

The prostate cancer gene 3 (PCA3) urine test in men with previous negative biopsies: does free-to-total prostate-specific antigen ratio influence the performance of the PCA3 score in predicting positive biopsies?

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OBJECTIVE

To determine the performance characteristics of the prostate cancer gene 3 (PCA3) score on the outcome of biopsy relative to different ranges of free-to-total prostate-specific antigen (PSA) ratio (f/tPSA) in men with a previous negative biopsy and a PSA level of 2.5–10 ng/mL, as urine tests like PCA3 are currently under investigation in order to improve prostate cancer diagnosis and to decrease the rate of unnecessary rebiopsies.

PATIENTS AND METHODS

Data from the previous prospective European multicentre study were reviewed. Only patients with a PSA level of 2.5–10 ng/mL were included in the present study. In all,

301 patients had complete data. The diagnostic accuracy of the PCA3 score for predicting a positive biopsy outcome was studied using sensitivity, specificity, negative and positive predictive values. The PCA3 performance was evaluated relative to three different subgroups of f/tPSA, as follows: >20% (group 1), 10–20% (group 2) and <10% (group 3).

RESULTS

The prostate cancer detection rates were 18.8%, 23.9% and 34.8% in groups 1, 2 and 3, respectively. The area under the receiver operating characteristic curve of the PCA3 score, total PSA and f/tPSA was 0.688, 0.553 and 0.571, respectively. The percentage of men with positive biopsies was 30.6%, 37.0% and 44.4% in those with a PCA3 score of >30, vs 10.3%, 15.5% and 28.6% when the PCA3 score was <30, in groups 1, 2 and 3, respectively. The difference was significant only in groups 1 and 2. In men with a f/tPSA

of ≤10% the difference in detection rates relative to the PCA3 score was not statistically significant regardless of which PCA3 threshold was used. A high PCA3 score was significantly associated with age, clinical T2 stage and positive biopsy ($P < 0.001$, 0.013 and <0.001, respectively). In bivariate analysis accounting for the PCA3 score and the f/tPSA, a PCA3 score of >30 was a significant independent predictor of positive biopsies (odds ratio 3.01; 95% confidence interval 1.74–5.23; $P < 0.001$).

CONCLUSIONS

PCA3 remained a better predictor of prostate cancer than f/tPSA. In men with a f/tPSA of >10%, the use of the PCA3 score was highly correlated with the risk of having cancer on re-biopsy, and could prevent unnecessary prostate biopsies if the value is low.

KEYWORDS

prostate cancer, biopsy, PCA3, detection rate

INTRODUCTION

Prostate cancer is the most common solid malignancy in men in the European Union, resulted in >87 000 deaths in 2006 [1]. The

prostate cancer detection rate has strongly increased since the use of PSA screening and of extended prostate biopsy protocols. Furthermore, ≈25% of prostate cancers remain undiagnosed after a single set of

biopsy cores [2]. In men with a negative first set of biopsies, a second set of extended biopsies is clearly recommended in men with persistent suspicion of cancer [3]. Despite such strategies, the false-negative rate

remains substantial, due to low positive predictive values (PPVs) in the 'grey' zone of PSA, i.e. 2.5–10 ng/mL. Thus, the identification of biomarkers capable of providing a reliable molecular diagnostic test for prostate cancer is highly desirable.

Urine biomarkers predicting pathological biopsy findings and prostate cancer have been studied [4,5]. Recently, the prostate cancer gene 3 (PCA3) assay has been hypothesized to better identify men at high risk of a positive biopsy and to clarify the best candidates for a repeat biopsy strategy [6–12]. Particularly, the probability of a positive repeat biopsy increased with increasing PCA3 score [10,11]. In men with at least one negative biopsy, the urinary PCA3 score was superior to serum PSA for predicting the biopsy outcome and its diagnostic accuracy was independent of total PSA level. Of clinical relevance would be to propose such a test in each candidate for a repeat strategy. Interestingly, in the European series, the best diagnostic accuracy was obtained in the 'grey' zone in which the yield of the free-to-total PSA ratio (f/tPSA) is maximal [10]. In that series, a high PSA3 score overall had a greater diagnostic accuracy than f/tPSA, but separate analyses stratified by f/tPSA were missing.

The goal of the present analysis was to determine the performance characteristics of the PCA3 score on the biopsy outcome relative to the different ranges of f/tPSA in men with previous negative biopsy and a PSA level of 2.5–10 ng/mL.

PATIENTS AND METHODS

Data from the previous prospective European multicentre study were reviewed [10]. This reference study included men with one or two previous negative prostate biopsies who had a repeat biopsy in six centres, excluding men with medical therapy known to affect PSA, UTI or invasive treatment for BPH. Urine specimens were collected between August 2006 and July 2007 before biopsy. The PCA3 score was calculated as (PCA3 mRNA)/(PSA mRNA) × 1000 [13]. Biopsy protocols included at least 10 peripheral zone biopsy cores and were reviewed by local pathologist. Age, PSA, f/tPSA, general and biopsy medical history, DRE findings and prostate volume were collected prospectively into the database. Only patients with a PSA level of 2.5–10 ng/mL were included in the present study;

Variable	Mean (range, median) or %	TABLE 1 The characteristics of the 301 men included in the study
Age, years	64.6 (43.3–83.4, 65.4)	
PSA, ng/mL	6.35 (2.5–10.0, 6.30)	
f/tPSA, %	0.19 (0.04–0.67, 0.18)	
Suspicious DRE,	17.4	
Prostate volume, mL	53.7 (15.0–217.0, 50.0)	
Number of previous negative biopsies		
1	76	
2	24	
Pathological findings		
Cancer	23.6	
Atypical small acinar proliferation	4.0	
Number of positive cores	3.0 (1.0–17.0, 2.0)	
Biopsy Gleason score		
≤6	65.5	
7	30.2	
8	4.3	
PCA3 score	43.4 (1.7–906.3, 24.2)	
>25	49.2	
>30	40.2	
>35	33.2	

in all, 301 had complete data, especially for f/tPSA.

The diagnostic accuracy of the PCA3 score for predicting a positive biopsy outcome was studied using sensitivity, specificity, negative (NPV) and PPVs. The PCA3 performance was evaluated relative to: (i) three different thresholds of PCA3 score (25, 30 and 35); and to (ii) three different subgroups of f/tPSA, i.e. >20% (group 1), 10–20% (group 2) and <10% (group 3). Odds ratios (ORs) were calculated in univariable analyses. Areas under the curve (AUC) of the receiver operating characteristics (ROC) were also computed. A multivariable analysis was used to compare the diagnostic accuracy of PCA3 score (threshold 30) and f/tPSA (threshold 10%) in predicting cancer on repeat biopsy. Finally, correlations between PCA3 score, f/tPSA, prostate volume, age, clinical stage and biopsy Gleason score were assessed. A Mann–Whitney nonparametric test was used for continuous variables, and qualitative data were tested using a chi-square test or Fisher's exact test as appropriate. The limit of statistical significance was defined as $P < 0.05$.

RESULTS

Of the 301 patients, 71 had prostate cancer on repeat biopsy (detection rate 23.6%); the characteristics are listed in Table 1. The mean age was 64.6 years, 24% of men had a second/

third set of biopsies and the clinical stage was T2 in 17.4% of men. The pathological biopsy Gleason score was 7 in 30.2% and ≥8 in 4.3% of men. The mean and median PCA3 score was 43.4 and 24.2, respectively.

The f/tPSA was >20% in 117 men (38.9%, group 1), 10–20% in 138 (45.8%, group 2) and ≤10% in 46 (15.3%, group 3). The prostate cancer detection rates were 18.8%, 23.9% and 34.8% in groups 1, 2 and 3, respectively. Only the rates between group 1 and 3 were statistically significant ($P = 0.030$).

Results in terms of sensitivity, specificity, NPV and PPV are shown in Table 2. The prostate cancer detection rate in patients with a PCA3 score of <25 and <35 was 12.4% and 16.9%, respectively, compared with 35.1% and 37.0% in men with a score of >25 and >35 ($P < 0.001$). The sensitivity was 68.8–77.3% among the three groups when a threshold score of 25 was used, and the specificity was 66.7–79.1% when the threshold score was 35.

NPVs were high in groups 1 and 2, regardless of how the PCA3 score was defined. In group 1, the NPV for a threshold score of >25 reached 91.1%. The PPV was 27.9–45.8% relative to the threshold and the group studied.

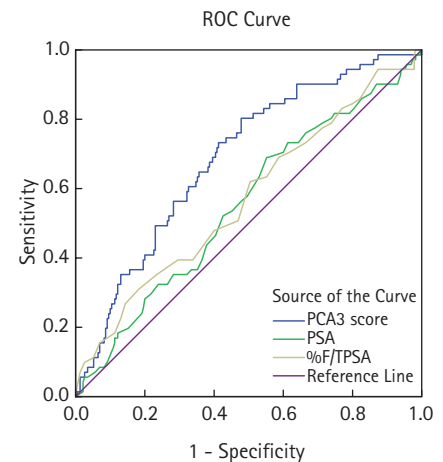
Performance value in terms of OR and AUC are also listed in Table 2. The AUC of the PCA3 score, total PSA and f/tPSA was 0.688, 0.553

	f/tPSA, %		
	>20	10–20	≤10
No. of men	117	138	46
PCA3 >25			
Sensitivity	77.3	72.7	68.8
Specificity	53.7	62.9	56.7
PPV	27.9	38.1	45.8
NPV	91.1	88.0	77.3
PCA3 >30			
Sensitivity	68.2	60.6	50.0
Specificity	64.2	67.6	66.7
PPV	30.6	37.0	44.4
NPV	89.7	84.5	71.4
PCA3 >35			
Sensitivity	59.1	51.5	43.8
Specificity	67.4	79.1	66.7
PPV	29.6	43.6	41.2
NPV	87.7	83.8	69.0
AUC	0.695	0.712	0.617
PCA3 >25			
OR	3.94	4.51	2.88
P	0.009	<0.001	0.10
95% CI	1.34–11.56	1.91–10.69	0.80–10.35
PCA3 >30			
OR	3.85	3.21	2.00
P	0.006	0.004	0.27
95% CI	1.43–10.35	1.43–7.22	0.58–6.91
PCA3 >35			
OR	2.98	4.00	1.56
P	0.021	0.001	0.49
95% CI	1.15–7.73	1.75–9.18	0.45–5.41

TABLE 2

Performance of the PCA3 score relative to f/tPSA as the sensitivity, specificity PPV and NPV, and as the AUC and OR

FIG. 1. ROC curves and AUC of PCA3 score, f/tPSA and total PSA.



clinical T2 stage and positive biopsy ($P < 0.001$, 0.013 and <0.001 , respectively). There was no difference in PCA3 score with prostate volume and biopsy Gleason score. For cancer aggressiveness, the PCA3 score was not significantly associated with biopsy Gleason score or number of positive cores, in univariable and multivariable analysis ($P = 0.604$ and 0.552 , respectively). The f/tPSA was significantly associated only with prostate volume and age.

In bivariate analysis considering the PCA3 score and f/tPSA, a PCA3 score of >30 was a significant independent predictor of positive biopsies (OR 3.01; 95% CI 1.74–5.23; $P < 0.001$). A f/tPSA of $\leq 10\%$ was not a significant predictor (OR 1.80; 95% CI 0.85–3.82; $P = 0.125$).

In multivariable analysis considering PCA3 score, f/tPSA, total PSA level, prostate volume, number of previous sets of biopsies and DRE finding, a PCA3 score of >30 remained a significant independent predictor of positive biopsies ($P = 0.027$). Abnormal DRE findings and prostate volume were also significant ($P < 0.001$ and 0.035 , respectively). The f/tPSA, total PSA and number of previous biopsies were not significant ($P = 0.096$, 0.256 and 0.941 , respectively).

DISCUSSION

The discovery and the development of novel biomarkers for prostate cancer diagnosis and prognosis remains a challenge, despite the widespread use of PSA screening. Despite a PSA-based screening strategy, the false-

Variable	PCA3 score	P	f/tPSA	P
Positive biopsy	63.8	<0.001	0.18	0.073
Negative biopsy	37.1		0.20	
Normal DRE	85.0	0.013	0.19	0.596
Suspicious DRE	34.7		0.19	
Gleason score <7	67.0	0.505	0.19	0.079
Gleason score ≥ 7	61.3		0.15	
Prostate volume <40	48.4	0.821	0.17	<0.001
Prostate volume ≥ 40	41.5		0.21	
Age <65 years	29.8	<0.001	0.18	0.001
Age ≥ 65 years	56.4		0.21	

TABLE 3

The mean PCA3 score and f/tPSA according to clinical and pathological variables

and 0.571, respectively (Fig. 1). In groups 1, 2 and 3, the AUC was 0.695, 0.712 and 0.617, respectively.

The OR evaluating the risk of having positive cores for prostate cancer was 3–4.5, relative to the PCA3 score used, and was significant ($P 0.021$ – <0.001). The OR was 1.6–2.9 in group 3, but was not significant. Using a PCA3 threshold of 30 (Fig. 2), the percentage of men

with positive biopsies was 30.6%, 37.0% and 44.4% for a PCA3 score of >30 , compared with 10.3%, 15.5% and 28.6% with a PCA3 score of <30 , in groups 1, 2 and 3, respectively.

Table 3 compares the distributions between PCA3, f/tPSA, pretreatment variables and biopsy pathological findings. A high PCA3 score was significantly associated with age,

negative rate for detecting prostate cancer remains substantial, due to low PPVs in the 'grey' zone of PSA, i.e. 2.5–10 ng/mL. The determination of f/tPSA can help clinicians to better identify candidates for biopsy. There was an interval of >10 years between the discovery of PSA and its clinical application [5]. The PCA3 gene was discovered at the end of the 1990s and methods of quantitative measurement of its expression are now well-standardized [6,13–15]. Current trends in research have shown the superiority of the PCA3 score over PSA level in terms of specificity and predictive values, although with slightly inferior sensitivity [10–12]. The quantitative PCA3 score was clearly correlated with the probability of a positive biopsy, independently of a previous biopsy history. Data were also consistent with the PCA3 score being independent of prostate volume and PSA level [10–12].

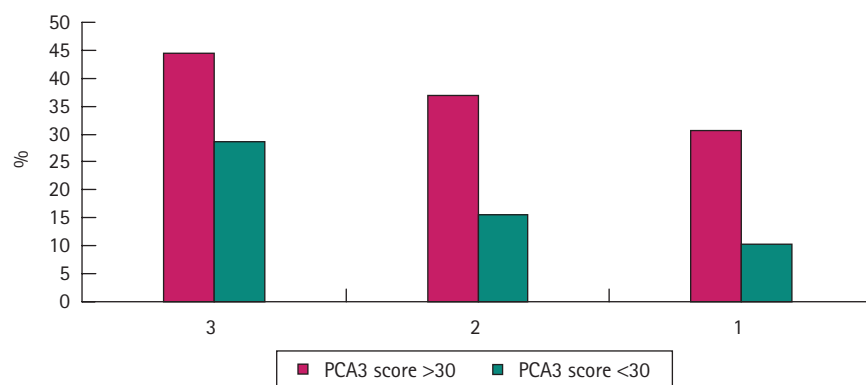
European and American repeat-biopsy studies have provided evidence that PCA3 values can add specificity to a diagnostic algorithm for prostate cancer in men with previous negative biopsies [10,11]. Haese *et al.* [10] emphasized that the PCA3 score had a greater diagnostic accuracy than f/tPSA when a threshold of 35 and 25% were used for each determinant, respectively.

The cost of the PCA3 urine test limits the widespread use of such a test for all patients with a previous negative set of biopsies. f/tPSA is a well-established and inexpensive tool that can help urologist to plan to take, or not, subsequent sets of biopsies in men with a PSA level in the grey zone [16–19].

Overall, the findings of the present study showed that, regardless of f/tPSA level, the probability of a positive repeat biopsy increased with higher PCA3 scores. As shown previously, using lower PCA scores as the threshold resulted in greater sensitivity and NPV at the expense of specificity and PPV. Overall results tended to show that the PCA3 score was independent of f/tPSA for predicting the repeat biopsy outcome.

However, the diagnostic yield of the PCA3 score was greater in patients with a f/tPSA of >10%, regardless of the PCA3 threshold used. The diagnostic performance of the PCA3 score was high in men with a f/tPSA of 10–20%, when the AUC was 0.712. The OR for having positive cores on biopsy reached 4.5 when the PCA3 threshold was 25 ($P < 0.001$). Only 9%

FIG. 2. The percentage of positive biopsies for prostate cancer relative to the PCA3 score (threshold 30) in each group; group 1 (f/tPSA >20%; $P = 0.006$); group 2 (f/tPSA 10–20%; $P = 0.004$); group 3 (f/tPSA <10%; $P = 0.270$).



of men with a PCA3 score of <25 had cancer on biopsies (NPV 91.1% and 88.0% in groups 1 and 2, respectively).

In patients with a f/tPSA of ≤10% the diagnostic yield of PCA3 remains interesting but appeared to be more limited than in those with a high f/tPSA. Overall the prostate cancer detection rate in this subgroup was high (34.8%). Using the PCA3 score in men with a f/tPSA of ≤10% resulted in lower sensitivity and NPVs; particularly, the NPV was only 69.0 when a threshold PCA3 score of 35 was used. Thus, 31% of men with a PCA3 score of <35 had positive cores on repeat biopsy. The loss of diagnostic accuracy for the PCA3 score for these f/tPSA ranges was essentially reported in terms of sensitivity and predictive values. Nevertheless, the specificity was maintained at an interesting level. In men with a f/tPSA of ≤10% the difference in detection rates relative to the PCA3 score was not statistically significant, regardless of the PCA3 threshold used. In men with a f/tPSA of >10% this difference was always significant.

As previously described, the PCA3 score was independent of prostate volume in men with a PSA level of 2.5–10 ng/mL. On the contrary, a low f/tPSA was strongly associated with low prostate volume. Thus, interpreting the f/tPSA can be difficult in patients with a large prostate, whereas the PCA3 score did not vary relative to it.

In the analysis of the overall cohort of the European study (463 patients), Haese *et al.* [10] found that a high PCA3 score was significantly associated with high Gleason score on biopsies (Gleason ≥7). Our findings

did not confirm this. This discrepancy can be explained by the smaller sample of men and by the restricted population studied in the present report (only men in the 'grey' zone of PSA level). Men at lower risk of aggressive and high Gleason score prostate cancer were studied in the present series (men with a PSA level of >10 ng/mL excluded). This resulted in a decrease of statistical power. However, other series also failed to show a statistical difference in PCA3 scores for biopsy Gleason score 6 vs ≥7 [12,20]. The PCA3 score did not appear as a predictor of Gleason score on biopsy in all series. Nevertheless, the PCA3 score has been hypothesized to be significantly associated with tumour volume, Gleason score and extraprostatic extension in radical prostatectomy specimens [20,21]. Multicentre data from the European series will be collected to determine such a correlation in the present cohort [10].

In conclusion, in men with previous negative biopsies and a PSA level of 2.5–10 ng/mL, the PCA3 score was strongly associated with the risk of prostate cancer on repeat biopsy. Interestingly, the diagnostic value appeared to be independent of f/tPSA level in a multivariable model and outperformed f/tPSA in predicting cancer on repeat biopsy. The highest diagnostic yield was obtained for patients with a f/tPSA of >10%. For these men, the use of PCA3 score could avoid unnecessary subsequent biopsies.

CONFLICT OF INTEREST

Hendrik van Poppel is a member of the advisory board of Gen-Probe. Alexandre de la Taille is an investigator with Gen-Probe.

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Abbreviations: PCA3, prostate cancer gene 3; f/tPSA, free/total PSA; ROC, receiver operating characteristic; AUC, area under the ROC curve; NPV, PPV, negative, positive predictive value; OR, odds ratio.