PCA3 permutation increases the prostate biopsy yield

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Background: A direct correlation between the preoperative prostate cancer antigen 3 (PCA3) gene and total tumor volume in postprostatectomy specimens has recently been reported. This suggests that the PCA3 score could serve as a surrogate for tumor burden in patients with prostate cancer. Accordingly, the PCA3 density (that is, the ratio of the PCA3 score to prostate volume) is representative of the degree of prostate volume occupied by tumor.

Objective: To show that the PCA3 density would be directly related to the likelihood of finding cancer on prostate biopsy, given that larger tumors in smaller glands would be more likely to be detected through prostate biopsy.

Methods: We identified 288 men referred for prostate biopsy for an elevated prostate-specific antigen (PSA) level, high PSA velocity, low free- to total-PSA ratio, or suspicious digital rectal exam. All of the patients had had a urinary PCA3 test performed no more than 4 weeks before biopsy, and prostate volume was recorded by transrectal ultrasound determination at the time of biopsy. The diagnostic yield of PSA level, PSA density (PSAD), PCA3 score, and PCA3 density in detecting cancer was evaluated using a receiver operating characteristic (ROC) curve.

Results: Of the 288 patients included for analysis, 183 (63.5%) underwent an initial prostate biopsy and 105 (36.5%) had at least 1 previous negative biopsy. Cancer was detected in 74 (25.7%) patients. The area under the curve was 0.486 for PSA level, 0.590 for PSAD, 0.687 for PCA3 score, and 0.717 for PCA3 density.

Conclusion: PCA3 density is strongly correlated with cancer detection and may be useful in selecting patients for biopsy.

Serum prostate-specific antigen (PSA) testing and digital rectal exam (DRE) have been the primary tools for assessing a patient's risk for harboring prostate cancer.[1] Permutations of the former—including PSA velocity, PSA density (PSAD), and free- to total-PSA ratio—have all been used to guide clinicians in assessing an individual patient's risk of cancer. Despite these refinements in the early detection process, thousands of negative biopsies occur annually. The morbidity of prostate biopsy has been well documented.[2]

The prostate cancer antigen 3 (PCA3) gene is highly overexpressed in prostate cancer cells in comparison with benign prostatic cells.[3] A urine assay for the expression of this PCA3 protein after the DRE has been shown to be useful in detecting prostate cancer.[4,5] More recently, investigators have reported a direct correlation between the preoperative serum PCA3 score and the total tumor volume in postprostatectomy specimens,[6,7] which suggests that the PCA3 score may serve as a surrogate for total tumor volume in patients who harbor prostate cancer.

On the basis of that finding, we hypothesized that PCA3 density (that is, the ratio of the PCA3 score to total prostate volume) may represent the percentage of prostate volume occupied by tumor. Accordingly, since transrectal ultrasound (TRUS)–guided biopsy is a random sampling of a small proportion of total prostate tissue, there would be a greater likelihood of finding cancer in the biopsy specimen because the PCA3 density increased. We tested our hypothesis in a cohort of men undergoing prostate biopsy.

Materials and methods

From the beginning of February 2008 to the end of February 2010, we identified 288 consecutive men who presented for TRUS-guided prostate biopsy because of a laboratory or clinical suspicion

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of prostate cancer and enrolled them prospectively into our study. The decision to biopsy was made if a patient had an elevated age-adjusted PSA value, a PSA velocity greater than 0.4 ng/dL a year, a low free-PSA level, or an abnormal DRE. We used a 12-core biopsy scheme in all of the patients.

The patients underwent a vigorous DRE on the day of their biopsies, followed by collection of a 20-mL aliquot of urine for a PCA3 assay, in accordance with a previously published protocol.[8] Prostate volume was estimated by conducting a TRUS-guided biopsy and using the conventional prolate ellipsoid formula (length x height x width x [π/6]).

PSA density and PCA3 density values were calculated by dividing the indicated measure by the prostate volume. The diagnostic yield of PSA, PSAD, PCA3, and PCA3 density in detecting cancer was evaluated through a receiver operating characteristic (ROC) curve analysis. We performed Pearson chi-square tests to determine if there was a relationship between biopsy positivity rates and the three PCA3 density categories. Statistical analyses were performed using the SPSS statistical software program (version 16.0.2, 2008). *P* values less than .05 were considered a priori to indicate statistical significance.

Results

Of the 288 patients who were enrolled in the study (Table), 187 patients (64.9%) had PSA values ranging from 4.1 to 10.0 ng/dL, and 73 patients (25.5%) had a positive biopsy.

The ROC curves for each of the clinical variables tested are shown in Figure 1. The areas under the curve (AUC) were 0.486 for PSA level ($P = .74$; 95% CI, 0.40-0.57), 0.590 for PSA density ($P = .04$; 95% CI, 0.50-0.68), 0.687 for PCA3 score ($P < .01$; 95% CI, 0.64-0.79), and 0.717 for PCA3 density ($P < .01$; 95% CI, 0.67-0.81). We detected a statistically significant difference in the AUC between PCA3 density and PSA level, and between PCA3 density and PSA density, but not between PCA3 score and PCA3 density ($P > .20$).

Patients were further categorized as those who were undergoing an initial biopsy (n = 183) and those who had had a previous biopsy with negative results (repeat biopsy, n = 105). Within those categories, men were grouped by PCA3 density scores of less than or equal to 1.0 (initial biopsy, n = 105; repeat biopsy, n = 64), 1.01-2.0 (initial biopsy, n = 48; repeat biopsy, n = 22), and greater than 2.0 (initial biopsy, n = 30; repeat biopsy, n = 19). There was a statistically significant relationship between the number of patients with a positive biopsy and the 3 groups categorized by PCA3 density values, such that more patients in the higher PCA3 density categories had positive biopsy results, both for the initial biopsy ($P < .004$) and for the repeat biopsy ($P = .013$). Across the 3 categories, 25, 15, and 23 patients, respectively, had initial biopsy–positive results, whereas 4, 4, and 6 patients, respectively, had repeat biopsy–positive results across the same categories. The percentage of men within each group with a positive biopsy is depicted in Figure 2. In

TABLE Patient characteristics

Characteristic	Value[a]
Patients, total no.	288
Age, y (range)	66 (40-87)
First biopsy, no. patients (percentage of total)	183 (63.5)
Repeat biopsy, no. patients (percentage of total)	105 (36.5)
Positive biopsies, no. patients (percentage of total)	73 (25.5)
First biopsy, no. patients (percentage of 183)	59 (32.2)
Repeat biopsy no. patients (percentage of 105)	14 (13.3)
Mean, median PV, cm^3 (range)	52.7,45.0 (10.3-240)
PSA level, ng/mL (no. patients [percentage of total])	
< 2.5	19 (6.6)
2.5-4.0	43 (15.0)
4.1-10.0	187 (64.9)
> 10	39 (13.6)

Abbreviations: cm^3, cubic centimeter; ng/dL; nanograms per deciliter; no., number; PSA, prostate-specific antigen; PV, prostate volume; y, years.
[a] Percentages may not add up to 100 because of rounding.

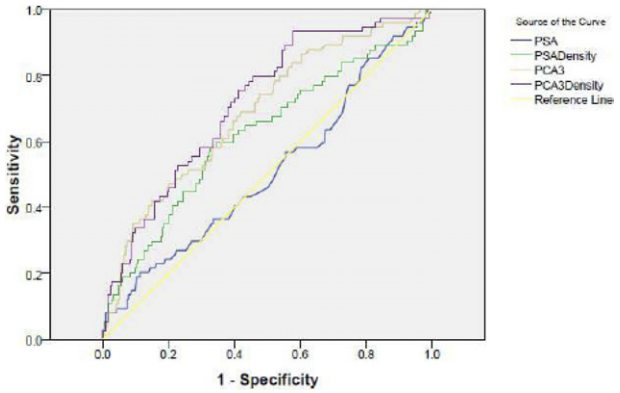

FIGURE 1 The receiver operating characteristic curve analysis for each of the clinical variables. PCA3 indicates prostate cancer antigen 3; PSA, prostate-specific antigen; and PSAD, prostate-specific antigen density.

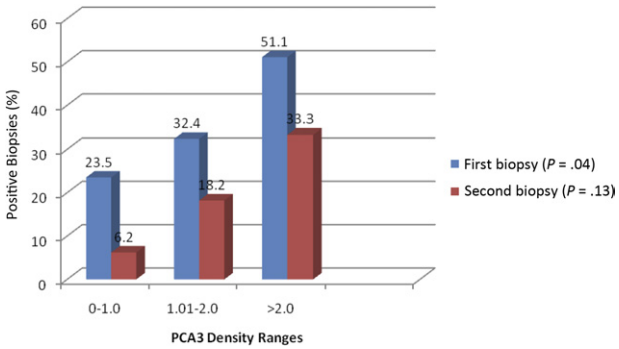

FIGURE 2 Percentage of men in each group with a positive biopsy. PCA3 indicates prostate cancer antigen 3.

both categories, the risk of being diagnosed with prostate cancer increased as a function of PCA3 density.

Discussion

In the PSA test era, most prostate cancer diagnoses are made by TRUS-guided biopsy. There are multiple permutations of PSA to guide physicians in discerning which patients are at the highest risk of harboring prostate cancer. PSA density, for example, was developed as an attempt to correct for the degree of PSA elevation that may be attributable to benign prostatic hyperplasia.

PCA3 is a PSA-independent variable that investigators have used successfully in identifying patients with an elevated risk of prostate cancer.[5] Messenger ribonucleic acid (mRNA) from the PCA3 gene is abundantly expressed in prostate cancer cells, but is found in low levels in normal prostate cells.[9] A urine assay relates the quantity of PCA3 mRNA to PSA mRNA, allowing for the calculation of a PCA3 score. A PCA3 score of 35 has been established as the cutoff above which the test is considered positive, suggesting the presence of prostate cancer. In patients who undergo radical prostatectomy, the PCA3 score has been directly correlated with the tumor volume in the pathology specimen.[6,7] By intuition, if the PCA3 score is truly a surrogate for tumor burden in patients with known prostate cancer, then the PCA3 density should correlate with the proportion of tumor volume and predict the chance of having a positive biopsy.

Our overall positive biopsy rate was 25.5%, which is lower than that in a previously published report.[10] We attribute this finding to the high proportion of men in our study cohort undergoing a repeat biopsy (36.6%). In this population, the positive biopsy rate was markedly lower than that of men undergoing initial biopsy (13.3% vs. 32.2%), a finding that has been previously reported.[11]

We found a very low AUC for PSA of 0.486, which is essentially not different from chance (50%). It's not clear what contributed to the very low sensitivity and specificity of PSA as a screening tool in our population. One possibility is that a strict PSA threshold for biopsy was not mandated in our study design, as PSA velocity and DRE were also considered in the decision to biopsy. As a result, 62 of the 288 (21.5%) patients underwent prostate biopsy with a total serum PSA of < 4.0 ng/dL. This may have affected the performance of the PSA test as a stand-alone screening tool relative to studies in which a biopsy threshold of a PSA level of ≥ 4.0 ng/dL is strictly applied.

The AUC for PCA3 alone was 0.687, which is consistent with previously published, similarly designed studies that examined the ability of the PCA3 score to predict biopsy outcome.[4,5] We are unaware of previous reports exploring the concept of PCA3 density. If the PCA3 score varies directly with tumor volume in patients with prostate cancer but is independent of prostate volume, then it may be useful in predicting which patients are likely to have a positive biopsy. Because the TRUS-guided biopsy samples only a small amount of prostate tissue, we hypothesized that those patients with greater tumor-to gland-volume ratios would be more likely to have a positive biopsy with the standard 12-core biopsy pattern.

It is worth noting that many studies that review the utility of the PCA3 score have identified no relationship between prostate volume and PCA3 score. Our study does not dispute those findings. Rather, we expound on the established relationship between PCA3 score and tumor volume to propose that normalization of the PCA3 score to prostate volume reflects the proportion of gland involved by tumor and, therefore, the likelihood of finding tumor with TRUS biopsy.

In our study, both the PCA3 score and PCA3 density showed improved diagnostic accuracy of biopsy outcome, compared with either PSA level or PSA density. Although the AUC for PCA3 density was slightly greater than that for PCA3 score, this difference did not achieve statistical significance. It is possible that future studies including a larger sample size may reveal a statistically significant difference.

We further noted a distinct difference in the percentage of positive biopsies when patients were categorized into groups on the basis of their PCA3 density values. We observed an increased risk of a positive biopsy with an increase in PCA3 density in patients undergoing first biopsies, as well as in those undergoing repeat biopsy, although the overall risk was lower in the latter group. The determination of cutoff values is an inherently imperfect and somewhat arbitrary process. Nevertheless, based on our findings, we proceeded to select a cutoff level for PCA3 density above which patients would have a higher likelihood of having a positive prostate biopsy. In

selecting this value, the priority was to maximize specificity. Using a PCA3 density of 2, this test had a specificity of 88.8% for finding cancer on prostate biopsy (sensitivity, 35.0%). By the normalization of the PCA3 score to the prostate volume, PCA3 density offers a modest improvement in specificity, compared with the commonly used PCA3 cutoff value of 35 (specificity, 83.4%) while achieving equivalent sensitivity. This information may be useful in counseling men who have a PCA3 density greater than 2 on the need to undergo biopsy.

A limitation of our study is that the validity of the PCA3 density value can only be confirmed by pathologic examination of patients' prostates. In addition, the test does not discriminate between patients with and without cancer; it only predicts the likelihood of a positive biopsy. Although these preliminary results are encouraging, its usefulness to the selection of men who should undergo prostate biopsy remains to be determined in future studies.

Conclusion

We showed that the PCA3 density (that is, the ratio of PCA3 score to prostate volume) correlates with finding prostate cancer on TRUS-guided prostate biopsy, and may be a surrogate for the degree of prostate volume occupied by tumor.

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