

Predicting Prostate Biopsy Outcome Using a PCA3-based Nomogram in a Polish Cohort

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Abstract. *Background: Prostate Cancer Gene-3 (PCA3) is highly prostate cancer (PCa)-specific and its application holds promise in identifying men with PCa. Aim: To determine whether the PCA3 score can be used relative to PCa clinical variables to predict biopsy outcome. Patients and Methods: PCA3 scores were assessed in a group of 80 patients using the ProgenSA assay (Gen-Probe, San Diego, CA, USA). The logistic regression algorithm was used to combine PCA3 results with the established biopsy risk factors including: age, prostate-specific antigen (PSA), digital rectal examination (DRE) and prostate volume (Pvol). Results: In univariate analyses, the ProgenSA PCA3 score outperformed all biopsy risk predictors. A logistic regression algorithm using: age, PCA3, PSA, DRE and Pvol increased the area under the Receiver Operating Characteristic (ROC) curve from 0.72 for PCA3-alone to 0.85. Conclusion: Combining PCA3 results with PCa risk factors provides significant improvements over the use of PCA3- or PSA-alone in predicting the probability of a positive prostate biopsy.*

The performance of prostate-specific antigen (PSA) as a diagnostic test for prostate cancer (PCa) detection remains suboptimal, with the lack of specificity being the main drawback. Prostate Cancer Gene-3 (PCA3) is a non-coding RNA whose expression is restricted to the prostate tissue. Importantly, the gene is significantly up-regulated in cancer - Hessels and colleagues reported, on a median 66-fold up-regulation of PCA3 in PCa tissues compared with normal

prostate tissues (1). As a consequence, the PCA3 gene, being highly overexpressed in specific PCa cell lines and prostatic tumors, constitutes today the most PCa-specific marker (2).

The urine ProgenSA PCA3 test (Gen-Probe, San Diego, CA, USA) has been clinically available since 2006. The ProgenSA PCA3 assay is a non-invasive and highly specific test, which measures the expression of the PCA3 gene and PSA mRNA concentrations in post-DRE urine. In many studies, the PCA3 test was superior to PSA for the early detection of PCa. The assay has been shown to be particularly useful in patients with a previous negative biopsies and persistently elevated PSA levels (3, 4).

To further improve diagnostic accuracy, the ProgenSA PCA3 was incorporated into nomograms, which are being more and more frequently used in clinical practice. The nomograms include different variables *e.g.* age, PSA, DRE, Pvol, prior biopsy. To our knowledge, two principal risk estimators that include PCA3 have been published and validated: Chun's *et al.*'s nomogram (5); and The Prostate Cancer Prevention Trial (PCPT) risk calculator which was updated with the PCA3 score (6). In both nomograms the addition of the ProgenSA PCA3 led to a higher diagnostic accuracy in predicting biopsy outcome, with an AUC ranging from 0.70 to 0.85.

The aim of this study was to assess whether the inclusion of the ProgenSA PCA3 score with other clinical variables could adequately predict the risk of a positive prostate biopsies for cancer in a prospective study on a Polish patient cohort. Furthermore, we sought to determine the value of the predictive model versus measuring the PCA3 score and/or serum PSA level alone.

Patients and Methods

In the prospective study involving 80 consecutive men (mean age=66 years, range=50-81 years) with an elevated serum PSA level (4-10 ng/ml) who did not undergo any previous prostate biopsy, first-catch urine samples after DRE (three strokes per lobe) were collected by the same urologist to measure for PCA3 RNA concentration and to calculate the PCA3 score using the ProgenSA PCA3 assay (7). On the same day, a prostate biopsy was performed. At least 10

The procedures were performed with ethical standards. Permission for the present study (RNN/61/11/KB) was granted by the Ethics Committee of the Medical University of Łódź on 18 January 2011.

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Table I. Patients' characteristics.

	Men with negative biopsy n=56	Men with positive biopsy n=24	Total study population n=80	p-Value
AGE, years				
Mean±SD.	65.7±7.0	67.5±6.5	66.2±6.8	0.2990
Range	50.0-81.0	56.0-81.0	50.0-81.0	
Median	64.5	67.5	66.0	
95% CI	[63.8; 67.6]	[64.7; 70.2]	[64.7; 67.8]	
PSA [ng/ml]				
Mean±SD.	7.4±2.0	7.9±1.9	7.5±1.9	0.2293
Range	4.0-10.0	4.4-10.0	4.0-10.0	
Median	7.3	8.4	7.5	
95% CI	[6.8; 7.9]	[7.1; 8.7]	[7.1; 8.0]	
PCA3 score				
Mean±SD.	43.6±39.0	89.9±67.2	57.5±53.2	0.0019
Range	1.0-158.0	5.0-263.0	1.0-263.0	
Median	31.5	72.5	38.0	
95% CI	[33.1; 54.0]	[61.5; 118.3]	[45.6; 69.3]	
Prostate volume [cm ³]				
Mean±SD.	43.6±23.1	32.0±21.8	40.1±23.2	0.0100
Range	10.0-140.0	8.9-110.0	0.0-140.0	
Median	38.0	29.0	37.5	
95% CI	[37.4; 49.8]	[22.8; 41.3]	[35.0; 45.3]	
PCA3 score >35	24/42.9%	18/75.0%	42/52.5%	0.0083
PCA3 score >10	8/14.3%	1/4.2%	9/11.3%	0.1893
Positive digital rectal exam	26/46.4%	20/83.3%	46/57.5%	0.0022

standardized peripheral zone biopsy cores were obtained and the specimens were evaluated by a pathologist. The ProgenSA PCA3 test was carried out at a specialized laboratory of NovioGendix, the Netherlands. The performance of the PCA3 assay alone and in combination with other independent clinical variables for predicting PCa, including patient age, Pvol, DRE and PSA, was evaluated in terms of sensitivity, specificity, positive (PPV) and negative predictive values (NPV) by comparing the results to biopsy outcome. The predictive accuracy of the biopsy outcome was quantified using the AUC of the ROC analysis in models with and without PCA3/PSA. Logistic regression (LR) was performed to generate a new multivariate predictive model to evaluate the potential of PCA3 score in use with other diagnostic indicators of PCa. LR and ROC analyses were carried out using the statistical package Statistica Statsoft, Inc. data analysis software system, version 10.0.

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Results

The patients' characteristics are shown in Table I. In our group of 80 men, all biopsied, the positive biopsy rate was 30% and a mean PCA3 score was 57.5. The probability of a cancer-positive prostate biopsy increased as the PCA3 score increased. The median PCA3 score was 72.5 in men with a positive biopsy and 31.5 in men with a negative biopsy. The mean PCA3 score was significantly higher in patients

diagnosed with cancer (89.9) compared to men who had a negative biopsy (43.6; $p=0.0019$). Mean Pvol, assessed by transrectal ultrasound (TRUS) in the whole study group was 40.1 cm³. In patients with PCa, the volume of the prostate was significantly lower (32.0 cm³) compared to men without cancer (43.6 cm³) ($p=0.01$). We found an inverse correlation between the PCA3 score and Pvol in the total study cohort but such correlation was not found in patients with a positive biopsy. As expected, there were significantly more patients with abnormal DRE among patients with cancer ($p=0.0022$).

The diagnostic accuracy of the PCA3 urine assay differed depending on the applied PCA3 score cut-offs (Table II). The recommended PCA3 score cut-off of value of 35 provided a sensitivity of 75%, specificity of 55%, PPV of 42% and NPV of 84%.

The independent predictor variables were age, suspicious DRE result, serum PSA levels and Pvol on TRUS. In univariate analyses, all variables were significant predictors of cancer at biopsy ($p\leq 0.05$) and the PCA3 assay outperformed all of the assessed risk factors in predicting the probability of positive prostate biopsies. In the LR model, the addition of ProgenSA PCA3 to the described variables resulted in the greatest achieved diagnostic accuracy. The AUC of PCA3 combined with age, Pvol, DRE and PSA was 0.85 compared to 0.72 for the PCA3-alone score (Figure 1). The LR model with the PCA3 score removed (age, Pvol,

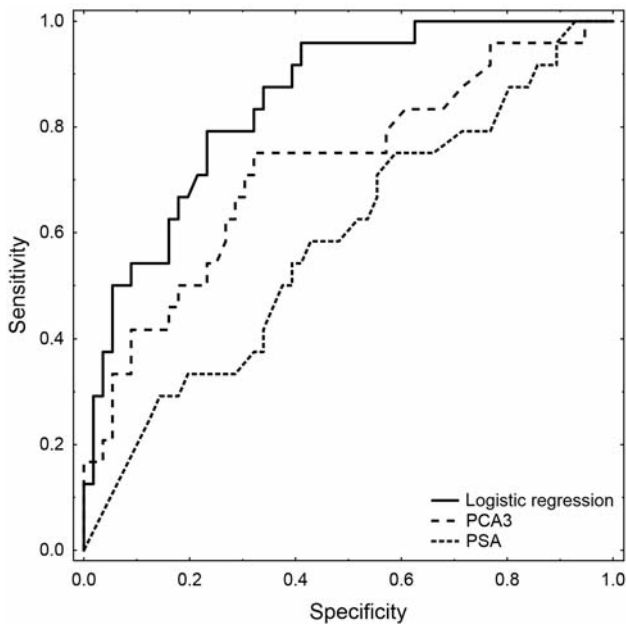


Figure 1. Receiver operating characteristic (ROC) curve analysis of serum PSA, PCA3 score and logistic regression model PCA3+PSA+Pvol+DRE+age in all men biopsied (n=80). The areas under the curve (AUC) for the logistic regression model was 0.85, for PCA3 was 0.72 and for PSA was 0.59.

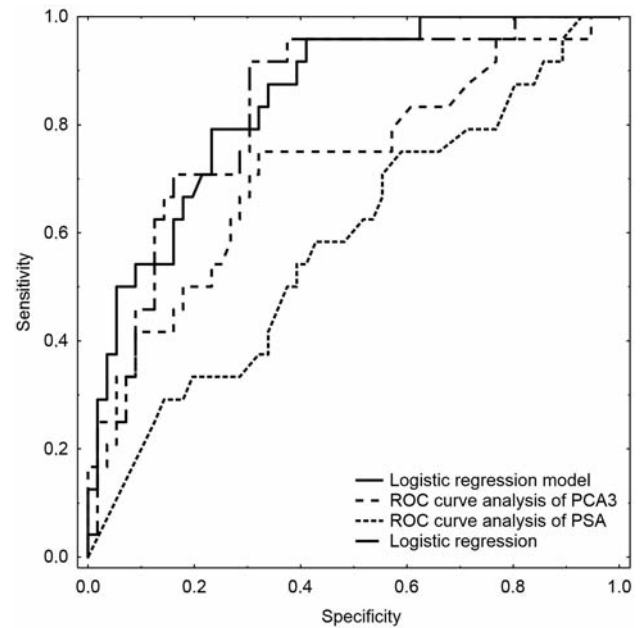


Figure 2. Receiver operating characteristic (ROC) curve of PSA, PCA3, logistic regression model PCA3+PSA+Pvol+DRE+age, and logistic regression with PSA removed PCA3+Pvol+DRE+age. The areas under the curve (AUC) for the logistic regression model was 0.85, for logistic regression with PSA removed was 0.83, for PCA3 was 0.72 and for PSA was 0.59.

DRE and PSA) resulted in an AUC of 0.71. The serum PSA test by itself showed less diagnostic accuracy in this data set with an AUC of 0.59, thus failing to achieve a satisfactory predictive accuracy (Figures 1 and 2). In comparison, the LR model with PSA removed (age, Pvol, DRE and PCA3) resulted in an AUC of 0.83 (Figure 2).

Discussion

Considering the high diagnostic accuracy of the PCA3 assay, the test has been recently incorporated into the clinically applied PCa nomograms. Chun *et al.*'s nomogram combines the established risk factors of PCa (age, Pvol, DRE, PSA and data on prior biopsy) with ProgenSA PCA3 (5). The authors observed that the incorporation of PCA3 into the base model described by Kattan and co-workers (8) significantly improves the accuracy of the nomogram by 2-4.6%. Among the analyzed variables, the PCA3 score had the highest accuracy at predicting the presence of PCa at first- and repeat-biopsy regardless of the cut-off used. Moreover, a PCa risk estimator based on the original PCPT risk calculator comprising of six variables: age, race, family history, DRE, prior biopsy, Pvol and PCA3 score, was developed, also leading to an increase in the diagnostic accuracy (6). The objective of these nomograms is to predict the probability of a positive biopsy for men with suspicion of PCa. Ankerst and

Table II. Diagnostic accuracy of PCA3 score at various cut-offs.

PCA3 cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
10	96	14	32	89
20	83	34	35	82
35	75	55	42	84
65	54	77	50	80
100	42	91	67	78

PPV: Positive predictive value; NPV: negative predictive value.

colleagues demonstrated that the area under the ROC curve of the PCPT risk calculator incorporating PCA3 was statistically significantly higher (0.696) than that of the original PCPT risk calculator (0.653) and of PSA (0.607), but not significantly different from that of PCA3-alone (0.665) (Table III). Further studies by Perdoni (9) and Au-prich (10) also showed higher diagnostic accuracy when the PCA3 result was added to the previously developed nomograms. Perdoni and co-workers observed that discriminative power of the updated PCPT calculator with PCA3 was superior to that of the nomogram of Chun *et al.* - the AUC of the ROC for predicting the first biopsy outcome was similar for the updated PCPT risk calculator (0.840) and PCA3 score alone

Table III. Comparison of the areas (AUC) under the receiver operating characteristic curve (ROC) for the updated Prostate Cancer Prevention Trial risk calculator [PCPT + prostate cancer gene-3 (PCA3)], Chun *et al.*'s nomogram, PCPT, PCA3 and prostate-specific antigen (PSA).

	Ankerst (6)	Perdona (9)	Auprich (10)	Deras (11)
Diagnostic test	AUC ROC	AUC ROC	AUC ROC	AUC ROC
PCPT+PCA3	0.696	0.840	N/A	N/A
Chun <i>et al.</i> 's nomogram	N/A	0.706	0.730-0.750	0.752
PCPT	0.653	N/A	N/A	N/A
PCA3 score	0.665	0.873	N/A	0.686
PSA	0.607	0.660	N/A	0.547

N/A, Not available.

(0.873) but significantly higher compared to Chun *et al.*'s nomogram (0.706) (Table III). According to the authors, both the updated PCPT calculator and Chun *et al.*'s nomogram appear helpful in making biopsy decisions especially in men with PSA<10 ng/ml. It is noteworthy that, both PCA3-based estimators allowed us to avoid unnecessary biopsies without missing aggressive cancer. Auprich *et al.* by externally validating the Chun *et al.*'s nomogram showed its high accuracy in predicting biopsy outcome with AUC ranging from 0.73 to 0.75 for various PCA3 score cut-offs used (10).

Similarly to previous studies, in order to increase the diagnostic accuracy of cancer detection, we combined four commonly-assessed independent PCa variables (patient age, Pvol, suspicious DRE result and serum PSA) with the ProgenSA PCA3 score. Our intention was to use the PCA3-based nomogram, to our knowledge, for the first time in a Polish population. In our study, we only analyzed patients undergoing first biopsy. According to Deras and colleagues (11), who analyzed the data of 570 consecutive men, the PCA3 score was not dependent on whether the man underwent first- or repeat-biopsy. We also did not take into account some of the variables included in PCPT nomogram *i.e.* race, family history of PCa and prior biopsy. Many risk factors included in the nomogram (seven in the PCPT calculator) make the nomogram impractical in daily clinical practice.

Our findings are similar to the observations of Deras *et al.* who reported an AUC of 0.752 for the same combination of PCa variables. Compared to PCPT and Chun *et al.*'s nomogram, the LR model applied in our study, led to even better diagnostic performance in predicting biopsy outcome. The increase of age, PCA3 score, PSA, positive DRE and decrease in Pvol were associated with an increased probability of cancer. Thus, the results of our study corroborate with previous findings demonstrating that the ProgenSA PCA3 can be combined with other diagnostic indicators of PCa for an improved prediction of biopsy outcome. Unfortunately, our group was relatively small, especially with regards to the low number of positive events (*i.e.* positive prostate cancer biopsies), which constitutes the main limitation of our analysis.

Importantly, unlike serum PSA, which can be confounded by an enlarged prostate, the PCA3 score was not affected by increasing Pvol on TRUS. Conversely, we even observed an inverse correlation between PCA3 score and Pvol in the total study population.

Although PCA3 testing is predominantly used in patients with increased PSA and a previous negative biopsy, data indicate that the PCA3 score could be useful to increase diagnostic accuracy in the first biopsy scenario, especially as an adjunct to other clinical variables.

The study was not performed on a pre-screened population. In conclusion, PCA3 can be considered a highly-specific PCa marker. The combination of PCA3 score with other established PCa risk factors has the potential to enhance diagnostic accuracy compared to use of each single factor alone. The results of the first study on the application of PCA3 nomogram in a Polish population are in line with previous findings. Furthermore, multicenter studies with more participants are necessary to externally validate the performance of the nomogram in predicting prostate biopsy outcomes.

Conflicts of Interest

The Authors have nothing to disclose. We confirm that all Authors have made a substantial contribution to the manuscript preparation. They have read and approved the final manuscript. They have no direct or indirect commercial financial incentive associated with publishing the article. We confirm that it has not been published previously in its present form and it may not pose conflict of interest.

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References

- Hessels D, Klein Gunnewiek JM, van O I, Karthaus HF, van Leenders GJ, van BB, Kiemeny LA, Witjes JA and Schalken JA: DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 44: 8-15, 2003.

- 2 Hessels D and Schalken JA: The use of PCA3 in the diagnosis of prostate cancer. *Nat Rev Urol* 6: 255-261, 2009.
- 3 Haese A, de la Taille A, van PH, Marberger M, Stenzl A, Mulders PF, Huland H, Abbou CC, Remzi M, Tinzi M, Feyerabend S, Stillebroer AB, van Gils MP and Schalken JA: Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur Urol* 54: 1081-1088, 2008.
- 4 Marks LS, Fradet Y, Deras IL, Blase A, Mathis J, Aubin SM, Cancio AT, Desaulniers M, Ellis WJ, Rittenhouse H and Groskopf J: PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* 69: 532-535, 2007.
- 5 Chun FK, de la Taille A, van PH, Marberger M, Stenzl A, Mulders PF, Huland H, Abbou CC, Stillebroer AB, van Gils MP, Schalken JA, Fradet Y, Marks LS, Ellis W, Partin AW and Haese A: Prostate cancer gene 3 (PCA3): Development and internal validation of a novel biopsy nomogram. *Eur Urol* 56: 659-667, 2009.
- 6 Ankerst DP, Groskopf J, Day JR, Blase A, Rittenhouse H, Pollock BH, Tangen C, Parekh D, Leach RJ and Thompson I: Predicting prostate cancer risk through incorporation of prostate cancer gene 3. *J Urol* 180: 1303-1308, 2008.
- 7 Groskopf J, Aubin SM, Deras IL, Blase A, Bodrug S, Clark C, Brentano S, Mathis J, Pham J, Meyer T, Cass M, Hodge P, Macairan ML, Marks LS and Rittenhouse H: APTIMA PCA3 molecular urine test: Development of a method to aid in the diagnosis of prostate cancer. *Clin Chem* 52: 1089-1095, 2006.
- 8 Kattan MW: Judging new markers by their ability to improve predictive accuracy. *J Natl Cancer Inst* 95: 634-635, 2003.
- 9 Perdoni S, Cavadas V, Di LG, Damiano R, Chiappetta G, Del PP, Franco R, Azzarito G, Scala S, Arra C, De SM and Autorino R: Prostate cancer detection in the grey area of prostate-specific antigen below 10 ng/ml: Head-to-head comparison of the updated PCPT calculator and Chun's nomogram, two risk estimators incorporating prostate cancer antigen 3. *Eur Urol* 59: 81-87, 2011.
- 10 Aufrich M, Haese A, Walz J, Pummer K, de la Taille A, Graefen M, de RT, Fisch M, Kil P, Gontero P, Irani J and Chun FK: External validation of urinary PCA3-based nomograms to individually predict prostate biopsy outcome. *Eur Urol* 58: 727-732, 2010.
- 11 Deras IL, Aubin SM, Blase A, Day JR, Koo S, Partin AW, Ellis WJ, Marks LS, Fradet Y, Rittenhouse H and Groskopf J: PCA3: A molecular urine assay for predicting prostate biopsy outcome. *J Urol* 179: 1587-1592, 2008.

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