open reading frame indicates that

PCA3 does not encode a protein and functions as a non-coding RNA.

Diagnostic utility of *PCA3*

The strong association between *PCA3* overexpression and malignant transformation of prostate epithelium has identified its potential use as a biomarker for the diagnosis of prostate cancer; however, in the absence of a protein product, the only substrate or target molecule that can be used is mRNA. RNA is prone to degradation, especially in biological fluids, which can lead to a decreased sensitivity of any RNA-based test. Thus, a good clinical RNA-based test will rely on the robustness of sample collection and having a high degree of sensitivity for its target.

In 2002, a real-time RT-PCR analysis for the quantification of *PCA3* mRNA in tissue specimens was developed.⁷ In 2003, Hessels *et al.*⁸ established a sensitive quantitative RT-PCR technique that used dual time-resolved fluorescence, an exogenous internal control and an external calibration curve. Both techniques confirmed that *PCA3* mRNA expression is restricted to the prostate and that it is present in normal prostate and BPH tissue at low, quantifiable levels. The median upregulation of *PCA3* mRNA in prostate cancer was 66-fold compared to normal prostate tissue. Using receiver operating characteristic (ROC) curves, both techniques revealed that *PCA3* has a high sensitivity and specificity for prostate cancer in tissue specimens (area under curve [AUC]) of 0.94 and 0.98, respectively) (Figure 2a).^{7–9} Moreover, in prostate tissue specimens that contained <10% prostate cancer cells, the median upregulation of *PCA3* was 11-fold. This demonstrates that *PCA3* is able to detect a small number of prostate cancer cells in a background of predominantly nonmalignant cells, without the need for microdissection.⁸ The combined data and the fact that

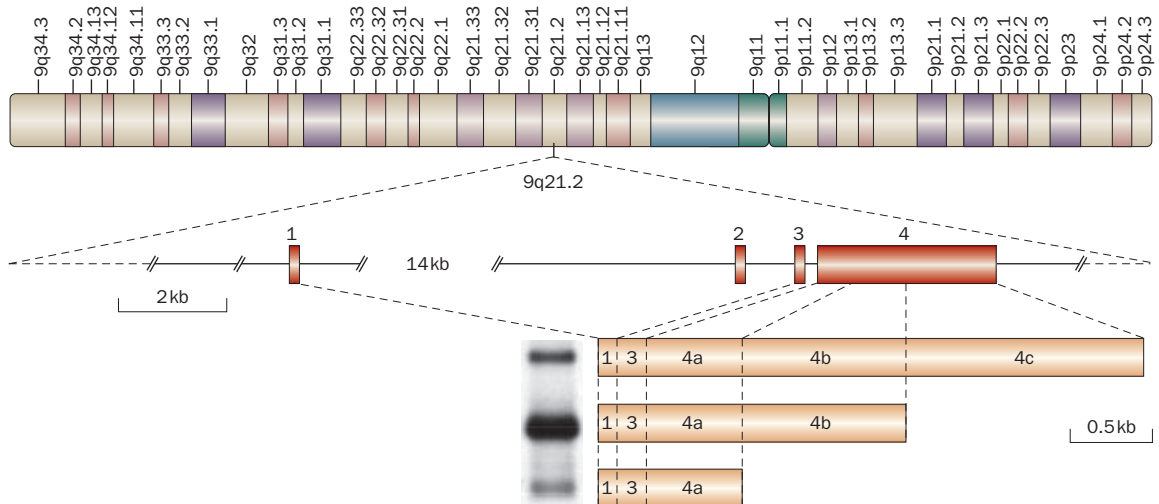


Figure 1 | Structure of the *PCA3* transcription unit. The gene encoding *PCA3* maps to chromosome 9q21–22 and consists of four exons. Alternative polyadenylation at three different positions in exon 4 (indicated 4a, 4b, and 4c) gives rise to three different-sized transcripts. Alternative splicing occurs, by which exon 2 (present in only 5% of the transcripts) is skipped. The most frequently found transcript contains exons 1, 3, 4a and 4b.⁶

PCA3 is not expressed in leukocytes (often present in body fluids) indicate the great utility of *PCA3* as a minimally invasive diagnostic tool to detect prostate cancer cells in body fluids.⁷

The RT-PCR-based *PCA3* test developed by Hessels and colleagues⁸ was used to evaluate the utility of *PCA3* to detect prostate cancer cells in urinary sediments after DRE. The reasoning behind this was that manipulation of the prostate would transport the cancer cells via the prostatic ductal system into the urethra. The first portion of voided urine following DRE contains the highest concentration of prostatic and urethral secretions.¹⁰ However, urinary sediments contain non-neoplastic prostate cells and urothelial cells as well as tumor cells. PSA mRNA was used to normalize for the number of prostate cells, as its expression was shown to be relatively constant in both normal prostate cells and prostate cancer cells.¹¹ Both *PCA3* and PSA mRNA levels were quantified in urinary sediments following DRE.⁸ The *PCA3* score is the ratio of *PCA3*:PSA mRNAs multiplied by 1,000. In a cohort of 108 men admitted for prostate biopsy based on serum PSA levels ≥ 3 ng/ml, 24 men were found to have prostate cancer upon biopsy. Using prostate biopsy as the gold standard, ROC analysis yielded an AUC of 0.72 (95% CI 0.58–0.85). The RT-PCR-based *PCA3* assay had 67% sensitivity and 83% specificity for detecting prostate cancer.⁸ For comparison, the serum PSA test specificity was 22%. This study was the first to demonstrate the potential of a quantitative *PCA3*-based urine test to aid in the prediction of biopsy outcome. This test can also be performed on prostatic fluid after DRE, resulting in similar sensitivity and specificity data to that obtained from urine.¹² In a Dutch multicenter study that examined urinary sediments following DRE in 583 men with serum PSA levels in the range 3–15 ng/ml, the AUC was 0.66 (95% CI 0.61–0.71) for *PCA3* and 0.57 (95% CI 0.52–0.63) for serum PSA (Figure 2b).¹³ Here, the test had a sensitivity of 65% and a specificity of 66% (versus 65% sensitivity and 47% specificity for serum PSA), confirming that *PCA3*-testing can improve the specificity of prostate cancer diagnosis.

A TMA-based *PCA3* test

Although the urinary RT-PCR-based test demonstrated the potential of *PCA3* in the diagnosis of prostate cancer, the methodology used was too intensive and time-consuming for widespread implementation in clinical laboratories, restricting its use to research laboratories. Moreover, the procedure for collecting and stabilizing specimens under standardized conditions had to be defined. Gen-Probe Inc, therefore, translated the initial RT-PCR-based urine *PCA3* test to its transcription-mediated amplification (TMA) platform.¹⁴ This technology is simple, fast and sensitive enough to be used in a clinical laboratory. The TMA platform is commercially available for several FDA-approved products, and its equipment is already present in many laboratories worldwide.

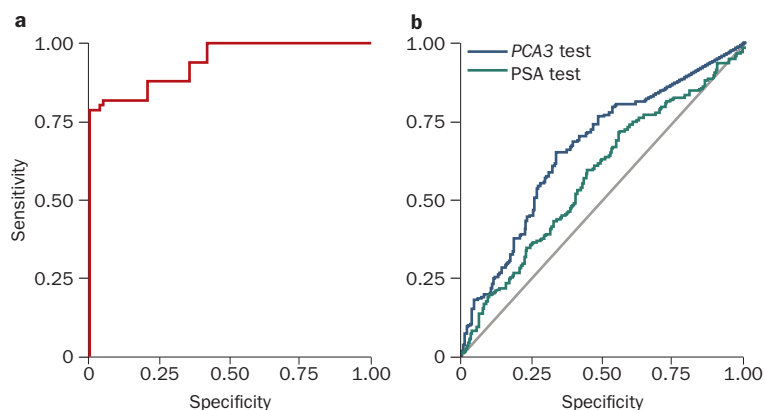


Figure 2 | Sensitivity and specificity of *PCA3* testing for detecting prostate cancer. **a** | ROC analysis demonstrated that *PCA3* has a high sensitivity and specificity for prostate cancer in tissue specimens (AUC=0.94 [95% CI 0.89–0.99]). **b** | ROC analysis was used to visualize the diagnostic efficiency of the quantitative RT-PCR-based *PCA3* test (AUC=0.66 [95% CI:0.61–0.71]) and serum PSA level (AUC=0.57 [95% CI 0.52–0.63]) in urinary sediments collected after DRE. Abbreviations: AUC, area under curve; ROC, receiver operator characteristic; RT-PCR, reverse-transcriptase polymerase chain reaction. Part a, reprinted from *Urology*, **62** (Suppl. 1), Schalken, J. A. et al. New targets for therapy in prostate cancer: differential display code 3 (DD3 [PCA3]), a highly prostate cancer-specific gene, 34–43 © 2003 with permission from Elsevier.⁹ Part b permission obtained from The American Association for Cancer Research © van Gils, M. P. et al. The time-resolved fluorescence-based *PCA3* test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. *Clin. Cancer Res.* **13**, 939–943 (2007).¹³

Sampling and methods

The TMA platform involves sample preparation, amplification and detection. This technology uses the first voided urine that is collected after a DRE consisting of three strokes per prostate lobe. The specimen-processing procedure is simplified using urine samples instead of urinary sediments. Cells are lysed and RNA is stabilized by mixing the urine sample with an equal volume of detergent-based stabilization buffer. The processed urine sample can be shipped overnight at room temperature to testing laboratories, or can be stored frozen for longer time periods. The *PCA3* and PSA mRNAs are quantified using similar protocols and reagents, with components specific for the two analytes. The target mRNAs are purified via capture onto magnetic particles coated with target-specific oligonucleotides, amplified using TMA, and the amplification products are detected with chemiluminescent DNA probes in a hybridization protection assay. All assay steps occur in a single tube, and the test can be completed within 6 h.¹⁴

Assay results

The analytical performance of the TMA-based *PCA3* assay has been extensively studied. Post-DRE specimens provided informative rates (number of specimens with sufficient RNA for analysis) of >95% in several studies, compared to 80% using the first morning voided urine or 74% using pre-DRE specimens.¹⁵ This demonstrates that any manipulation of the prostate will shed enough cells in

Table 1 | Results for *PCA3*-based urine testing using different methodologies in predicting biopsy outcome

Study	<i>PCA3</i> -based test methodology	Number of patients	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hessels <i>et al.</i> (2003) ⁸	QRT-PCR	108	0.72	67	83	53	90
Van Gils <i>et al.</i> (2007) ¹³	QRT-PCR	583	0.66	65	66	48	80
Groskopf <i>et al.</i> (2006) ¹⁴	TMA	70	0.75	69	79	50	89
Marks <i>et al.</i> (2007) ¹⁷	TMA	233	0.68	58	72	43	83
Deras <i>et al.</i> (2008) ¹⁸	TMA	570	0.69	54	74	58	74
Haese <i>et al.</i> (2008) ¹⁹	TMA	470	0.66	47	72	39	78

Abbreviations: AUC, area under curve; NPV, negative predictive value; PPV, positive predictive value; QRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; TMA, transcript-mediated amplification.

the urine to provide an informative specimen. The intra-run and inter-run coefficients of variance for *PCA3* and PSA mRNA quantification were low (<13% and <12%, respectively).¹⁴ These data have been confirmed, and the variation between research sites was also shown to be low (<9%), demonstrating a robust performance of the test both within and across sites.^{15,16}

The accuracy and ease of use of the TMA-based *PCA3* test will be key factors in its implementation in clinical practice. The yield of RNA (that is, having sufficient prostate cells) in a sample determines the utility of the test and is, therefore, also very important. The success of the TMA-based test is due to the simplified specimen processing procedure that utilizes the whole urine instead of urine sediments, as well as improvements to mRNA capture (which removes inhibitory substances in the first step of the assay) and amplification technology.¹⁴ If, however, the RNA yield in the specimen is insufficient, patients must return to provide another urine sample. This is inconvenient for the health care system and the patient, who will also need a repeat DRE.

***PCA3* as a predictor of biopsy outcome**

In 2006, the quantitative TMA-based *PCA3* assay was applied to urine samples collected after DRE from men scheduled for prostate biopsy (pre-biopsy population), men who had undergone radical prostatectomy, and healthy men with no risk factors for prostate cancer.¹⁴ The median *PCA3* scores for men in the pre-biopsy group found to have positive biopsies, those in the pre-biopsy group with negative biopsies and healthy men were significantly different ($P < 0.01$), confirming previous data.^{8,13} The greatest diagnostic accuracy in the pre-biopsy population was obtained using a *PCA3* score cutoff of 50. This assay had 69% sensitivity and 79% specificity for prostate cancer detection. At the same sensitivity, the specificity of serum PSA measurement was 60% in this cohort. Although a different methodology was used, the clinical performance was similar to the previously reported quantitative RT-PCR-based *PCA3* test (Table 1).^{8,13} Three independent studies confirmed these results using a *PCA3* score of 35 as the cutoff.^{17–19} Furthermore, they demonstrated that, for men with elevated serum PSA levels and one previous

negative biopsy, the risk of positive biopsy findings correlated with *PCA3* scores (Figure 3). Deras *et al.*¹⁸ demonstrated that men with a *PCA3* score <5 had a positive biopsy rate of 14%, whereas 69% of men with a *PCA3* score >100 had prostate cancer upon biopsy. The analytical performance and diagnostic accuracy of *PCA3* was independent of the serum PSA level and of whether the individual underwent a first biopsy or repeat biopsy. *PCA3* scores were independent of prostate volume and showed no correlation with biopsy Gleason score.

The ratio of uncomplexed PSA to total PSA (%fPSA) is often used to improve the specificity of prostate cancer detection. In the study by Haese and colleagues,¹⁹ *PCA3* had a better diagnostic accuracy than %fPSA for predicting repeat biopsy outcome. Using a *PCA3* score cutoff of 35, *PCA3* had a sensitivity of 47% and a specificity of 72%. In comparison, the specificity of %fPSA (cutoff 25%) was only 23%. Three independent studies demonstrated that, in men undergoing repeat biopsy, *PCA3* was superior to either serum PSA or %fPSA testing for predicting biopsy outcome.^{17–19}

Combination of *PCA3* with other factors

The combination of new prostate cancer biomarkers such as *PCA3* with other diagnostic factors might help to more accurately predict whether cancer is found on prostate biopsies. Logistic regression analysis has shown that the combination of *PCA3* with factors such as serum PSA, prostate volume and DRE findings could increase the diagnostic accuracy to an AUC of 0.75, compared with 0.69 for *PCA3* alone and 0.55 for serum PSA alone.¹⁸ Thus, a combination of biomarkers with other diagnostic indicators of prostate cancer can indeed increase diagnostic accuracy. This was confirmed by Ankerst *et al.*²⁰ by using the Prostate Cancer Prevention Trial (PCPT) risk calculator. In the PCPT risk calculator, six risk factors (serum PSA, DRE, first-degree family history of prostate cancer, biopsy history, age and black ethnicity) are combined for estimating the risk of developing prostate cancer and the risk of developing high-grade disease (Gleason score ≥ 7). Incorporation of *PCA3* into this risk calculator resulted in a significant improvement in the diagnostic accuracy of the original model. When applied to individual patients,

the incorporation of *PCA3* into the risk calculator refined the estimate of prostate cancer risk; a low *PCA3* score indicated a decreased risk for the patient, and a high *PCA3* score indicated an increased risk of developing prostate cancer. Using the case of a 65-year-old man, the original PCPT risk calculator estimated a risk of 26.5%. When a low *PCA3* score was included, the risk decreased to 16.9%, whereas incorporating a high *PCA3* score increased the risk to 43.1%.²⁰ These data show that new biomarkers like *PCA3* can be successfully incorporated into risk calculators, resulting in improvements in prostate cancer detection.

***PCA3* and *TMPRSS2-ERG* gene fusions**

Because prostate cancer is a heterogeneous disease, the use of a panel of biomarkers can further improve diagnostic accuracy. Fusions of the 5' untranslated region of the *TMPRSS2* gene with the *ETS* transcription factors *ERG*, *ETV1*, and *ETV4* have been reported in prostate cancer.^{21,22} Hessels *et al.*²³ showed that noninvasive detection of *TMPRSS2-ERG* fusion transcripts is feasible in urinary sediments obtained after DRE using an RT-PCR-based research assay. Owing to the high specificity of the test (93%), the combination of *TMPRSS2-ERG* fusion transcripts with *PCA3* improved the sensitivity from 62% (*PCA3* alone) to 73% (combined) without compromising the specificity for detecting prostate cancer.

Laxman *et al.*²⁴ demonstrated that *SPINK1*, *GOLPH2* and *TMPRSS2-ERG* were, like *PCA3*, independent predictors of prostate cancer upon repeat biopsy. By combining *PCA3* with these markers in a quantitative, multiplexed RT-PCR analysis, the ROC AUC value improved from 0.66 (*PCA3* alone) to 0.76. This multiplexed, urine-based assay had 66% sensitivity and 76% specificity for detecting prostate cancer in repeat biopsies.

***PCA3* as a prognostic indicator**

The association of *PCA3* score with prostatectomy tumor volume and other clinical and pathological features was assessed by Van Gils *et al.* in 2008.²⁵ They correlated the *PCA3* score in urinary sediments after DRE in 62 men with the prognostic parameters that are assessed in radical prostatectomy specimens; no significant correlation was found between *PCA3* score and Gleason score ($P=0.90$), pathological tumor stage ($P=0.59$), or total tumor volume ($P=0.96$).

More encouraging results were found by Nakanishi *et al.*²⁶ Their study population consisted of 59 men scheduled for prostate biopsies because of serum PSA values >2.5 ng/ml and/or an abnormal DRE, and 83 men diagnosed with prostate cancer who were scheduled for a radical prostatectomy. In the biopsy population, no significant difference was found between *PCA3* score and biopsy Gleason score 6 versus Gleason score ≥ 7 tumors, confirming previous results.¹⁸ However, the *PCA3* score was significantly correlated with tumor volume ($P=0.008$) and Gleason score (6 versus ≥ 7) in prostatectomy specimens ($P=0.005$). Furthermore, the

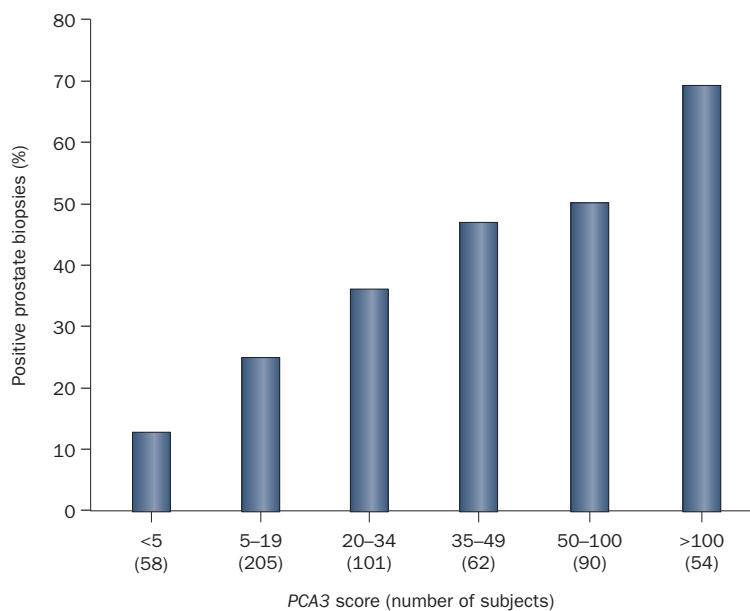


Figure 3 | The correlation of *PCA3* score (determined with the TMA-based *PCA3* test) with the percentage of men with positive prostate biopsies. The probability of a positive prostate biopsy increases with *PCA3* scores. Reprinted from *J. Urol.* **179**, Deras, I. L. *et al.* *PCA3*: a molecular urine assay for predicting prostate biopsy outcome. 1587–1592, © 2008 with permission from Elsevier.¹⁸

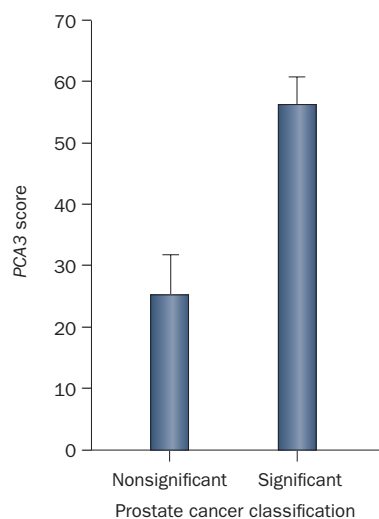


Figure 4 | *PCA3* score and clinical relevance of prostate cancers. The *PCA3* score was found to be significantly higher in patients with clinically significant prostate cancers ($n=85$; Gleason score ≥ 7 , and/or volume >0.5 ml) versus nonsignificant prostate cancers ($n=11$; Gleason score ≤ 6 and volume <0.5 ml, $P=0.007$). Error bars represent standard error of average.²⁶ Reprinted from *J. Urol.* **179**, Nakanishi, H. *et al.* *PCA3* molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. 1804–1809 © 2008 with permission from Elsevier.

PCA3 score was significantly lower in men with clinically insignificant prostate cancer (dominant tumor volume <0.5 ml and Gleason score ≥ 6) compared to the *PCA3*

Table 2 | The possibilities of the TMA-based *PCA3* test in clinical practice

<i>PCA3</i> score + prostate biopsy result	Course of action to consider	Established prognostic factor
Low + negative	Conservative follow-up	PSA kinetics
High + negative	Advanced imaging (e.g. contrast-enhanced MRI)	PSA kinetics
Low + positive	'Active surveillance'	Clinical stage and grade; PSA kinetics
High + positive	Intervention	Clinical stage and grade

score in clinically significant prostate cancer ($P=0.007$) (Figure 4). These data indicate that the *PCA3* score could be effective in helping to determine which men are candidates for active surveillance.

A European multicenter study of 463 men with one or two negative biopsies confirmed that patients with clinically significant prostate cancer had higher *PCA3* scores than those with clinically insignificant prostate cancer ($P=0.0059$).¹⁹ In this cohort, men with clinical stage T2 tumors had a significantly higher *PCA3* score than men with clinical stage T1c tumors ($P=0.005$). *PCA3* has also been found to be an independent predictor of extracapsular extension ($P=0.01$) and of a low tumor volume (<0.5 ml) ($P=0.04$).²⁷ Combined with Gleason score >6 and preoperative serum PSA level, the ROC AUC improved from 0.73 for *PCA3* alone to 0.90 for predicting extracapsular extension. A *PCA3* score cutoff of 47 resulted in 57% sensitivity, 94% specificity and a positive predictive value of 80% for predicting extracapsular extension. Thus, *PCA3* may be indicative of clinical stage and prostate cancer significance.²⁶

The results indicating a correlation between *PCA3* and cancer aggressiveness should be regarded as preliminary. The inconsistency in results between *PCA3* and pathological tumor features might be explained by differences among the study population or in the pathological evaluation of the specimens. The only way to find out its true predictive value is through validation in definitive trials.

***PCA3*: clinical and future applications**

A summary of the potential uses of the *PCA3* score in clinical practice is given in Table 2. *PCA3* measurement can be a useful test when considering the need for a repeat biopsy, especially in men with chronically elevated serum PSA values owing to chronic inflammation or prostatitis. *PCA3* can help to stratify men according to their risk of developing prostate cancer. A negative biopsy and a low *PCA3* score might indicate that a more conservative follow-up is appropriate. Based on preliminary data regarding the prognostic value of *PCA3*, a negative prostate biopsy accompanied by a high *PCA3* score might indicate the need for advanced imaging modalities to locate a clinically significant prostate cancer. If a patient has prostate cancer but the *PCA3* score is low, active surveillance might be considered for a clinically insignificant tumor.

When the biopsy is positive and the *PCA3* score is high, there is a high likelihood of finding clinically significant prostate cancer, and the urologist might decide to recommend intervention.

The information in Table 2 raises the question of what *PCA3* score should be used as the cutoff value in these different clinical applications. With regard to the identification of men with an increased probability of having a positive repeat biopsy, a *PCA3* score cutoff of 35 provided the optimal balance between sensitivity and specificity in a pre-biopsy population in several studies.^{17–19} A low *PCA3* score indicates a low probability of cancer; however, it does not exclude clinically significant prostate cancer in the biopsy. This was shown in the study by Haese and colleagues:¹⁹ a *PCA3* score cutoff of 35 avoided 67% of unnecessary biopsies, while 21% of clinically significant cancers would have gone undetected. If a *PCA3* score of 20 had been used, 44% of the unnecessary biopsies would have been avoided while only 9% of clinically significant cancers would not have been detected. In their cohort, the *PCA3* score cutoff of 20 worked best for both avoiding unnecessary biopsies and minimizing the risk of leaving significant prostate cancer undetected. Whitman and colleagues²⁷ found a median *PCA3* score of 26 in their pre-prostatectomy cohort. Based on the defined *PCA3* score cutoff of 35, half of the prostate cancers in their patient cohort would not have been detected in a pre-biopsy population. Whether this difference in observation is due to the high percentage (25%) of black men in their patient cohort is unclear. Black men represent a group at a particularly high risk for developing prostate cancer. Significant differences in the frequency of risk alleles in these men have been identified and might, in part, explain an increased susceptibility to prostate cancer; however, this observation indicates that *PCA3* score might be more useful in both diagnostic and prognostic applications as a continuous variable in combination with other clinical and/or pathological data.^{18,20}

Efforts to further validate and expand the clinical utility of the TMA-based *PCA3* test continue. Potential applications include the use of *PCA3* prior to a first prostate biopsy, detecting local recurrence following radical prostatectomy or radiation therapy, or monitoring patients receiving drug therapies that affect serum PSA levels (for example, 5 α -reductase inhibitors).

The challenge remains to identify markers that can help to identify potentially life-threatening prostate cancer at a curable stage. Prostate cancer is a heterogeneous disease and, therefore, a panel of biomarkers, including *PCA3* and *TMPRSS2-ERG* gene fusions, will yield more clinical information than any single test.

At the tissue level, *PCA3* is almost completely specific for prostate cancer because of its high overexpression in prostate cancer cells. Studies attempting to delineate the range of transcription factors that interact with the *PCA3* promoter, and to determine the function of *PCA3*, are ongoing.

Conclusion

The CE-marked version of the TMA-based *PCA3* test was launched at the end of 2006, and is now commercially available in Europe and the US under the trade name ProgenSA™ *PCA3* (Gen-Probe Inc., San Diego, CA). In contrast to serum PSA measurement, the TMA-based *PCA3* test directly detects prostate cancer cells in urine. Several studies have shown that *PCA3* score is superior to serum PSA testing for predicting biopsy outcome.^{8,14,17–19} Furthermore, the combination of *PCA3* with *TMPRSS2-ERG* gene fusions improved the sensitivity for prostate cancer diagnosis without compromising the specificity.²³ The diagnostic accuracy can also be increased when *PCA3* is combined with other diagnostic factors (for example, serum PSA level, DRE, first-degree family history of prostate cancer, biopsy history, age and black ethnicity).^{18,20} Preliminary data demonstrate a correlation between *PCA3* and Gleason score, tumor size, clinical significance of tumors, and extracapsular extension.^{18,19,26,27}

The TMA-based *PCA3* test is the first fully translated RNA-based molecular diagnostic assay for prostate cancer in body fluids that is available to urologists. It can aid in the diagnosis of prostate cancer, and its role as a prognostic indicator seems promising. Other genetic markers, such as *TMPRSS2-ERG* gene fusions, are currently under evaluation in urine. Noninvasive biomarker-based assays for the diagnosis of prostate cancer have now become reality.

Review criteria

PubMed was searched with the terms “*PCA3*” and “prostate cancer”. From the citations identified during these searches, publications were selected on the basis of relevance to the subject matter, as well as scientific and clinical value. Only papers published in English were reviewed.

- Jemal, A. *et al.* Cancer statistics, 2008. *CA Cancer J. Clin.* **58**, 71–96 (2008).
- Ferlay, J. *et al.* Estimates of the cancer incidence and mortality in Europe in 2006. *Ann. Oncol.* **18**, 581–592 (2007).
- Roddam, A. W. *et al.* Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2–10 ng/ml: systematic review and meta-analysis. *Eur. Urol.* **4**, 386–399 (2005).
- Hessels, D. *et al.* Applicability of biomarkers in the early diagnosis of prostate cancer. *Expert Rev. Mol. Diagn.* **4**, 513–526 (2004).
- Hessels, D. *et al.* Molecular Diagnostics in Prostate Cancer. *EAU Update Series* **3**, 200–213 (2005).
- Bussemakers, M. J. *et al.* DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res.* **59**, 5975–5979 (1999).
- de Kok, J. B. *et al.* DD3 (*PCA3*), a very sensitive and specific marker to detect prostate tumors. *Cancer Res.* **62**, 2695–2698 (2002).
- Hessels, D. *et al.* DD3 (*PCA3*)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur. Urol.* **44**, 8–15 (2003).
- Schalken, J. A. *et al.* New targets for therapy in prostate cancer: differential display code 3 (DD3 [*PCA3*]), a highly prostate cancer-specific gene. *Urology* **62** (5 Suppl. 1), 34–43 (2003).
- Iwakiri, J. *et al.* An analysis of urinary prostate specific antigen before and after radical prostatectomy: evidence for secretion of prostate specific antigen by the periurethral glands. *J. Urol.* **149**, 783–786 (1993).
- Meng, F. J. *et al.* The expression of a variant prostate-specific antigen in human prostate. *Cancer Epidemiol. Biomarkers Prev.* **11**, 305–309 (2002).
- van Gils, M. P. *et al.* Molecular *PCA3* diagnostics on prostatic fluid. *Prostate* **67**, 881–887 (2007).
- van Gils, M. P. *et al.* The time-resolved fluorescence-based *PCA3* test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. *Clin. Cancer Res.* **13**, 939–943 (2007).
- Groskopf, J. *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).
- Sokoll, L. J. *et al.* A multicenter evaluation of the *PCA3* molecular urine test: pre-analytical effects, analytical performance, and diagnostic accuracy. *Clin. Chim. Acta* **389**, 1–6 (2008).
- Shappell, S. B. *et al.* *PCA3* urine mRNA testing for prostate carcinoma: patterns of use by community urologists and assay performance in reference laboratory setting. *Urology* **73**, 363–368 (2008).
- Marks, L. S. *et al.* *PCA3* molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* **69**, 532–535 (2007).
- Deras, I. L. *et al.* *PCA3*: a molecular urine assay for predicting prostate biopsy outcome. *J. Urol.* **179**, 1587–1592 (2008).
- Haese, A. *et al.* Clinical utility of the *PCA3* urine assay in European men scheduled for repeat biopsy. *Eur. Urol.* **54**, 1081–1088 (2008).
- Ankerst, D. P. *et al.* Predicting prostate cancer risk through incorporation of prostate cancer gene 3. *J. Urol.* **180**, 1303–1308 (2008).
- Tomlins, S. A. *et al.* Recurrent fusion of *TMPRSS2* and *ETS* transcription factor genes in prostate cancer. *Science* **310**, 644–648 (2005).
- Tomlins, S. A. *et al.* *TMPRSS2:ETV4* gene fusions define a third molecular subtype of prostate cancer. *Cancer Res.* **66**, 3396–3400 (2006).
- Hessels, D. *et al.* Detection of *TMPRSS2-ERG* fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin. Cancer Res.* **13**, 5103–5108 (2007).
- Laxman, B. *et al.* A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. *Cancer Res.* **68**, 645–649 (2008).
- van Gils, M. P. *et al.* Detailed analysis of histopathological parameters in radical prostatectomy specimens and *PCA3* urine test results. *Prostate* **68**, 1215–1222 (2008).
- Nakanishi, H. *et al.* *PCA3* molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J. Urol.* **179**, 1804–1809 (2008).
- Whitman, E. J. *et al.* *PCA3* score before radical prostatectomy predicts extracapsular extension and tumor volume. *J. Urol.* **180**, 1975–1978 (2008).

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