

available at www.sciencedirect.com
journal homepage: www.europeanurology.com



European Association of Urology



Platinum Priority – Prostate Cancer

Editorial by Vincenzo Ficarra, Giacomo Novara and Filiberto Zattoni on pp. 482–484 of this issue

Performance of the Prostate Cancer Antigen 3 (PCA3) Gene and Prostate-Specific Antigen in Prescreened Men: Exploring the Value of PCA3 for a First-line Diagnostic Test

Monique J. Roobol^{*}, Fritz H. Schröder, Pim van Leeuwen, Tineke Wolters, Roderick C.N. van den Bergh, Geert J.L.H. van Leenders, Daphne Hessels

Erasmus MC, University Medical Centre, Department of Urology, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Article info

Article history:

Accepted June 29, 2010

Published online ahead of print on July 9, 2010

Keywords:

PCA3

Prostate specific antigen (PSA)

Prostate cancer

Detection

Performance characteristics



www.eu-acme.org/
[europeanurology](http://europeanurology.com)

Please visit

www.eu-acme.org/

[europeanurology](http://europeanurology.com) to read and

answer questions on-line.

The EU-ACME credits will

then be attributed

automatically.

Abstract

Background: The performance characteristics of serum prostate-specific antigen (PSA) as a diagnostic test for prostate cancer (PCa) are poor. The performance of the PCa antigen 3 (PCA3) gene as a primary diagnostic is unknown.

Objective: Assess the value of PCA3 as a first-line diagnostic test.

Design, setting and participants: Participants included men aged 63–75 who were invited for rescreening in the period from September 2007 to February 2009 within the European Randomised Study of Screening for Prostate Cancer, Rotterdam section.

Interventions: Lateral sextant biopsies were performed if the serum PSA value was ≥ 3.0 ng/ml and/or the PCA3 score was ≥ 10 .

Measurements: Measurements included distribution and correlation of PSA value and PCA3 score and their relation to the number of cases and the characteristics of PCa detected. Additional value of PCA3 was included in men with previous negative biopsy and/or PSA < 3.0 ng/ml.

Results and limitations: In 721 men, all biopsied, 122 PCa cases (16.9%) were detected. Correlation between PSA and PCA3 is poor (Spearman rank correlation: $\rho = 0.14$; $p < 0.0001$). A PSA ≥ 3.0 ng/ml misses 64.7% of the total PCa that can be detected with the sextant biopsy technique and 57.9% of serious PCa (T2a or higher and/or Gleason grade ≥ 4 , $n = 19$), and 68.2% of biopsies could have been avoided; the respective data for PCA3 ≥ 35 are 32%, 26.3%, and 51.7%. Performance of PCA3 in men with low PSA (area under the curve [AUC]: 0.63) and/or previous negative biopsy (AUC: 0.68) is unclear but has limited reliability due to small numbers.

Conclusions: PCA3 as a first-line screening test shows improvement of the performance characteristics and identification of serious disease compared with PSA in this prescreened population.

© 2010 European Association of Urology. Published by Elsevier B.V. All rights reserved.

^{*} Corresponding author. Tel. +31 10 703 2240; Fax: +31 10 703 5315.

E-mail address: m.roobol@erasmusmc.nl (M.J. Roobol).

1. Introduction

Messenger RNA (mRNA) of the prostate cancer antigen 3 (PCA3) gene, formerly known as DD3, was identified in 1999 and was found to be strongly overexpressed in >95% of primary prostate cancer (PCa) tissue specimens and PCa metastases [1]. In the absence of a protein, a nucleic acid-based test was developed to investigate the potential of PCA3 mRNA as the basis for a diagnostic test [2]. In a cohort of 108 men admitted for prostate biopsies based on serum prostate-specific antigen (PSA) levels >3.0 ng/ml and/or an abnormal digital rectal examination (DRE), PCa was diagnosed in 24 of the 108 men (22.2%). Using a cut-off value of 200×10^{-3} , this test has a sensitivity of 67% and a specificity of 83%. The relatively low sensitivity of 67% left doubts about the value of PCA3 as a first-line biopsy indicator [2]. The claimed PCa specificity was confirmed in another study [3], and diagnostic performance was validated in several studies using both urine and prostatic fluid as a substrate [4,5]. Gen-Probe Inc. (San Diego, CA, USA) translated this quantitative PCA3-based test to the company's proprietary technology platform under the trade name ProgenSA PCA3 test [6]. The first studies concentrated on the value of this test in men who had a previous negative biopsy [7].

No single study to date has addressed the value of the PCA3 test as a primary indication for biopsy. All available studies primarily use the PSA test at different cut-off values with or without an abnormal DRE as a biopsy indication. Consequently, these studies are subject to assignment or attribution bias because any PSA cut-off value will miss many PCa cases, including aggressive ones, as has been shown [8,9].

The objective of the present study is to evaluate the performance characteristics of the PCA3 test with a cut-off score of ≥ 10 in comparison to a biopsy indication driven by PSA values of ≥ 3.0 ng/ml. Two issues of great clinical interest will also be addressed: (1) Is PCA3 helpful in improving the detection, specifically, of serious PCa in the low PSA ranges? (2) Is PCA3 helpful in improving the detection of PCa in men with a previous negative biopsy?

The evaluation is based on men presenting for rescreening within the ongoing European Randomised Study of Screening for Prostate Cancer (ERSPC), Rotterdam section [10].

2. Materials and methods

During the period from September 2007 to February 2009, men were invited for rescreening within ERSPC Rotterdam. Next to the protocol-based serum PSA level, the ProgenSA PCA3 score was measured. To avoid attribution bias, we chose a biopsy indication resulting in biopsying of as many men as was reasonably possible.

Men with a PSA ≥ 3.0 ng/ml or a PCA3 score ≥ 10 were invited to undergo DRE, transrectal ultrasound, and lateralised sextant biopsy. In case of a hypoechoic lesion, a seventh biopsy was taken.

The PSA test (Hybritech; Beckman Coulter Inc., Fullerton, CA, USA) was carried out in a standard fashion at the clinical laboratory of the Erasmus University Medical Centre. The ProgenSA PCA3 test was done at the laboratory of experimental urology at Radboud University Nijmegen

Medical Centre. Histologic examinations of the biopsy specimens were handled by one specialised pathologist (AvL).

We assessed the distribution and the relation of the serum PSA and PCA3 scores and the number of cases and the characteristics of PCa detected in relation to the PSA value and the PCA3 score. PCa was considered to be serious if the clinical stage exceeded T2a and/or if a Gleason pattern of ≥ 4 was seen. It is important to realise that understaging and undergrading are present using biopsy-derived data.

2.1. Statistical analyses

Performance characteristics of both PCA3 and PSA in the total study cohort were evaluated by determining sensitivity, specificity, and related parameters. Correlation between PSA and PCA3 was assessed with the Spearman rank correlation. Receiver operating characteristic (ROC) analysis was carried out using the statistical package SPSS v.16 (SPSS Inc., Chicago, IL, USA).

Permission for the present study (ISBN 978-90-5549-653-2) was granted by the Netherlands Ministry of Health on 24 July 2007.

3. Results

Our data are derived from a prescreened population of 965 men aged 64–77 and screened for the third time ($n = 451$), the fourth time ($n = 502$), or the fifth time ($n = 12$). One-third of the men had a history of previous negative biopsies. Of the 965 men, 721 (74.7%) were biopsied utilising either a PSA cut-off value of ≥ 3.0 ng/ml, following the ERSPC protocol, or a PCA3 score ≥ 10 with the intention of evaluating the performance characteristics of both markers in a setting where most men would present with a biopsy indication. A total of 122 PCa cases (16.9%) were detected.

Table 1 summarises the baseline characteristics for the total cohort and for men who were actually biopsied. The differences between the two cohorts are very small and have not been tested for statistical significance.

In Table 2, the distribution of biopsy indications by PCA3 (cut-off score: ≥ 10) and PSA (cut-off ≥ 3.0 ng/ml) are shown for the total cohort and for those men who were actually biopsied. With this algorithm, 87.5% ($n = 844$) of all men had a biopsy indication and 74.7% ($n = 721$) actually underwent a prostate biopsy.

Only 32 of the 721 men actually biopsied had a biopsy indication based on PSA alone, and 492 were based on PCA3 alone. Compliance with biopsy recommendations was very similar in all subgroups and varied between 84.3% and 88.3%. Correlation between the two markers was poor ($\rho = 0.14$; $p < 0.0001$).

Data on sensitivity and specificity of PSA (Table 3) are very much in line with published information [8,11]. A PSA cut-off value of 3.0 ng/ml, as utilised in the ERSPC study, identifies 35.2% of all PCa cases and correctly identifies 69% of those men who do not have PCa. Compared with the recommended cut-off value of 35 for the PCA3 score, the sensitivity of PSA turns out to be higher; the specificity is comparable to the PSA cut-off value of 2.0 ng/ml. No cut-off value can be identified that matches a high sensitivity and specificity, with the possible exception of the PCA3 score of 35. To maximise detection to >80% of the detectable PCa, one would have to choose a PSA cut-off of >1.0 ng/ml and a

Table 1 – Baseline characteristics of the total study cohort (N = 965) and of men actually biopsied (n = 721)

	Total cohort (N = 965)		Men actually biopsied (n = 721)	
	Mean/median	Range	Mean/median	Range
Age, yr	70.0/70.2	63.6–77.5	70.07/70.23	63.7–74.0
PSA, ng/ml	2.5/1.6	0.1–23.0	2.74/1.8	0.2–23.0
PCA3 score	45.7/28.0	0–984	51.9/33.0	0–984
Prostate volume, cm ³	44.3/39.7	12.7–179.0	44.6/39.7	12.7–179.0
	Yes, No. (%)	No, No. (%)	Yes, No. (%)	No, No. (%)
PSA ≥3.0 ng/ml	260 (26.9)	705 (73.1)	229 (31.8)	492 (68.2)
PCA3 ≥10	807 (83.6)	158 (16.4)	689 (95.6)	32 (4.4)
DRE abnormal	126 (13.1)	839 (86.9)	118 (16.4)	603 (83.6)
TRUS abnormal	23 (2.4)	942 (97.6)	21 (2.9)	700 (97.1)
Previous negative biopsy	245 (25.4)	720 (74.6)	212 (29.4)	509 (70.6)
PCa detected	122 (12.6)	843 (87.4)	122 (16.9)	599 (83.1)

DRE = digital rectal examination; PCa = prostate cancer; PCA3 = prostate cancer antigen 3 gene; PSA = prostate-specific antigen; TRUS = transrectal ultrasound.

Table 2 – Distribution of biopsy indications by PCA3 (cut-off: ≥10) and prostate-specific antigen (cut-off: ≥3.0 ng/ml): (a) total study cohort (N = 965); (b) men actually biopsied (n = 721)

	PSA ≥3.0 ng/ml	PSA <3.0 ng/ml	Total
A:			
PCA3 ≥10	223	584	807
PCA3 <10	37	121	158
Total	260	705	965
B:			
PCA3 ≥10	197	492	689
PCA3 <10	32	0	32
Total	229	492	721

PCA3 = prostate cancer antigen 3 gene; PSA = prostate-specific antigen.

Table 3 – Performance characteristics of prostate-specific antigen and PCA3 in 721 men biopsied with 122 prostate cancer cases detected

PSA cut-off, ng/ml	Sensitivity, %	Specificity, %
≥1.0	86.1	26.9
≥2.0	57.4	53.8
≥3.0	35.2	69.0
≥4.0	23.8	80.5
≥10.0	3.3	97.2
PCA3 cut-off		
≥10	69.7	4.7
≥20	84.4	28.2
≥35	68.0	55.7
≥60	38.5	75.8
≥100	22.9	89.7

PCA3 score >20. The areas under the curve (AUCs) are indicated in Fig. 1. Based on the ROC analyses, PCA3 performs marginally better than PSA (Fig. 1A; *p* = 0.143).

In Table 4, PCa detection with different PCA3 cut-off scores was compared with several PSA cut-off values. The low prevalence of serious disease (*n* = 19; 15.6%), of which almost half was present at low PSA values, reflects the prescreened study population. The positive predictive value

(PPV) of the PCA3 test rises slightly with an increasing cut-off, contrary to PSA cut-offs, as can be expected in any PSA-based prescreened population. It is remarkable, however, that 62 of the 90 men (68.9%) with a PCA3 score ≥100 did not have a positive biopsy. A PCA3 score ≥20 identifies 18 of 19 serious PCa cases and avoids 26.1% of biopsies. A PCA3 score ≥35 misses five serious PCa cases, but 48.3% of all

Table 4 – Prostate cancer detection with different prostate-specific antigen and PCA3 cut-off values as biopsy indication*

	Biopsied men, No. (%)	PCa cases, No. (%)	PPV	Missed PCa, No. (%) (n = 122)	Missed serious PCa, No. (%) (n = 19)	Biopsies saved, No. (%) (n = 721)
No cut-off	721	122	16.9	–	–	–
PSA, ng/ml						
≥2.0	347 (48.1)	70 (57.4)	20.2	52 (42.6)	9 (47.4)	374 (51.9)
≥3.0	229 (31.8)	43 (35.3)	18.8	79 (64.7)	11 (57.9)	492 (68.2)
≥4.0	146 (20.3)	29 (23.8)	19.9	93 (76.2)	13 (68.4)	575 (79.8)
≥10.0	21 (2.9)	4 (3.3)	19.1	118 (96.7)	18 (94.7)	700 (97.1)
PCA3 score						
≥10	689 (95.6)	118 (96.7)	17.1	4 (3.3)	0 (0.0)	32 (4.4)
≥20	533 (73.9)	103 (84.4)	19.3	19 (15.6)	1 (5.3)	188 (26.1)
≥35	348 (48.3)	83 (68.0)	23.9	39 (32.0)	5 (26.3)	373 (51.7)
≥100	90 (12.5)	28 (23.0)	31.1	94 (77.0)	12 (63.2)	631 (87.5)

PCa = prostate cancer; PCA3 = prostate cancer antigen 3 gene; PPV = positive predictive value; PSA = prostate-specific antigen.

* 721 men biopsied with 122 PCa cases detected, of which 19 cases were classified as serious (T2a or higher and/or Gleason score >3 + 3).

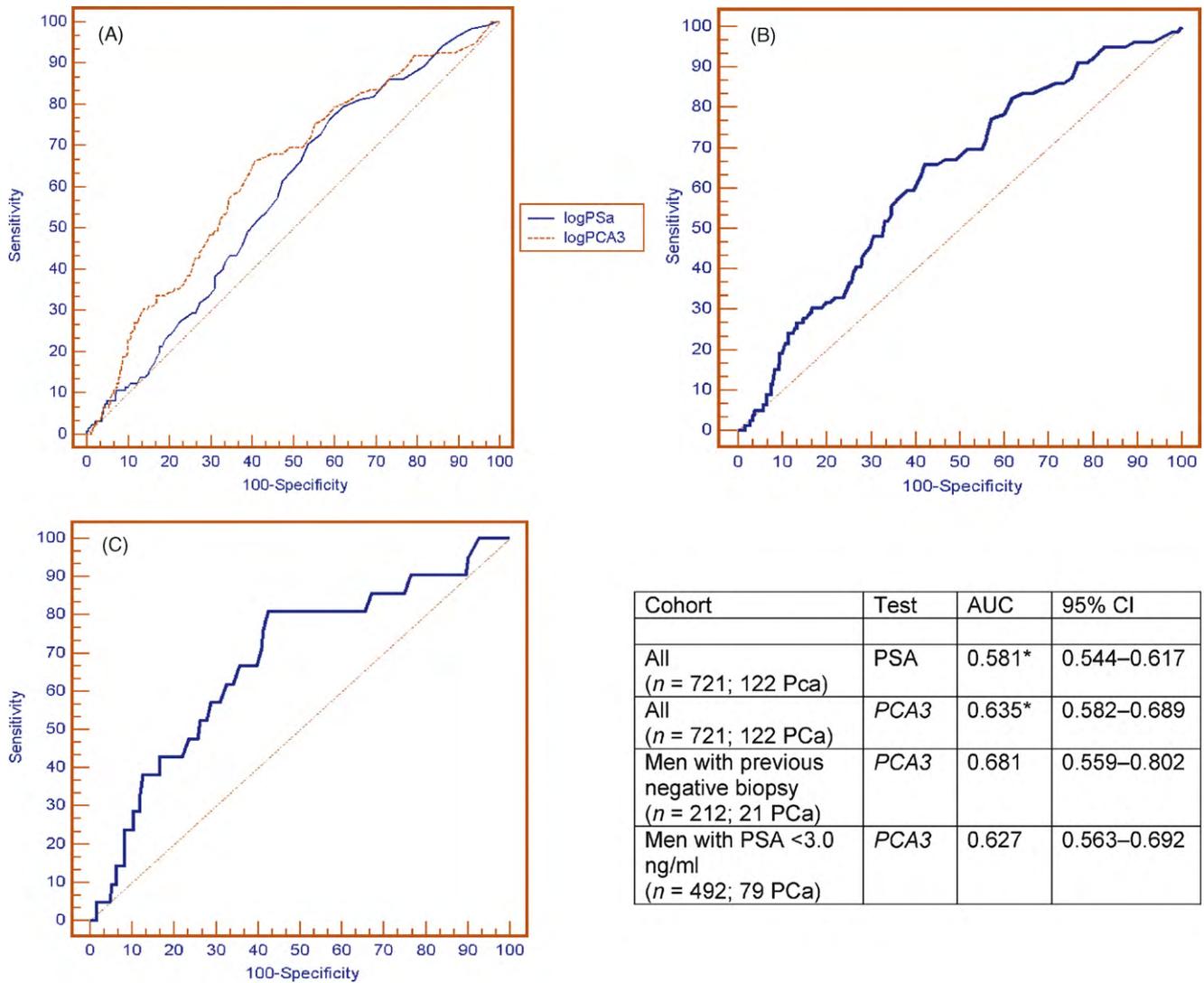


Fig. 1 – Receiver operating characteristic (ROC) curves of (A) prostate-specific antigen (PSA) and the prostate cancer antigen 3 (PCA3) gene in all men biopsied (*n* = 721), (B) PCA3 in men with PSA <3.0 ng/ml (*n* = 492), and (C) PCA3 in men with previous negative biopsy (*n* = 212). AUC = area under the curve; CI = confidence interval; PCa = prostate cancer; PCA3 = prostate cancer antigen 3 gene; PSA = prostate-specific antigen. **p* = 0.143 for PSA versus PCA3.

biopsies could have been avoided. It obviously remains unknown whether the missed serious cases still would have been curable and whether those cases might have escaped notice with all screening efforts anyway.

The data evaluating the performance of PCA3 in men with low PSA values and in men with a previous negative biopsy are presented in Table 5. If the small numbers could be considered trustworthy, 4 of the 11 serious PCa cases would be missed with a PCA3 score ≥ 35 and 10 would be missed with a PCA3 score ≥ 100 . These results, together with the only marginal increase in the PPV with increasing PCA3 scores, do not support our working hypothesis that PCA3 might be useful in identifying aggressive cancers in the low PSA ranges, where only 13% of men were previously biopsied. However, it is encouraging to see rising PPVs with different PCA3 cut-off values and to see that most serious PCa cases are detected with a cut-off of 35.

Looking at 212 men who had undergone previous biopsy driven by a PSA ≥ 3.0 ng/ml, findings do not seem to be more encouraging. The numbers, however, are small and need to be confirmed.

4. Discussion

There is now general agreement that the performance of PSA as a diagnostic test for PCa leaves much to be desired. It has been shown that PSA's performance can be improved by adding other risk factors such as prostate volume and the outcome of DRE [12]. Similar improvements in performance have also been noted for the same predictors in connection with PCA3 scoring [9,13]. Still, a direct head-to-head comparison of PSA and PCA3 has not been available until now and is necessary to understand the relationship between the two markers and to better understand the

Table 5 – Prostate cancer detection with different PCA3 cut-off values as biopsy indication in men previously biopsied (n = 212*) and in men presenting with PSA <3.0 ng/ml (n = 492)**

Men previously biopsied	Biopsied, No. (%)	PCa cases, No. (%)	PPV	Missed PCa, No. (%) (n = 21)	Missed serious PCa, No. (%) (n = 3)	Biopsies saved, No. (%) (n = 212)
Total cohort	212	21	9.9	–	–	–
<i>PCA3 score</i>						
≥10	190 (89.6)	19 (90.5)	10.0	2 (9.5)	0 (0.0)	22 (10.4)
≥20	157 (74.1)	18 (85.7)	11.5	3 (14.3)	0 (0.0)	55 (25.9)
≥35	102 (48.1)	18 (85.7)	17.7	3 (14.3)	0 (0.0)	110 (51.9)
≥100	26 (12.3)	6 (28.6)	23.1	15 (71.4)	1 (33.3)	186 (87.7)
Men with PSA <3.0 ng/ml	Biopsied, No. (%)	PCa, No. (%)	PPV	Missed PCa, No. (%) (n = 79)	Missed serious PCa, No. (%) (n = 11)	Biopsies saved, No. (%) (n = 492)
Total cohort†	492	79	16.1	–	–	–
<i>PCA3 score</i>						
≥20	380 (77.2)	69 (87.3)	18.2	10 (12.7)	0 (0.0)	112 (22.8)
≥35	245 (49.8)	53 (67.1)	21.6	26 (32.9)	4 (36.4)	247 (50.2)
≥100	56 (11.4)	15 (19.0)	26.8	64 (81.0)	10 (90.9)	436 (88.6)

PCa = prostate cancer; PCA3 = prostate cancer antigen 3 gene; PPV = positive predictive value; PSA = prostate-specific antigen.
 * 21 PCa cases, 3 of which were serious.
 ** 79 PCa cases, 11 of which were serious.
 † Total cohort numbers are equal to PCA3 cut-off ≥10 due to study design.

performance of PCA3 in a setting where almost all men of a large cohort were biopsied. Table 3 shows that a commonality of both markers is that there is no cut-off value at which sensitivity and specificity achieve a reasonable balance. In the setting of this prescreened population, a PSA cut-off of ≥2.0 ng/ml and the recommended score of 35 for PCA3 provide the best balance for the two parameters. However, a sensitivity of 57.4% for PSA ≥2.0 ng/ml and a sensitivity of 68% for a PCA3 score of 35 means that 42.6% and 32%, respectively, of all biopsy-detectable cancers are missed.

How then should PCA3 be applied, and which aspects are important in decision taking? As our data show, there is always a trade-off between sensitivity and specificity. If we wish to apply either one of the two tests with a higher sensitivity, then we have to accept a lower specificity, translating into a larger proportion of all men biopsied and a larger proportion of potentially unnecessary biopsies.

Alternatively, if we decide to work with lower sensitivities and accept missing larger proportions of cancer, then we need to know that those missed cancers are not potentially life threatening.

A PCA3 score ≥35, as generally recommended, shows a PPV of 23.9% and misses 39 of the 122 cancers, 5 of which are considered to have serious characteristics. Still, 51.7% of all biopsies are saved. The fact that a PSA cut-off of 3.0 ng/ml has been used two or more times in this population obviously creates a disadvantage for PSA; however, we are dealing with a prescreened population, and we wish to imitate a situation that is common in most countries where PSA screening is prevalent. A recent inventory of tumour characteristics and treatment in both arms of the ERSPC shows that PCa cases detected in recent years in the control arm resemble those detected with screening [14].

Table 6 provides a review of six studies [2,5,7,9,13,15] from the literature, of which two [2,5] report complete data

Table 6 – Performance characteristics of PCA3: Literature overview

Study	Men, No.	PCa, No.	Biopsy indication by PSA, ng/ml	PPV, %	PCA3 score applied	PPV, %	Sensitivity, %	Specificity, %	Serious PCa missed, No. (%)	AUC
Hessels et al [2]	108	24	≥3.0	22.2	200 × 10 ⁻³	53.3	67	83	38 (37.5)	0.72
Van Gils et al [5]	68	16	≥2.5 with or without DRE	23.5	50 × 10 ⁻³	50.0	69	79	Not known	0.74
Kranse et al [11]	534	174	≥3.0–15.0	33	58	48.1	65	66	Not known	0.66
Marks et al [7]	233*	60	≥2.5	27	35	42.2	58	72	Not known**	0.68
Deras et al [9]	570	206	≥2.5 with or without DRE	36	35	54.2	54	74	Not known**	0.70/0.68*
Van Gils et al [13]	463*	128	PSA with or without DRE	28	35	39.0	47	72	27 of 60 missed PCa (45%)	0.65/0.67**

AUC = area under the curve; DRE = digital rectal examination; PCa = prostate cancer; PCA3 = prostate cancer antigen 3 gene; PPV = positive predictive value; PSA = prostate-specific antigen.

* Men with previous negative biopsy.

** PCA3 scores not significantly different between Gleason 6 and Gleason score ≥7.

on performance characteristics of *PCA3*. Sensitivity and specificity show a remarkable similarity in all studies, including the present one. The AUCs indicate an overall performance of a test and, contrary to sensitivity and specificity, do not have direct clinical relevance.

In considering these studies, the reader must realise that all except our study use variable PSA cut-off values with or without DRE as the primary diagnostic tool. This approach results in attribution or assignment bias because, contrary to our data, cancers that can be found in large proportions in the PSA ranges below the ones utilised in each one of these studies are, by definition, excluded. The present study, in which 95.6% of all men were biopsied (70% were biopsied for the first time) on the basis of *PCA3* score, allows more conclusive evaluation of test performance.

Table 5 addresses the value of *PCA3* in the PSA range <3.0 ng/ml, showing that 66.2% of these men have a *PCA3* score ≥ 35 , necessitating biopsy in about half of the population. The PPV of 21.6% compares very favourably with the PPV of DRE in men with PSA values of <4.0 ng/ml, reported to be 10% [16].

The results obtained by authors who have studied the relationship between *PCA3* scores and parameters of cancer aggressiveness differ by outcome. Although some studies [9,17,18] have not established a positive relationship between *PCA3* scores and parameters of more serious disease (T2 or higher or Gleason ≥ 6), other authors show such relationships [13,19,20]. Several authors including Nakanishi et al [21] found a significant value for *PCA3* in predicting small prostates with volumes <0.5 cm³; however, Deras et al [9] could not confirm this finding. The reported and discussed data suggest that *PCA3* may benefit from combination with other predictive factors. The limited literature on this issue is developing. Chun et al [22] used a base model of clinical predictors, and *PCA3* scores were used to construct a nomogram recommended for use in conjunction with *PCA3* testing, leading to significant improvements of the AUC up to a maximum of 0.730. Ankerst et al [23] added the *PCA3* test to the National Cancer Institute risk calculator, which is based on the Prostate Cancer Prevention Trial [8], leading to a nonsignificant increase of the performance of this risk calculator. Adding the *PCA3* test results to the ERSPC risk calculator showed an improvement of the AUC for predicting a positive biopsy of 0.69–0.71. A similar increase of the AUC was seen when adding a previously described kallikrein panel [24,25].

In the available data sets, it is remarkable that the highest usually reported *PCA3* score of ≥ 100 has relatively low positive biopsy rates, such as the 54% reported by Deras et al [9] and the 47% reported by Van Gils et al [13]. This finding is confirmed in an exaggerated fashion in our data set. As Table 4 shows, of 90 men with a *PCA3* score ≥ 100 , only 28 (31%) had PCa. The available information in the literature does not offer a conclusive explanation for this phenomenon. One explanation is that PCa is missed by biopsy. Reports on the number of biopsies taken are incomplete in the cited literature. To exclude this possibility, a follow-up study of our present protocol has been

carried out in which repeat biopsy is offered to those men who had a *PCA3* score ≥ 100 and a negative biopsy (M.J. Roobol, unpublished data, 2010).

Strengths and weaknesses of our study are that we included a large cohort of men, recruitment and testing took place under well-controlled circumstances, all tests were carried out in one laboratory, and the prescreened nature of our study population reflects the current clinical situation in most countries. Our study allows the establishment of performance characteristics of *PCA3* because 95.6% of all men who were biopsied were also indicated by the *PCA3* cut-off ≥ 10 and, for the first time, allows evaluation of *PCA3* in men with low PSA values (<3.0 ng/ml). The fact that we are addressing a prescreened population could also be considered a weakness because data cannot be used to enhance our understanding of the performance characteristics of *PCA3* in previously unscreened men. Due to prior screening, the number of first biopsies above the PSA cut-off of 3.0 ng/ml is low, and a sextant biopsy technique was applied.

5. Conclusions

The present study allowed investigation, for the first time, of the performance characteristics of *PCA3* compared with those of PSA. The performance characteristics of PSA are much in line with previous investigations. Large proportions of cancer, including potentially aggressive disease, are missed with presently used cut-off values. A somewhat more favourable picture is seen for *PCA3*. PPV and sensitivity are improved, as is the identification of serious disease compared with PSA in this pre-screened population. The value of *PCA3* for improving detection in the low-PSA ranges and after previous negative biopsies, being hampered by small numbers, is unclear and needs to be explored further.

Author contributions: Monique J. Roobol had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Roobol, Schröder.

Acquisition of data: Roobol, van den Bergh, van Leeuwen, Wolters, van Leenders, Hessels.

Analysis and interpretation of data: Roobol.

Drafting of the manuscript: Roobol, Schröder.

Critical revision of the manuscript for important intellectual content: van den Bergh, van Leeuwen, Wolters, van Leenders, Hessels.

Statistical analysis: Roobol.

Obtaining funding: Schröder.

Administrative, technical, or material support: None.

Supervision: None.

Other (specify): None.

Financial disclosures: I certify that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: F.H. Schröder is an advisor to Glaxo Smith Kline, Ferring, and Schering. M.J. Roobol is an advisor for Glaxo Smith Kline, Beckman, and Genprobe. D. Hessels is associated with NovioGendix.

Funding/Support and role of the sponsor: The sponsor was involved in review and approval of the manuscript.

Acknowledgment statement: The authors thank the staff of the ERSPC Rotterdam screening unit for their administrative assistance and data collection.

References

- [1] Bussemakers MJ, van Bokhoven A, Verhaegh GW, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res* 1999;59:5975–9.
- [2] Hessels D, Klein Gunnewiek JMT, van Oort I, et al. DD3^{PCA3}-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 2003;44:8–16, discussion 15–6.
- [3] De Kok JB, Verhaegh GW, Roelofs RW, et al. DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. *Cancer Res* 2002;62:2695–8.
- [4] Tinzl M, Marberger M, Horvath S, Chypre C. DD3^{PCA3} RNA analysis in urine – a new perspective for detecting prostate cancer. *Eur Urol* 2004;46:182–7, discussion 187.
- [5] Van Gils MP, Cornel EB, Hessels D, et al. Molecular PCA3 diagnostics on prostatic fluid. *Prostate* 2007;67:881–7.
- [6] Groskopf J, Aubin SM, Deras IL, et al. APTIMA. PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin Chem* 2006;52:1089–95.
- [7] Marks LS, Fradet Y, Deras IL, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* 2007;69:532–5.
- [8] Thompson IM, Ankerst DP, Chi C, et al. Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. *JAMA* 2005;294:66–70.
- [9] Deras IL, Aubin SM, Blase A, et al. PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J Urol* 2008;179:1587–92.
- [10] Roobol MJ, Kirkels WJ, Schröder FH. Features and preliminary results of the Dutch centre of the ERSPC (Rotterdam, the Netherlands). *BJU Int* 2003;92(Suppl 2):48–54.
- [11] Kranse R, Beemsterboer PMM, Rietbergen JBW, Habbema D, Hugosson J, Schröder FH. Predictors for biopsy outcome in the European Randomized Study of Screening for Prostate Cancer (Rotterdam region). *Prostate* 1999;39:316–22.
- [12] Roobol MJ, Steyerberg EW, Kranse R, et al. A risk-based strategy improves prostate-specific antigen-driven detection of prostate cancer. *Eur Urol* 2010;57:79–85.
- [13] Van Gils MP, Hessels D, van Hooij O, et al. The time-resolved fluorescence-based PCA3 test on urinary sediments after digital rectal examination; a Dutch multicenter validation of the diagnostic performance. *Clin Cancer Res* 2007;13:939–43.
- [14] Boevee S, Venderbos LDF, Ranniko A, et al. Change of treatment over time in both arms of the ERSPC. *Eur J Cancer*. In press.
- [15] Haese A, de la Taille A, van Poppel H, et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur Urol* 2008;54:1081–8.
- [16] Catalona WJ, Smith DS, Ornstein DK. Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/ml and benign prostate examination. Enhancement of specificity with free PSA measurements. *JAMA* 1997;277:1452–5.
- [17] Hessels D, van Gils MP, van Hooij O, et al. Predictive value of PCA3 in urinary sediments in determining clinico-pathological characteristics of prostate cancer. *Prostate* 2010;70:10–6.
- [18] Schilling D, de Reijke T, Tombal B, de la Taille A, Hennenlotter J, Stenzl A. The prostate cancer gene 3 assay: indications for use in clinical practice. *BJU Int* 2010;105:452–5.
- [19] Whitman EJ, Groskopf J, Ali A, et al. PCA3 score before radical prostatectomy predicts extracapsular extension and tumor volume. *J Urol* 2008;180:1975–8, discussion 1978–9.
- [20] Van Gils MP, Hessels D, Hulsbergen-van de Kaa CA, et al. Detailed analysis of histopathological parameters in radical prostatectomy specimens and PCA3 urine test results. *Prostate* 2008;68:1215–22.
- [21] Nakanishi H, Groskopf J, Fritsche HA, et al. PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J Urol* 2008;179:1804–9, discussion 1809–10.
- [22] Chun FK, de la Taille A, van Poppel H, et al. Prostate cancer gene 3 (PCA3): development and internal validation of a novel biopsy nomogram. *Eur Urol* 2009;56:659–68.
- [23] Ankerst DP, Groskopf J, Day JR, et al. Predicting prostate cancer risk through incorporation of prostate cancer gene 3. *J Urol* 2008;180:1303–8, discussion 1308.
- [24] Roobol MJ, Hessels D, Vickers A, et al. Predicting prostate biopsy outcome in a screening setting. PSA, PCA3, a kallikrein panel, the risk calculator or a combination? ERSPC Rotterdam. Poster 974 presented at: European Association of Urology Annual Congress; April 16–20, 2010; Barcelona, Spain.
- [25] Vickers AJ, Cronin AM, Roobol MJ, et al. A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European Randomized Study of Screening for Prostate Cancer, Rotterdam. *Clin Cancer Res* 2010;16:3232–9.