PCA3 Molecular Urine Test as a Predictor of Repeat Prostate Biopsy Outcome in Men with Previous Negative Biopsies: A Prospective Multicenter Clinical Study

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Abbreviations and Acronyms

DRE = digital rectal examination
LR = logistic regression
NPV = negative predictive value
PCa = prostate cancer
PCA3 = PCa gene 3
PPV = positive predictive value
PSA = prostate specific antigen
SOC = standard of care
sPSA = serum PSA

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See Editorial on page 11.

Purpose: We evaluated the clinical usefulness of the PROGENSA® PCA3 Assay for predicting repeat prostate biopsy outcome.

Materials and Methods: Men with at least 1 prior negative prostate biopsy who were scheduled for repeat prostate biopsy based on best clinical judgment were enrolled at 14 centers. Whole blood and post-digital rectal examination urine samples were collected before extended template transrectal biopsy with 12 or more cores. Urinary PCA3 scores and biopsy outcomes were assessed by logistic regression analysis, which also included age, race, serum prostate specific antigen, clinical stage, family history of prostate cancer and the number of previous negative biopsy sessions.

Results: A total of 466 men were included in study and prostate cancer was identified in 21.9%. A PCA3 score cutoff of 25 yielded 77.5% sensitivity, 57.1% specificity, and negative and positive predictive values of 90% and 33.6%, respectively. On multivariable logistic regression men with a PCA3 score of less than 25 were 4.56 times as likely to have a negative repeat biopsy as men with a score of 25 or greater. PCA3 score significantly increased the predictive accuracy of the logistic regression model. At 90% sensitivity adding the PCA3 score to the model increased specificity, and positive and negative predictive values by 22.6%, 6.4% and 7.1%, respectively, relative to the model without the PCA3 score.

Conclusions: The PCA3 score supplements serum prostate specific antigen and other clinical information to provide more accurate prediction of repeat biopsy outcome. Thus, it provides clinicians and patients with independent, clinically useful information to make more informed repeat biopsy decisions.

Key Words: prostate; prostatic neoplasms; biopsy; prognosis; prostate cancer antigen 3, human

In 2012 the American Cancer Society estimated that there would be 241,740 new PCa cases and 28,170 deaths.[1] sPSA is the most widely used biomarker for identifying men at risk for PCa. While sPSA is prostate specific, it is not cancer specific. Thus, a continuum of PCa risk exists, such that high and low sPSA levels are not always associated with the presence/absence of PCa. This has led to significant controversy over the usefulness of sPSA to

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screen for PCa, although much of the concern has to do with the morbidity of diagnosis and treatment.[2]

Most initial prostate biopsies are negative for cancer.[3–5] However, because prostate biopsies are usually unguided and subject to false-negative results, many men undergo a second, third or more prostate biopsies after an initial negative biopsy. Repeat prostate biopsy is positive in approximately 20% to 35% of patients[6–8] but this means that as many as 80% repeat biopsies may be unnecessary.

Prostate biopsy is associated with significant morbidity, including pain, anxiety, hemospermia, hematuria, rectal bleeding, urinary retention, urinary tract infection and sepsis.[9,10] The hospital admission rate for complications after prostate biopsy has increased.[11] Physicians and patients would benefit from more accurate methods that could be used in conjunction with other clinical factors to stratify men by the risk of a positive repeat biopsy. Such a test could help avoid unnecessary repeat biopsies.

PCA3 is a noncoding, prostate tissue specific RNA that is over expressed in 95% of prostate tumors with median 66-fold up-regulation compared to benign prostate tissue.[12–14] The PROGENSA PCA3 Assay quantifies PCA3 RNA in post-DRE urine specimens to generate a PCA3 score. Several groups reported using a PCA3 score cutoff of 35,[15–19] while others explored the use of lower PCA3 score cutoffs.[20–23] We evaluated the clinical usefulness of the PCA3 assay to predict repeat biopsy outcome in a population based in the United States.

MATERIALS AND METHODS

Study Population

We prospectively obtained clinical and demographic information, pre-DRE/prebiopsy blood, post-DRE urine and biopsy samples from men recruited at 14 geographically diverse, community based urology clinics, group health organizations and academic institutions in the United States. The distribution of institution type and geographic diversity was intended to ensure that results could be widely generalized to the American population.

Participants were men without PCa with 1 or more previous negative prostate biopsy session who were recommended by their physician for repeat biopsy. Subjects were excluded if the most recent negative biopsy consisted of fewer than 8 cores or it was done within 42 days before post-DRE urine collection. Exclusion criteria also included PCa history, use within the previous 90 days of medications or hormones known to affect sPSA, including 5-α-reductase inhibitors, clinical symptoms of urinary tract infection, history of invasive therapy for benign prostatic hyperplasia or lower urinary tract symptoms within 180 days of enrollment and participation in treatment studies within 6 months of enrollment. The target population was men 50 years old or older.

Sample Collection

Blood, post-DRE urine and biopsy samples were collected in that order from each subject. Samples were generally collected within 24 hours of each other. If this was not possible, the post-DRE urine sample was collected within 7 days after the blood sample and biopsy samples were collected within 7 days after urine collection.

sPSA Testing

Whole blood samples were collected, processed to serum and tested based on the standard protocol at each site using institutionally validated sPSA tests in accordance with manufacturer specifications. Laboratory personnel were blinded to subject clinical status, PCA3 score and biopsy result.

Post-DRE Urine Sampling and PCA3 Measurement

First catch urine (20 to 30 ml) was collected after a DRE of 3 strokes per prostate lobe. Urine samples were processed within 15 minutes of collection or maintained at 2C to 8C or on ice and processed within 4 hours of collection. Urine (2.5 ml) was transferred to a PROGENSA PCA3 Urine Transport Tube (Gen-Probe®) and shipped to 1 of 3 designated testing sites. Upon receipt, samples were stored according to package insert instructions at 2C to 8C for up to 14 days. PCA3 testing was generally completed within that time. If samples could not be tested within 14 days, they were frozen at −20C or less and tested within 90 days of collection. Laboratory personnel were blinded to subject clinical status, and sPSA and biopsy results.

The PCA3 assay was described previously.[16,24] Briefly, the assay uses target RNA purification by hybridization to magnetic particles coated with target specific oligonucleotides, transcription mediated amplification of target RNA and a hybridization protection assay to specifically detect amplification products using chemiluminescent probes. The method separately quantifies PCA3 and PSA RNAs in urine. PSA mRNA serves as a prostate specific housekeeping gene to which PCA3 RNA copy numbers are normalized and it ensures that the RNA yield is sufficient to generate a reliable result. The assay final output is the PCA3 score, calculated using the formula, PCA3 score = [(PCA3 RNA copies per ml)/(PSA RNA copies per ml)] × 1,000.

Study Biopsies

Transrectal ultrasound guided biopsies of 12 cores or greater were performed according to the normal procedure at each study site. Each specimen was evaluated at the site pathology facility according to institutional procedures. All pathologists were blinded to PCA3 assay and other test results. Gleason scores, total cores obtained and the number of positive cores were recorded for each subject.

Statistical Analysis

A sample size of 100 biopsy positive subjects was required to show a statistically significant OR using a multivariable LR model. Assuming that the positive biopsy prevalence was 20%, an enrollment of 500 subjects was planned. PCA3 score was categorized as a binary variable with scores less than 25 considered negative. The cutoff was