Rational Approach to Implementation of Prostate Cancer Antigen 3 Into Clinical Care

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BACKGROUND: Prostate cancer antigen 3 (*PCA3*) encodes a prostate-specific messenger ribonucleic acid (mRNA) that serves as the target for a novel urinary molecular assay for prostate cancer detection. The objective of the current study was to evaluate the ability of PCA3, added to measurements of serum prostate-specific antigen (PSA), to predict cancer detection by extended template biopsy. **METHODS:** Between September 2006 and December 2007, whole urine samples were collected after attentive digital rectal examinations from 187 men before they underwent ultrasound-guided, 12-core prostate biopsy in a urology outpatient clinic. Urine PCA3/PSA mRNA ratio scores were measured within 1 month, and serum PSA was measured within 6 months prior to biopsy. Those measurements were related to cancer-positive biopsies. **RESULTS:** Overall, 87 of 187 biopsies (46.5%) were positive for cancer. The sensitivity and specificity of a PCA3 score $\geq$35 for positive biopsy were 52.9% and 80%, respectively, and the positive and negative predictive values were 69.7% and 66.1%, respectively. By using receiver operating characteristic curve analysis, PSA alone resulted in an area under the curve (AUC) of 0.63 for prostate cancer detection; whereas a combined PSA and PCA3 score resulted in an AUC of 0.71. The likelihood of prostate cancer detection rose with increasing PCA3 score ranges ($P > .0001$), providing possible PCA3 score parameters for stratification into groups at low risk, moderate risk, high risk, and very high risk for a positive biopsy. **CONCLUSIONS:** Adding PCA3 to serum PSA improved prostate cancer prediction. The use of PCA3 in a clinical setting may help to stratify patients according to their risk for biopsy and cancer detection, although a large-scale validation study will be needed to address assay standardization, optimal cutoff values, and appropriate patient populations. **Cancer 2009;115:3879–86. © 2009 American Cancer Society.**

KEY WORDS: prostatic neoplasms, genes, biopsy, urine.

Prostate-specific antigen (PSA) has revolutionized the evaluation and treatment of prostate cancer (PCa) since its initial discovery in 1979,[1] resulting in increased cancer detection and subsequent downward stage migration.[2] Yet there remains significant debate regarding optimal PSA thresholds for cancer detection.[3] To refine risk stratification, derivative measurements, such as percentage free PSA,[4,5] age-specific PSA ranges,[6] and PSA velocity,[7] have been proposed but are constrained by limitations similar to those of PSA itself. That is, nonmalignant conditions, such as benign prostatic hyperplasia and prostatitis, can cause

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PSA elevations[8] and can result in unnecessary and repeated prostate biopsies. These limitations of PSA as a prognostication tool have led to an intensive search for other PCa biomarkers.

PCa antigen 3 (PCA3) (also known as differential display code 3 [*DD3*]) is a prostate-specific gene that was present in 95% of initially studied samples of PCa[9] and was overexpressed significantly in cancer tissues versus benign tissues.[10] PCA3 is known as a noncoding messenger ribonucleic acid (mRNA) that has no resultant protein. Clinically, PCA3 mRNA is detectable in the urine and prostatic fluid of men with PCa. PCA3 mRNA levels are independent of prostate volume and serum PSA but may be higher in patients who have larger, more aggressive tumors.[11] PCA3 now serves as the target for a novel urinary molecular assay for PCa detection.[12-15] This clinical test requires the collection of urine after an attentive digital rectal examination (DRE) to increase the number of prostate cells shed into the urine,[6,7] and all versions of this assay are reported as a ratio of PCA3 mRNA/PSA mRNA.

Currently, several urinary PCA3 assays exist, and initial feasibility studies in Europe have relied on a time-resolved fluorescence-based (TRF), quantitative reverse transcriptase-polymerase chain reaction-based methodology. The only commercially available PCA3 assay in the United States uses whole urine rather than sediment and relies on magnetic microparticle capture, transcription-mediated RNA amplification, and hybridization protection assay detection of PSA and PCA3 mRNA. With an initial cutoff value set at 50, this assay demonstrated a sensitivity of 69% and specificity of 79%.[13] The TRF-based version has demonstrated sensitivities of 65% to 67%, specificities of 66% to 83%, and negative predictive values of 80% to 90%.[10,15] The nucleic acid sequence-based amplification urine uPM3 assay (DiagnoCure, Inc., Quebec City, Quebec, Canada) has demonstrated similar results; with a cutoff of 0.5, sensitivity ranged from 66% to 82%, specificity ranged from 76% to 89%, and negative predictive value ranged from 84% to 87%.[12,14] PCA3 is not intended to be used alone for PCa screening at this time; and, to date, all studies have investigated its utility in conjunction with PSA and other biomarkers.

Although initial results from these studies are promising, they cannot be generalized to all populations, because study cohorts were comprised of only prescreened patients who were undergoing biopsy for an elevated PSA. The unrestricted widespread use of novel biomarkers like PCA3 without consideration for the possible results may result in unanticipated consequences.[16] Our objectives were to describe the ability of urine PCA3, combined with serum PSA, to improve PCa detection on biopsy versus serum PSA alone and to discuss potential downstream effects of this new biomarker. In this report, we examine the available clinical evidence and illustrate the benefits and limitations of using PCA3 in clinical practice.

MATERIALS AND METHODS

Patient Selection and Sample Processing

From September 2006 to December 2007, whole urine specimens were collected from men after an attentive DRE and before ultrasound-guided, 12-core prostate biopsy according to a protocol approved by the Institutional Review Board at the University of Michigan. All men who presented for prostate biopsy were approached about participating in this prospective database study. All prostate biopsies were performed within 1 month of DRE and urine specimen collection, and both prostate examinations and biopsies were completed by a single surgeon (J.T.W.) at a urologic outpatient satellite clinic. Inclusion criteria included adult men who were undergoing prostate biopsy for any of the following reasons: elevated or rising PSA, <15% free PSA, PCa risk factors, previous atypical small acinar proliferation (ASAP) or high-grade prostate intraepithelial neoplasia (HGPIN), or abnormal DRE. Exclusion criteria included a history of PCa or previous prostate surgery, urine not collected after DRE and before prostate biopsy, inadequate prostate biopsy with <12 cores, or men who declined their consent to participate in the study.

An attentive DRE included firm pressure on the prostate from base to apex and from lateral to median lobe, with 3 strokes per lobe and enough pressure to slightly depress the prostate surface.[17] After DRE, patients collected their initial void of 20 mL to 30 mL of urine, and the urine was processed for the Gen-Probe assay performed off-site at Molecular Profiling Institute. Clinic specimen processing involved transferring urine into a transport tube that contained a detergent-based stabilization buffer and keeping all specimens at or below 30°C. PCA3 and PSA mRNAs were isolated from urine and

underwent transcription-mediated amplification; the products were detected with the hybridization protection Gen-Probe assay using target-specific, acridinium ester-labeled probes. PCA3 scores were reported as a quantitative PCA3/PSA mRNA ratio × 1000 to normalize PCA3 to the amount of prostate RNA present in the urine sample. Cases with insufficient PSA mRNA were considered inconclusive and were excluded. A PCA3 score ≥35 was considered positive (according to the laboratory standard), and serum PSA levels were measured within 6 months before prostate biopsy.

Prostate biopsy was performed within 1 month after urine collection for the assay. At minimum, all patients underwent a transrectal ultrasound-guided. 12-core biopsy using a sextant template, and patients underwent additional biopsies if they had lesions discovered on palpation or imaging or as clinically indicated. Prostate parameters and total volume were measured at the time of biopsy.

Data Analysis

Clinical data, including final prostate biopsy pathology results, were collected prospectively using explicit data collection tools. Prognostic ability (sensitivity, specificity, negative and positive predictive values) of the PCA3 test was determined by cross-tabulation. Logistic regression models were used to produce data for receiver operating characteristic (ROC) curves, with area under the curve (AUC) values determined from the model c-statistic. The Mantel-Haenszel chi-square test was used to test for associations between ordinal PCA3 groupings and the positive biopsy rate. Pearson correlation coefficients were used to explore the association between PCA3 and prostate volume. All tests were performed at the 5% significance level using SAS statistical software (version 9.1; SAS Institute, Inc., Cary, NC).

RESULTS

Overall, 192 men consented to participate in the research study and submitted whole urine samples for PCA3 analysis; of these, 187 samples yielded sufficient RNA for analysis, resulting in a specimen informative rate of 97.4%. The demographics of our patient population are detailed in Table 1. The mean age of the biopsied men was 62 years, and their mean prostate volume was 59.4 g. Biopsy was performed for elevated PSA in most patients, and 16% of patients had an abnormal DRE. Most patients underwent biopsy for the first time. The mean PSA was 8.7 mg/mL (standard deviation [SD], 12.4 ng/mL), and the mean PCA3 score was 41.1 (SD, 63.3). Overall, PCa was diagnosed by biopsy in 87 men (46.5%), most men had Gleason 6 or 7 PCa, and approximately 25% had Gleason ≥8 PCa. There was no relation between PCA3 score and prostate volume ($P = .26$). The Spearman rank-

Table 1. Demographics for 187 Men Undergoing Prostate Biopsy

Parameter	No. of Patients (%)
Total no.	187 (100)
Age, y	
Mean±SD	62±8.3
Range	44-86
Prostate volume, g	
Mean±SD	59.4±39.2
Range	9-241
Race	
Caucasian	171 (91.5)
Black	10 (5.3)
Other	6 (3.2)
Ethnicity	
Non-Hispanic	181 (97)
Hispanic	2 (1.1)
Unknown	4 (2.1)
Family history of PCa	
Yes	35 (18.7)
Reason for biopsy	
Elevated PSA	166 (88.8)
HGPIN	11 (5.9)
ASAP	4 (2.1)
Other	19 (10.2)
Previous biopsies	
None	136 (72.7)
≥1	51 (27.3)
Biopsy positive	87 (46.5)
Gleason 6	32 (36.8)
Gleason 7	33 (37.9)
Gleason ≥8	22 (25.3)
Biopsy stage	
T1c	60 (69)
T2a	19 (21.8)
T2b	4 (4.6)
T2c	1 (1.1)
≥T3	3 (3.4)

SD indicates standard deviation; PCa, prostate cancer; PSA, prostate-specific antigen; HGPIN, high-grade intraepithelial neoplasia; ASAP, atypical small acinar proliferation.

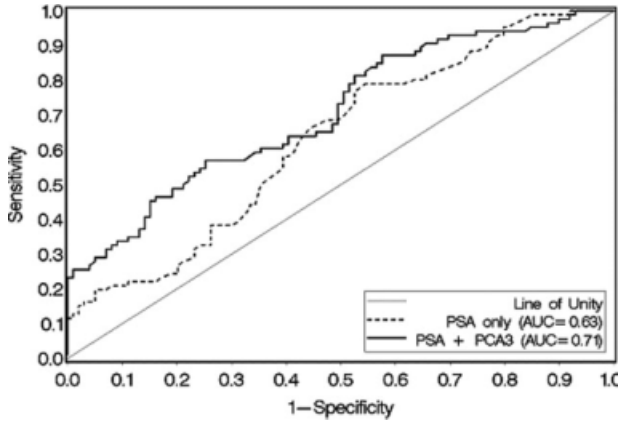

FIGURE 1. Receiver operating characteristic (ROC) curve analysis for serum prostate‐specific antigen (PSA) versus serum PSA and prostate cancer antigen 3 (PCA3) urine assay score. ROC curve analysis was used to compare serum PSA alone (dashed line) versus serum PSA plus urine PCA3 score (solid line) as a predictor of positive prostate biopsy. The area under the curve for serum PSA alone was 0.63 versus 0.71 for serum PSA plus PCA3 score.

correlation was 0.25 between PCA3 and biopsy Gleason score and 0.43 between PCA3 and the percentage of positive biopsy cores.

The overall sensitivity and specificity of a PCA3 score >35 for positive biopsy in this cohort were 52.9% and 80%, respectively. The positive predictive value of PCA3 score for PCa was 69.7%, and the negative predictive value was 66.1%. By using ROC curve analysis, serum PSA alone resulted in an AUC of 0.63 for a positive diagnosis of PCa, whereas the combination of PSA and PCA3 score resulted in an improved AUC of 0.71 (Fig. 1). Logistic regression analysis indicated that PCA3 ($P = .001$) was associated independently with a positive biopsy after adjusting for the effect of PSA ($P = .07$). In addition, PCA3 ($P = .003$) remained a significant predictor of PCa risk after adjusting for other clinical factors, including age, family history, number of previous biopsies, DRE results, and PSA. When the cohort was divided further into men who underwent an initial biopsy or a repeat biopsy, both PSA ($P = .02$) and PCA3 ($P = .004$) independently predicted a positive biopsy in the initial biopsy cohort (n = 136).

Regarding PSA breakdown, 151 patients had a PSA <10 ng/mL, and 34 patients had a PSA >10 ng/mL. The AUC for PCA3 plus PSA could be calculated for patients with a PSA <10 ng/mL (0.69) and for patients with a PSA >10 ng/mL (0.90). The performance characteristics in the group with PSA <10 ng/mL were similar to those for the overall group (AUC, 0.71), indicating that the addition of PCA3 still was useful in the cohort with PSA <10 ng/mL.

Figure 2 illustrates a significant rise in the proportion of positive biopsies with increasing PCA3 score ranges (Mantel-Haenszel chi-square test; $P>.0001$), providing clinically meaningful parameters for stratification into groups with a low risk (PCA3 score, <5; 10.5% positive biopsies), a moderate risk (PCA3 score, 5-34; 38.2% positive biopsies), a high risk (PCA3 score, 35-100; 64.7% positive biopsies), and a very high risk (PCA3 score, >100; 86.7% positive biopsies) of having a positive biopsy. From these data, we calculated that the overall utility rate for the PCA3 score was 45%, signifying that 85 of 187 men with either low or high PCA3 score ranges (eg, <5, 35-100, and >100) would experience a change in their cancer detection rate relative to their pre-PCA3 score probability of cancer detection (46.5% in this cohort).

DISCUSSION

The limitations of serum PSA alone for the detection and risk stratification of PCa have been explored extensively. Through conventional PSA surveillance, clinicians both are missing patients who have PCa with a nonelevated PSA and are performing a large number of unnecessary biopsies to detect a smaller proportion of questionably clinically significant tumors. By using a standard cutoff of 4.0 ng/mL, 15% of men who have a PSA <4.0 ng/mL have biopsy-proven PCa, and a subsequent 15% of those men harbor cancer with a Gleason score ≥7.[3] In addition, PSA has not been able to predict lethal PCa with precision.[18] These issues have remained points of controversy long after PSA was disseminated widely for screening and evaluation. This sequence of events should prompt careful planning for the next generation of PCa biomarkers to ensure their measured dissemination into clinical practice.

PCA3 currently is the only urinary PCa biomarker to progress past the initial discovery phases and to be translated into a commercial assay. Despite promising initial studies, the optimal clinical utility of PCA3 remains unclear. Reviewed here are several clinical scenarios that inevitably will arise as PCA3 is disseminated into practice.

Screening

PCa screening is performed currently with annual DREs and the serum PSA test. A threshold of 4 ng/mL for serum

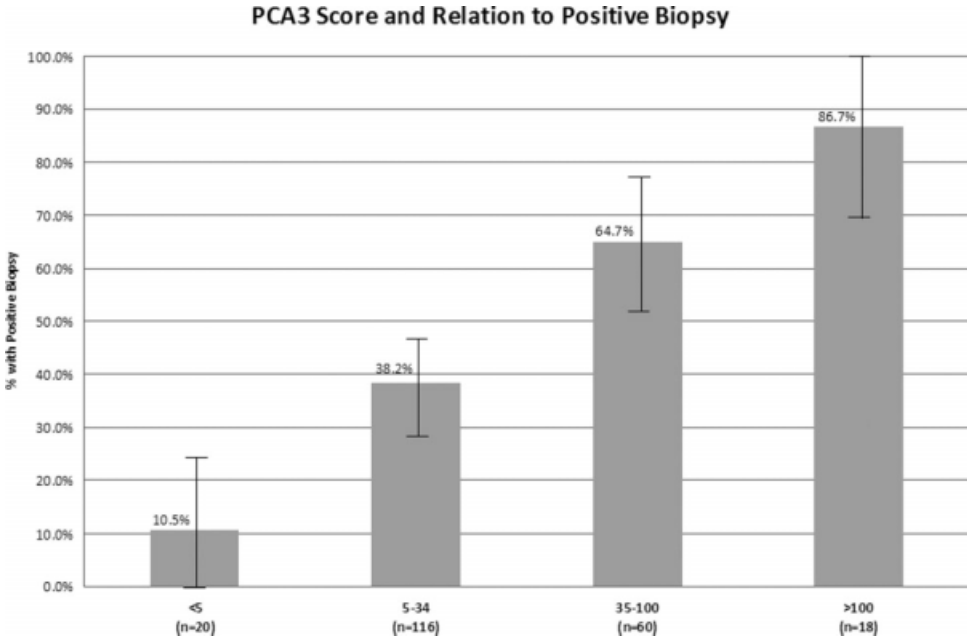

FIGURE 2. Prostate cancer antigen 3 (PCA3) urine assay scores were related to the percentage of positive biopsies. There was a significant rise in the proportion of positive biopsies with increasing PCA3 score ranges ($P > .0001$). A PCA3 score of 35 was used as a cutoff level.

PSA typically us used as the upper limit of normal, although optimal screening levels remain controversial.[3] Although a new biomarker ultimately could be adapted for screening, to our knowledge, there have been no studies to date investigating urine PCA3 as a screening tool. Similarly, all published PCA3 studies have investigated its performance in prescreened populations of men who were referred for biopsy, often for an elevated PSA, and there have been no head-to-head studies comparing PSA with PCA3 in a general population. Thus, there is no empiric evidence at this time to support supplanting serum PSA with the urine PCA3 assay in a screening context, and widespread use of PCA3 in the absence of PSA would be ill-advised. Moreover, the utility of PCa screening in general still is questioned; the results of the randomized, multicenter Prostate Lung Colon Ovarian trial are awaited anxiously to determine the benefit of PCa screening in decreasing cancer-specific mortality.[19]

Adjunct to Prostate-Specific Antigen for Initial Prostate Biopsy

An elevated serum PSA >4.0 ng/mL or a focal prostate nodule often prompts referral for transrectal ultrasound-guided prostate biopsy. Recent studies investigating PCA3 have demonstrated that, in a prescreened population referred for biopsy, the urine PCA3 score correlated well with the probability of cancer on biopsy. Several studies have demonstrated a significant difference in the median PCA3 score between healthy, biopsy-negative groups and biopsy-positive groups.[11,13] The PCA3 score also demonstrated a direct correlation with the probability of positive biopsy in a study by Deras et al[20]; those results also were consistent with what we observed in our cohort. These PCA3 risk groups may provide possible parameters for the risk of a positive biopsy when used as a PSA adjunct. In our cohort, the combination of PSA and PCA3 resulted in superior PCa prediction over PSA alone. In a logistic regression algorithm published by other authors, the incorporation of PCA3 into a model with serum PSA, prostate volume, and DRE resulted in an improved AUC from 0.69 for PCA3 alone to 0.75 ($P = .0002$).[20] In the current study, we also demonstrated that PCA3 remained an independent predictor of a positive biopsy after adjusting for multiple clinical factors.

Furthermore, PCA3 may be valuable, because its performance characteristics demonstrate stability across serum PSA levels and independence from prostate volume.[20] Thus, PCA3 potentially may be used to determine risk for positive biopsy across all PSA ranges. More

recently, it was observed that the PCA3 test was able to differentiate men with low-risk, low-volume PCa,[11] although these data have yet to be verified independently.

Adjunct to Prostate-Specific Antigen for Repeat Prostate Biopsy

Repeat prostate biopsy is indicated for patients who have a prior negative biopsy but continue to have an elevated serum PSA or abnormal DRE or for the follow-up of previous pathologic diagnoses of premalignant HGPIN or ASAP. In our series, >25% of all biopsies were repeat biopsies. It remains unclear when and how often to repeat a prostate biopsy. There is a documented decline in cancer detection with each successive biopsy[21]; and, for men with persistently elevated PSA who are undergoing repeat biopsy, Marks and colleagues demonstrated limited reliability of PSA in PCa prediction and a significant superiority of the urine PCA3 assay in 226 men who were undergoing repeat biopsy (AUC, 0.68 for PCA3 vs 0.52 for serum PSA; $P < .01$).[22] Another study indicated that, in a subanalysis, the diagnostic accuracy of PCA3 was similar between men who were undergoing their first biopsy versus men who were undergoing a repeat biopsy,[20] although there has been no similar study investigating PCA3 performance only among men who were undergoing repeat biopsy. Our own analysis of the predictive ability of PCA3 with PSA in the repeat biopsy cohort failed to confirm the findings of Marks et al, although this may have been secondary to the small repeat biopsy sample size. There is a suggestion that PCA3 may improve the pretest probability significantly for men who are considered for a repeat biopsy,[22] although this also remains unconfirmed.

Post-Treatment Cancer Surveillance

Clinically localized PCa often is treated with radical prostatectomy or radiotherapy (brachytherapy or external beam); serum PSA is the primary means of monitoring for biochemical recurrence after radical prostatectomy, tumor persistence, or treatment failure after radiotherapy. In the initial descriptive study of the PCA3 assay, PCA3 and PSA signals were detected at only background levels in 20 of 21 men who had undergone prostatectomy, and mRNA copy numbers were insufficient for analysis; the only postprostatectomy specimen that yielded measurable PCA3 and PSA signals was from a patient who developed a biopsy-positive recurrence.[13] Since then, to our knowledge, no study has investigated the use of PCA3 in a postprostatectomy cohort; and, unlike PSA in biochemical recurrence, there are no current data to support using PCA3 for post-treatment PCa surveillance, although this is biologically plausible.

Potential Impact and Limitations of Prostate Cancer Antigen 3 for Risk Stratification

The strongest evidence available supports the use of PCA3 as an adjunct to PSA to determine the risk of having a positive biopsy. For example, a man aged 56 years has an elevated PSA of 4.1 ng/mL on annual PSA testing and has no obvious nodule palpable on DRE. In this man, previous PSA levels have been <4 ng/mL, and he has had no episodes of prostatitis. His likelihood of having a positive prostate biopsy is 47% in our cohort. In this scenario, the PCA3 assay may be helpful in determining the risk for positive biopsy. If his PCA3 score returns <5, then our data suggest a positive biopsy rate of approximately 10%, significantly lower than our overall positive biopsy rate. Alternatively, if his PCA3 score returns to between 35 and 100 or >100, then his rate of positive biopsy increases to 65% or 87%, respectively. Even taking into account some variability in the overall positive biopsy rate from different cohorts, these changes in pre-PCA3 assay and post-PCA3 assay probability for approximately 50% of patients in this cohort can have a significant impact on the mutual decision to continue PSA surveillance or to proceed with repeat biopsy.

Although PCA3 appears to improve PCa detection, it has inherent limitations. There is no international standard for the urinary assay, and all methods rely on urine obtained immediately after an attentive DRE. This is not unlike PSA, for which there are several assays, and reported values vary based on the assay method of PSA measurement used.[23,24] Specimen informative rates generally are high, but a small proportion of men will have to provide repeat urine samples after an inadequate DRE to express a sufficient number of prostate cells. Furthermore, it is unclear whether a suboptimal DRE or a small peripheral tumor that produces a minimal number of shed cells

into the urine can result in a falsely negative PCA3 score; and, although no relation has been observed between PCA3 score and prostate volume, a recent report suggests that PCA3 RNA can be detected in HGPIN and benign tissue proximal to neoplastic glands, suggesting the existence of precursor molecular changes.[25] It has yet to be determined whether this can result in false-positive results with HGPIN pathology. Finally, although a PCA3 score $\geq$35 has been adopted as a preliminary positive cut-off point, 33.9% of men in our sample who had a PCA3 score $<$35 had PCa on biopsy, providing a reminder that risk remains a continuous rather than dichotomous variable.

Despite the evidence for PCA3, its exact role in clinical practice has yet to be truly validated. With regard to phases of biomarker validation,[26] PCA3 has passed the initial preclinical phase of laboratory exploratory study and clinical assay development and is being investigated currently in prospective trials. Before PCA3 becomes propagated widely as a novel biomarker for PCa detection or screening, a bona fide validation study should be conducted to confirm findings from limited, single-institution studies, to refine assay standardization, and to define the most relevant patient population for application. It should be recognized that existing studies of PCA3 are limited by their lack of multi-institution accrual, small sample sizes, and potential selection bias.

In conclusion, there is mounting evidence to suggest that a combination of urine PCA3 and serum PSA is superior to PSA alone for the detection of PCa, although those studies were limited to prescreened patients who had elevated or rising PSA levels. PCA3 may serve as a useful adjunct to determine the risk for a positive prostate biopsy and may be useful in counseling men who are contemplating a repeat biopsy; although the question remains whether we merely are contributing to the over-diagnosis of PCa that is not clinically significant. To date, there is no definitive evidence demonstrating that PCA3 can predict lethal PCa; and, in the absence of such evidence, these biomarkers may only contribute to the continued over-diagnosis of PCa. Nevertheless, if our objectives are to minimize unnecessary prostate biopsies and to optimize early PCa detection, then PCA3 appears to be worthy of systematic, large-scale validation to clarify its role as a passing trend or as a valuable next-generation biomarker for the detection of PCa.

Conflict of Interest Disclosures

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John T. Wei and Arul M. Chinnaiyan are consultants for Gen-Probe.

Kirk J. Wojno is employed by Ameripath and serves on the scientific advisory board for Gen-Probe and Onconome.

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