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ORIGINAL ARTICLE

Utility of PCA3 in patients undergoing repeat biopsy for prostate cancer

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BACKGROUND: Men with persistently elevated and/or rising PSA levels after negative prostate biopsy often undergo multiple repeat biopsies. Prostate cancer antigen 3 (PCA3) has emerged as a predictor of prostate cancer.

METHODS: We sought to define the utility of PCA3 in combination with other clinical data in predicting the risk of prostate cancer on repeat biopsy. We retrospectively obtained PCA3, PSA, PSA density (PSAD), digital rectal examination (DRE) and transrectal ultrasound (TRUS) findings from 103 patients at a single institution who had at least one prior negative prostate biopsy. The sensitivity and specificity of PCA3 in detecting prostate cancer was determined. Receiver operating characteristics curves were produced for each variable individually and in multivariable analysis, controlling for PCA3, PSAD, TRUS, PSA and DRE. A nomogram was created, internally validated and compared to another recently published nomogram.

RESULTS: Of the 103 patients, 37 (31%) had prostate cancer on repeat biopsy. The sensitivity and specificity of PCA3 (using a cut point of 25) was 0.67 and 0.64, respectively. In multivariable analyses, PCA3 was independently associated with prostate cancer (odds ratio: 1.02, 95% confidence interval: 1.01–1.04), with area under the curve (AUC) of 0.64. A multivariable model containing PCA3, PSAD, PSA, DRE and TRUS findings showed the most diagnostic accuracy (AUC: 0.82).

CONCLUSIONS: In the setting of prior negative biopsies, PCA3 was independently associated with prostate cancer in a multivariable model. In combination with other clinical data, PCA3 is a valuable tool in assessing the risk of prostate cancer on repeat biopsy.

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Keywords: nomogram; decision curve analysis; prostate cancer antigen 3; repeat prostate biopsy

Introduction

Men with persistently elevated or rising serum PSA levels despite a normal initial prostate biopsy pose a diagnostic challenge. In all, 10–39% of such patients may ultimately be found to have prostate cancer on repeat biopsy.[1,2] However, prostate biopsy is uncomfortable and can carry significant morbidity,[3,4] and most men who undergo repeat biopsy are ultimately found to be free of prostate cancer. Additionally, the probability of having a positive biopsy decreases with each subsequent biopsy.[5] Thus, it is important to define who is at greatest risk of having prostate cancer on repeat biopsy.

Prostate cancer antigen 3 (PCA3) is a non-coding gene, which has recently emerged as a strong predictor of prostate cancer.[6] Elevations in PCA3 gene transcripts in post-digital rectal examination (DRE) urine have been shown to be associated with prostate cancer in patients undergoing initial and repeat prostate biopsy.[7] In men undergoing repeat biopsy, studies have suggested that PCA3 may be superior to both PSA[8] and free PSA[9] in predicting the presence of prostate cancer. Furthermore, inclusion of PCA3 improved predictive accuracy of a multivariable model that evaluated probability of having prostate cancer in men with elevated PSA, with or without prior biopsy in a recent study by Chun *et al*.[10] Based on these findings, PCA3 was included in a multivariable nomogram designed to predict the presence of prostate cancer.

We sought to further define the ability of PCA3 along with other clinical factors to predict the presence of prostate cancer in patients who had previously undergone negative prostate biopsy.

Materials and methods

We retrospectively reviewed data from 188 consecutive men undergoing repeat prostate biopsy at our institution.

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All patients had previously undergone one or more negative prostate biopsies. Indications for repeat prostate biopsy were based on suspicious DRE, persistently elevated PSA, previous suspicious histology (such as high-grade prostatic intraepithelial neoplasia or atypical small acinar proliferation) and/or patient preference. All men underwent transrectal ultrasound (TRUS) and biopsy with $\geqslant$12 cores taken (two cores from each sextant of the prostate are taken plus additional cores from suspicious areas by TRUS and/or anterior prostate cores). All TRUS evaluations and biopsies were performed by the same clinician (KS).

For each patient, we performed medical record review and abstracted clinical data, including serum PSA at the time of repeat biopsy, DRE findings and the presence or absence of visible lesions on TRUS. PSA density (PSAD) was calculated by dividing serum PSA by prostate volume as determined on TRUS. Urinary PCA3 was assessed for each patient before repeat biopsy. First catch urine samples were collected following DRE with prostate massage as instructed by the laboratory. The urine was processed to determine PCA3–mRNA and PSA–mRNA concentrations (Bostwick Laboratories, Richmond, VA, USA). PCA3 scores were calculated as (PCA3–mRNA)/(PSA–mRNA) $\times$ 1000.

Only patients with complete data for PCA3, PSA, PSAD, DRE and TRUS were included in the study. For each variable, the sensitivity and specificity for diagnosing prostate cancer on repeat biopsy was determined. For PCA3, different cutoff values were used to identify the cut point, resulting in optimal sensitivity and specificity. The threshold of 35 is commonly used as the optimal cutoff for identifying prostate cancer.[7,8] In univariable analysis, receiver operating characteristic (ROC) curves were created and area under the curve (AUC) was determined.

We subsequently performed multivariable logistic regression analysis, incorporating PCA3, PSA, PSAD, TRUS and DRE data. PCA3 and PSA were taken as continuous variables, while PSAD was analyzed as a dichotomous variable with a cutoff of 0.15 ng ml^{-1} ml^{-1}. ROC curve analysis was performed for these multivariable models, and a nomogram was created. We performed internal validation of our nomogram using our same full cohort of patients. For comparison, we also performed external validation of the nomogram created by Chun *et al*.[10] using our data set. ROC curve analysis was performed for both of these nomograms. Next, using our nomogram and that provided by Chun *et al*.,[10] we performed decision curve analysis.[11] This method determines the net benefit, calculated as follows:

$$\text{Net benefit} = (\text{true positive count}/n) - (\text{false positive count}/n)(P_t/(1-P_t)),$$

where n is the study population and P_t is the threshold probability. The threshold probability is the probability of finding cancer where the expected benefit of repeat prostate biopsy is considered equal to the expected benefit of avoiding treatment. Using this method, the two nomograms were compared against each other.

Data analysis was performed using Stata version 11.0 (StataCorp, College Station, TX, USA) software. The nomogram was created using R (Free Software Foundation, GNU Project, Boston, MA; http://www.r-project.org). Approval of this study was obtained from our institutional review board before review or analysis of patient data.

Results

Of the 188 men evaluated for repeat biopsy at our institution, complete TRUS, PSA, PSAD, DRE and PCA3 data were available for 103 (54.7%) patients. Of the 103 patients, 91 (88.3%) underwent two or more prior biopsies and 40 (38.8%) underwent three or more prior biopsies. Reasons cited for repeat biopsy included the following: persistently elevated PSA (88% of patients), rising PSA (48%), prior biopsy with atypical small acinar proliferation (18%), free PSA $<$15% (15%), PCA3$>$35 (9%), prior biopsy with high-grade prostatic intraepithelial neoplasia (8%), abnormal DRE (7%), abnormal MRI (5%), family history of prostate cancer (2%) and prior abnormal TRUS (1%). Clinical data for these patients are shown in Table 1. Mean PCA3 was 30.4 (s.d. 33.1), with 31% of patients having a PCA3 of $>$35.

Of the 103 patients undergoing repeat prostate biopsy, 57 patients (55%) had normal biopsies, 37 patients (36) were found to have prostate cancer, 6 patients (6%) were found to have atypical small acinar proliferation and 3 patients (3%) had high-grade prostatic intraepithelial neoplasia. Of those that had prostate cancer, 14 patients (38%) were found to have Gleason score $\geqslant$7, including one patient with Gleason score 8 prostate cancer.

Table 1 Clinical data

Variable	*Number of patients (%)*	*Mean (s.d.)*
Age (years)		
$<$50	2 (2)	63.5 (7.4)
50–60	39 (38)	
$>$60	62 (60)	
PSA (ng ml^{-1})		
$<$10	64 (62)	11.0 (8.5)
10–20	31 (30)	
$>$20	8 (8)	
PCA3		
$<$10	31 (30)	30.4 (33.1)
10–25	25 (24)	
25–35	15 (15)	
$>$35	32 (31)	
PSAD (ng ml^{-1} ml^{-1})		
$<$0.1	23 (22)	0.21 (0.17)
0.1–0.15	30 (29)	
$>$0.15	50 (49)	
Number of prior negative biopsies		
1	12 (12)	2.7 (1.4)
2	51 (50)	
3	20 (19)	
$\geqslant$4	20 (19)	
TRUS volume (ml)		
$<$40	31 (30)	65.0 (36.0)
$>$40	72 (70)	
DRE abnormality		
No	90 (87)	
Yes	13 (13)	
TRUS lesion		
No	70 (68)	
Yes	33 (32)	

Abbreviations: DRE, digital rectal examination; PCA3, prostate cancer antigen 3; PSAD, PSA density; TRUS, transrectal ultrasound.

Table 2 Sensitivity, specificity, PPV, NPV and area under the curve of common variables for predicting prostate cancer on repeat biopsy

	Sensitivity	*Specificity*	*PPV*	*NPV*	*ROC AUC (95% CI)*
PCA3 (>25)	0.67	0.64	0.52	0.78	0.64 (0.53–0.75)
PCA3 (>35)	0.38	0.77	0.50	0.66	
DRE abnormality (nodule/induration)	0.22	0.88	0.53	0.64	0.55 (0.47–0.62)
PSA (<10 versus $\geqslant$10 ng ml^{-1})	0.40	0.61	0.40	0.62	0.51 (0.40–0.63)
PSA density (>0.15 ng ml^{-1} ml^{-1})	0.66	0.6	0.51	0.74	0.68 (0.57–0.79)
TRUS lesion (no HEL versus yes HEL)	0.61	0.82	0.68	0.77	0.71 (0.62–0.80)

Abbreviations: AUC, area under the curve; CI, confidence interval; DRE, digital rectal examination; HEL, hypoechoic lesion; NPV, negative predictive value; PCA3, prostate cancer antigen 3; PPV, positive predictive value; PSAD, PSA density; ROC, receiver operating characteristics; TRUS, transrectal ultrasound.

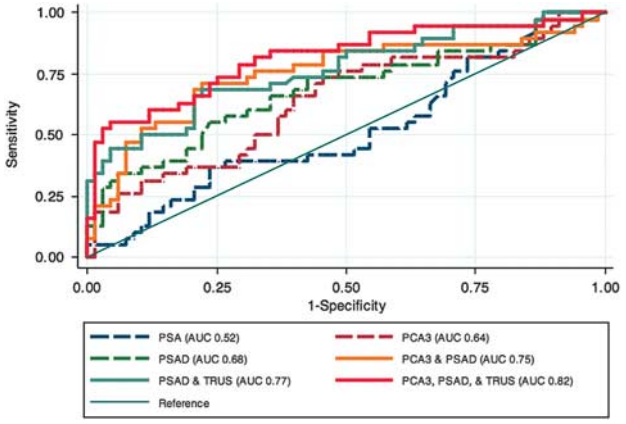

Figure 1 Receiver operating characteristic curves for univariable analysis (dashed lines) and multivariable analysis (solid lines). AUC, area under the curve; PCA3, prostate cancer antigen 3; PSAD, PSA density; TRUS, transrectal ultrasound.

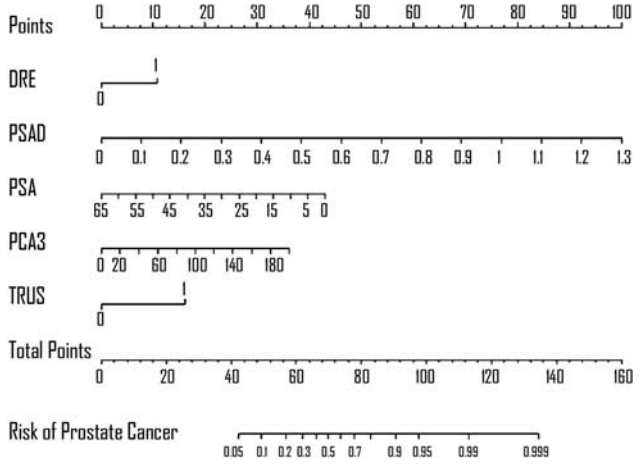

Figure 2 Nomogram including digital rectal examination (DRE), PSA density (PSAD), PSA, prostate cancer antigen 3 (PCA3) and transrectal ultrasound (TRUS).

The sensitivity, specificity, positive predictive value and negative predictive value (NPV) of the variables studied are shown in Table 2. PSA and PCA3 did not correlate, with a correlation coefficient, $r=-0.064$. By ROC analysis, a PCA3 cutoff of 25 had the best diagnostic accuracy. The sensitivity of PCA3 was considerably improved using a PCA3 cutoff of 25 (sensitivity 0.67) as compared with 35 (sensitivity 0.38). Using a cutoff of 25, consequently, improved NPV of PCA3 (0.78 for PCA3 >25 compared with 0.66 for PCA3 >35). With a cutoff of 25 for PCA3, PCA3 had the highest sensitivity and NPV of the clinical factors assessed on univariable analysis. Overall, DRE had the highest specificity (0.88), but the lowest sensitivity (0.22).

Thirteen patients with positive biopsy results had false negative results on PCA3, using a cutoff of 25. These patients did not differ significantly from patients who had a biopsy positive for prostate cancer and a positive PCA3 result with regard to PSA (10.6 versus 13.1 ng ml^{-1}, $P=0.53$, false negative versus true positive patients), PSAD (0.23 versus 0.31 ng ml^{-1} ml^{-1}, $P=0.39$), or volume on TRUS (59.9 versus 50.1 ml, $P=0.38$). While not reaching statistical significance, patients with false negative results were generally younger (61.2 versus 66.1 years, $P=0.08$), had more positive DREs (38.5% versus 12.5%, $P=0.08$) and more positive TRUS results (69.2% versus 50%, $P=0.22$). Similarly, 22 patients with false negative PCA3 results, using a cutoff of 35, also did not differ in a statistically significant manner from those with true positive results.

In univariable analysis, ROC curves were created for PSA, PSAD and PCA3 (Figure 1). AUC was highest for PSAD (0.68), followed by PCA3 (0.64). The lowest AUC was found with PSA. However, the AUC did not differ significantly between these variables (Table 2). In multivariable analysis, PCA3, PSAD and TRUS were found to be significantly associated with prostate cancer on repeat prostate biopsy. PCA3 taken as a continuous variable was found to have an odds ratio (OR) of 1.02 (95% confidence interval (CI): 1.003–1.03). PSAD as a dichotomous variable with a cutoff of 0.15 ng ml^{-1} ml^{-1} was found to have an OR of 2.3 (95% CI: 1.4–4.0). Patients with hypoechoic lesions on TRUS had 5.4 times greater odds of having prostate cancer on repeat biopsy (95% CI: 1.9–15.5). In this model, serum PSA (OR: 0.93, 95% CI: 0.86–1.01) was found to be a negative predictor of prostate cancer, though this result did not reach statistical significance. DRE findings (OR: 6.75, 95% CI: 0.60–75.5) did not significantly associate with the presence of prostate cancer on repeat prostate biopsy. Using PCA3, PSAD and TRUS, an ROC curve with an AUC of 0.82 was obtained. This is improved compared with an ROC curve for just PSAD and TRUS, which had an AUC of 0.77 (Figure 1).

Based on multivariable analysis, a nomogram was created (Figure 2). The equation for the risk of finding prostate cancer on repeat biopsy is shown below.

$$P_{\text{cancer}} = \frac{1}{1+e^{-2.86+0.019(\text{PCA3})-0.071(\text{PSA})+1.15(\text{DRE})+8.19(\text{PSAD})+1.72(\text{TRUSvol})}}$$

In this model, increased PSAD and PCA3 increased the probability of having prostate cancer on repeat biopsy.

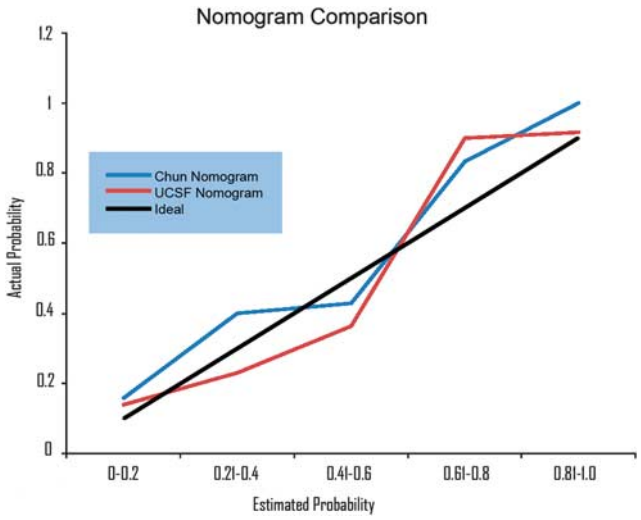

Figure 3 Comparison of the estimated probability determined by the nomogram with the actual probability for each nomogram. UCSF, University of California, San Francisco.

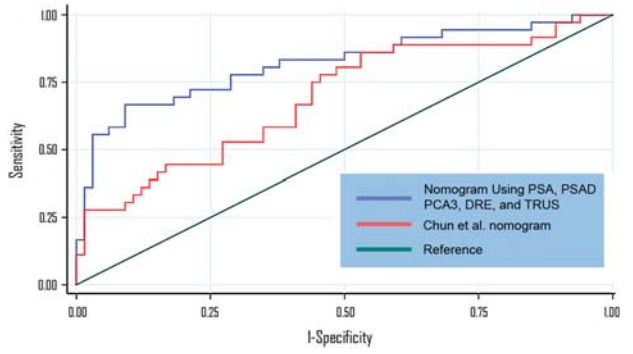

Figure 4 Receiver operating characteristic analysis for each nomogram. The nomogram by Chun *et al*. has an area under the curve (AUC) of 0.71, while the nomogram presented here has an AUC of 0.82. DRE, digital rectal examination; PCA3, prostate cancer antigen 3; PSAD, PSA density; TRUS, transrectal ultrasound.

Additionally, positive DRE and TRUS demonstrated positive association with finding prostate cancer. Conversely, a higher PSA was associated with a lower risk of prostate cancer. Validation of the nomogram created in this study and one created by Chun *et al*.[10] was undertaken using our data (Figure 3). Both curves show generally good calibration to the ideal curve across the range of risk levels. A comparison of the two nomograms using ROC analysis revealed significantly better diagnostic accuracy for our nomogram (AUC: 0.82) compared with that of Chun *et al*. (AUC: 0.71) ($P<0.05$) (Figure 4).

Decision curve analysis was used to further compare the two nomograms. The net benefit was calculated for variable threshold probabilities for each nomogram (Figure 5). This demonstrates that at low threshold probabilities (<20%), the net benefit for each nomogram is similar to that of adopting a plan to perform repeat biopsy on all patients. Beyond a threshold probability of 20%, the net benefit is distinctly higher for the nomogram presented here than it is for that described by Chun *et al*.

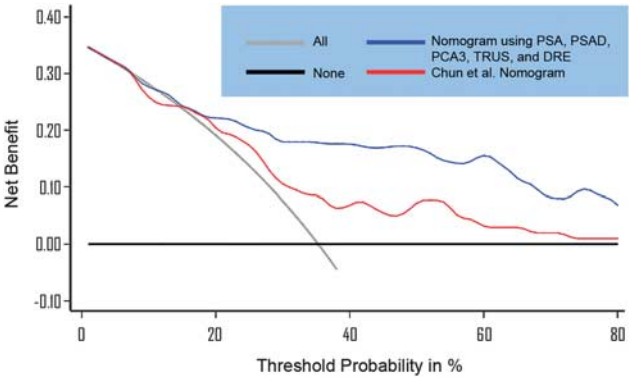

Figure 5 Decision curve analysis. The 'all' line shows the net benefit if all patients were taken for repeat prostate biopsy. The 'none' line shows the net benefit if no patients were taken for repeat prostate biopsy. As the threshold probability increases, the net benefit for each nomogram declines. DRE, digital rectal examination; PCA3, prostate cancer antigen 3; PSAD, PSA density; TRUS, transrectal ultrasound.

Discussion

The indications for repeat prostate biopsy are not always clear. While a significant number of patients may be found to have prostate cancer on repeat biopsy,[1,2] the potential morbidity of prostate biopsy must be considered.[3] A recent population-based study of Canadian men reported a 30-day hospital admission rate of 4.1% and a mortality rate of 0.9% due to biopsy-related complications.[4] We present an evaluation of PCA3 as a univariable predictor of prostate cancer on repeat biopsy and as a component of a multivariable nomogram. These data show that PCA3 alone is comparable or superior to other previously defined markers when predicting prostate cancer risk. In a multivariable model controlling for PSA, PSAD, DRE results and TRUS findings, PCA3 was shown to be independently and significantly associated with the risk of prostate cancer on repeat biopsy.

Expectedly, higher PSAD, positive DRE, positive TRUS and higher PCA3 all correlated with higher risk of prostate cancer in our nomogram. Less intuitive is that higher PSA was found to be associated with decreased risk of prostate cancer in our nomogram. While PSA was not a statistically significant predictor of prostate cancer, its 95% CI (0.86–1.01) nearly excluded 1, and was thus included in the nomogram. We suspect that PSA may have an inverse risk association in our model because of the inclusion of PSAD. Patients with high PSA and high volume (and consequently low or normal PSAD) may actually be at less risk than those with high PSAD. Additionally, TRUS performed better in this study than in prior analyses.[12] The relative contribution of TRUS is likely dependent on the experience of the ultrasonographer.

PCA3 has shown promise when assessing the risk of prostate cancer in men with prior negative biopsies. Marks *et al*.[8] found in 226 patients undergoing repeat biopsy that PCA3 had an AUC of 0.68 for prostate cancer detection, and had superior diagnostic accuracy compared with serum PSA, which had an AUC of 0.52. Haese *et al*.,[9] using data from 463 patients, were able to report comparable diagnostic accuracy of PCA3, with an AUC of 0.66, which was superior to that of percent free

PSA (AUC: 0.58). Both studies found lower rates of prostate cancer on repeat biopsy (27% and 28%) as compared with our rate of 36%, but similar to our study, most cancers found were Gleason score 6–7.

The optimal interpretation of PCA3 score results is a matter of debate. In clinical practice, the PCA3 score is reported as positive/negative if it is above/below a threshold of 35. Deras *et al*.[7] found this value to provide the optimal balance with a sensitivity of 54% and a specificity of 74%. In repeat biopsy patients, Haese *et al*.[9] also recognized a PCA3 cutoff of 35 as the optimal balance between sensitivity and specificity. The data presented here suggest that a PCA3 cutoff of 25 is actually a value with more optimal balance than a cutoff of 35. Additionally, with this cutoff, the NPV of PCA3 is greatly enhanced. A strong NPV is important when deciding who no longer needs repeat biopsy. Chun *et al*.[10] found in their study an optimal cutoff value of 17. In fact, in our analysis, PCA3 was predictive as a continuous variable, with risk of cancer increasing steadily with increasing PCA3 score. Thus, it may well be the case that the score should not be dichotomized, at least not in the setting of prior negative biopsy.

Even with an optimal balanced cutoff, PCA3 may not be enough to decide whether or not to repeat a biopsy. Multiple prediction models with and without PCA3 have been created for predicting the outcome of repeat prostate biopsy.[10,13–16] Ankerst *et al*.[15] used a cohort of 521 patients to incorporate PCA3 into the Prostate Cancer Prevention Trial (PCPT) calculator, and validated these two models using a separate cohort of 443 patients. They found that the addition of PCA3 improved the AUC of the predictive model from 0.653 for the PCPT calculator alone to an AUC of 0.696 with PCA3. Using 1072 patients from the Reduction by Dutasteride of Prostate Cancer Events trial, Aubin *et al*.[16] found that PCA3 improved the AUC of a multivariable model, including PSA, percent free PSA, prostate volume, age and family history, from 0.72 to 0.75. Chun *et al*.[10] used data from 809 men undergoing both repeat and first-time prostate biopsies to create a nomogram for prostate cancer risk. They found that the PCA3 score enhanced the diagnostic accuracy of their nomogram by up to 4.6%, raising the AUC from 0.679 to 0.725. These studies all suggest that PCA3 is an important addition to our ability to predict risk through multivariable models.

Perdona *et al*.[17] have previously compared the Chun *et al*. nomogram with that of the updated Prostate Cancer Prevention Trial calculator. The latter calculator performed better than the Chun *et al*. nomogram on ROC curve analysis (AUC: 79.6% versus 71.5%, $P = 0.043$), but the Chun *et al*. nomogram performed better on decision curve analysis. The nomogram from this study also is compared with the Chun *et al*. nomogram. In both ROC curve analysis and decision curve analysis, the nomogram presented here outperformed that of Chun *et al*. in our cohort of patients. The improved diagnostic accuracy with our nomogram in repeat biopsy patients might reflect inherent differences between first-time and repeat biopsy patients as the Chun *et al*. nomogram was created using both repeat biopsy and first-time biopsy patients. For example, in our nomogram, PSA is a negative predictor of finding prostate cancer, and this association may be unique to the subset of patients who are undergoing repeat prostate biopsy. Additionally, the use of PCA3 as a continuous variable instead of a dichotomous variable (defined as $\leqslant 17$ or >17 in the Chun *et al*. nomogram) within the nomogram may be important as there is no clear consensus on the optimal PCA3 score cutoff. Indeed, when the Chun *et al*. nomogram was externally validated by Auprich *et al*.[18] AUC values did differ, albeit not in a statistically significant manner, depending on whether PCA3 was treated as a continuous variable or as a dichotomous variable with different cutoffs.

There are several limitations to this study. First, the retrospective nature of these data may create bias as not all individuals receiving a PCA3 assay underwent repeat biopsy. Additionally, several other prostate cancer markers including percent free PSA, PSA velocity and PSAD of the transition zone have been suggested as highly sensitive and specific for prostate cancer.[19–22] These values were unavailable in these data, but addition of these factors to the nomogram may have further improved its diagnostic accuracy. Additionally, validation of our nomogram in the same cohort in which it was developed may be problematic with regard to overfit bias, and the need for external validation is clear. Comparison between the Chun *et al*. nomogram and that presented here will also favor the nomogram presented here as a consequence of the cohort used for validation. If these two nomograms were compared using cohorts foreign to both nomograms, then the results may be different. With just 37 prostate cancer events, the study may be underpowered for proper multivariable analysis of PSA, PSAD, TRUS, DRE and PCA3. Finally, most of the patients in the cohort were referred to our institution following initial negative biopsy, and the incremental value of TRUS and/or PSAD may be less if the same urologist who did the original biopsy is the one considering a repeat biopsy.

PCA3 is a useful tool in identifying patients in need of repeat prostate biopsy. Very low PCA3 values may identify patients who can avoid biopsy, with good NPV. However, PCA3 is optimally used with consideration of other clinical data. We were able to present a multivariable predictive model with strong diagnostic accuracy. Further investigation is warranted for further validation and refinement of this model.

Conflict of interest

The authors declare no conflict of interest.

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