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Predicting prostate biopsy outcome: prostate health index (phi) and prostate cancer antigen 3 (PCA3) are useful biomarkers

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ABSTRACT

Indication for prostate biopsy is presently mainly based on prostate-specific antigen (PSA) serum levels and digital-rectal examination (DRE). In view of the unsatisfactory accuracy of these two diagnostic exams, research has focused on novel markers to improve pre-biopsy prostate cancer detection, such as phi and PCA3. The purpose of this prospective study was to assess the diagnostic accuracy of phi and PCA3 for prostate cancer using biopsy as gold standard.

Phi index (Beckman coulter immunoassay), PCA3 score (Progensa PCA3 assay) and other established biomarkers (tPSA, fPSA and %fPSA) were assessed before a 18-core prostate biopsy in a group of 251 subjects at their first biopsy.

Values of %p2PSA and phi were significantly higher in patients with PCa compared with PCa-negative group ($p<0.001$) and also compared with high grade prostatic intraepithelial neoplasia (HGPIN) ($p<0.001$). PCA3 score values were significantly higher in PCa compared with PCa-negative subjects ($p<0.001$) and in HGPIN vs PCa-negative patients ($p<0.001$). ROC curve analysis showed that %p2PSA, phi and PCA3 are predictive of malignancy.

In conclusion, %p2PSA, phi and PCA3 may predict a diagnosis of PCa in men undergoing their first prostate biopsy. PCA3 score is more useful in discriminating between HGPIN and non-cancer.

1. Introduction

Results of prostate biopsies are currently dichotomized in presence and absence of cancer. Biopsy outcomes that are neither benign nor malignant are diagnosed, in most of cases, as high grade prostatic intraepithelial neoplasia (HGPIN) or atypical small acinar proliferation (ASAP). Among these, HGPIN shares morphologic and genetic changes with cancer, but do not invade the basement membrane of prostatic gland [1]. The incidence of isolated HGPIN on needle biopsy ranges from 0 to 24.6% and the risk of cancer on re-biopsy is 22% [2,3].

PSA serum level and DRE are the main tools to select subjects for prostate biopsy [4], even if there are assay-dependent variations in PSA [5] and inter-observer variability of DRE [6]. Several studies address this issue and new biomarkers that may improve the detection of prostate cancer (PCa) have been proposed [7,8]. Among these, prostate health index (phi) and prostate cancer antigen 3 (PCA3) appear extremely promising [9].

PCA3 is a urine biomarker useful to select candidates for a repeat biopsy strategy [10–12]. In first biopsy, previous report indicated that PCA3 is able to improve PSA diagnostic performance [13]. We previously reported that PCA3 can aid in predicting cancer in patients with PSA levels in the "grey" area [14].

Abbreviations: phi, prostate health index; PCA3, prostate cancer antigen 3; PSA, prostate-specific antigen; DRE, digital-rectal examination; tPSA, total PSA; fPSA, free PSA; p2PSA, [−2]proPSA; f/t PSA, free/total PSA; HGPIN, high grade prostatic intraepithelial neoplasia; ASAP, atypical small acinar proliferation; TRUS, transrectal-ultrasound; n.s., not significant; AUC, Area Under the Roc Curve.

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Recently, the phi index, resulting from the combination of total PSA (tPSA), fPSA and [−2]proPSA (p2PSA) was developed [15]. ProPSA represents a cancer-associated form of free PSA detectable in the circulation [16]. Preliminary studies showed that p2PSA, %p2PSA and phi are higher in malignant than in benign prostatic conditions and their use can significantly improve cancer detection with respect to other biomarkers such as tPSA and f/t PSA ratio [15–17].

Aim of this prospective study was to assess accuracy of phi and PCA3 to predict benign, malignant and HGPIN diagnosis in men undergoing first biopsy.

2. Materials and methods

2.1. Study design

Between May and December 2010, two hundred and fifty male subjects were referred to a major oncologic center (IRCCS Fondazione G. Pascale, Naples, Italy) to undergo first prostate biopsy. They provided informed consent and were screened to be enrolled in a prospective study. Hospital ethics committee was obtained and Standards for the Reporting of Diagnostic Accuracy guidelines were followed.

Blood specimens were collected according to predetermined standard operating procedure [18]. Among these, only those meeting eligibility criteria according to the study protocol were ultimately enrolled: age over 50 years, no prior prostate surgery and biopsy, no bacterial acute or chronic prostatitis, no use of 5-α reductase inhibitors in the previous six months, PSA values included between 2 and 20 ng/ml, negative digital rectal examination (DRE), availability of serum and urine samples and corresponding clinical data.

2.2. Specimens and laboratory analysis

Whole blood was allowed to clot before serum was separated by centrifugation. Serum aliquots were stored at −80 °C until samples were processed, according to Semjonow et al. [19]. Specimens were analyzed in blinded fashion for PSA, fPSA and p2PSA by Access2 Immunoassay System analyzer (Beckman Coulter, Brea, CA, USA).

First catch urine samples were collected following an attentive DRE (three strokes per lobe) as described by Grospkopf et al. [20] immediately before biopsy was performed; urine samples were processed and tested to quantify PCA3-messenger RNA(mRNA) and PSA-mRNA concentrations using the Progensa PCA3 assay (Gen-Probe, San Diego, CA, USA). The PCA3 score was calculated as mRNA PCA3 / mRNA PSA × 1000.

All patients underwent a 18-core transrectal-ultrasound (TRUS) guided prostate biopsy according to a standardized scheme [21]. Primary and secondary Gleason scores were assigned by a single genitourinary pathologist blinded to the biomarkers values, according to the 2005 consensus conference of the International Society of Urological Pathology definitions [22]. Patients diagnosed with high-grade prostatic intraepithelial neoplasia (HGPIN) were compared to benign and malignant biopsy outcome.

2.3. Study endpoints

The primary aim of the study was to compare the identifying ability of PCa-negative, PCa-positive and HGPIN of Beckman coulter phi [(p2PSA/fPSA) × √tPSA] and PCA3 score [(PCA3 mRNA/PSA mRNA) × 1000].

2.4. Statistical analysis

Statistical analyses were performed with the statistical computing environment R (version 2.12.1; R Foundation for Statistical Computing, Vienna, Austria). For all analyses, we used two-sided tests, with p values less than 0·05 denoting statistical significance. Results are expressed as Median [Min–Max] for numeric variables and as percentages for categorical factors.

The Kruskal–Wallis test was used to assess the presence of differences among groups (no PCa, HGPIN and PCa); if the results were significant, multiple comparisons were made according to the Mann–Whitney nonparametric procedure.

Diagnostic validity of the different biomarkers was evaluated by ROC curve analysis. The diagnostic accuracy was measured using the Area Under the Roc Curve (AUC). In order to reach a statistical power (1-β) of 80% at a significance level (α) of 5%, a total of 141 subjects (47 with positive status and 94 with a negative status) were needed to detect a difference between two correlated AUC of ±15% (when this difference truly exists), assuming a sample allocation rate of 1/2 and a correlation between the two test variables of 0.4. The evaluation of the statistical significance of the difference between two correlated AUC was carried out according to the DeLong method [23] whenever the direction of the two testing variables was equal; otherwise a bootstrap procedure was carried out with B = 2000 bootstrap samples.

Multivariable statistical modeling to assess whether the synthesis of several biomarkers into a diagnostic score would have produced a significant increase in their discriminatory ability was carried out according to standard statistical practice [24,25].

3. Results

Overall, one hundred and fifty-one patients met inclusion criteria and were enrolled. Demographic and clinical characteristics of the study population are listed in Table 1. HGPIN was found in 24% of subjects, prostate biopsy-detected cancer in 32%, of which about 90% were clinically significant according to Epstein criteria, based on 1) PSA density ≥0.15 ng/ml, 2) biopsy Gleason score >6, 3) the presence of tumor in more than two cores, and 4) more than 50% involvement by tumor in any single core [26].

As shown in Table 2 the median age (range) of the subjects included in this study was 64.5 ± 7.3 years. Mean age was not significantly different in cancer, pre-cancer and non-cancer groups. Values of %p2PSA and phi were significantly higher in patients with PCa compared with PCa-negative group (median values: 1.86 vs 1.45 and 53.38 vs 36.21 respectively, p<0.001) and also compared with

Table 1
Clinical characteristics of study population.

Characteristics	BPH n = 67 (44%)	HGPIN n = 36 (24%)	PCa n = 48 (32%)	All n = 151
Prostate volume (cc)				
≤40	51 (76)	24 (67)	37 (77)	112 (74)
>40	16 (23)	12 (33)	11 (23)	39 (26)
PSAd (ng/ml/cc)				
≤0.15	40 (59)	23 (64)	22 (46)	85 (56)
>0.15	27 (40)	13 (36)	26 (54)	66 (44)
PSA (ng/ml)				
0–4	13 (19)	9 (25)	3 (6)	25 (16)
4.1–10	43 (64)	25 (69)	33 (69)	101 (67)
10.1–20	11 (16)	2 (5)	12 (25)	25 (16)
%f-PSA				
1–10	4 (6)	1 (3)	12 (25)	17 (11)
10.1–15	10 (15)	5 (14)	12 (25)	27 (18)
15.1–20	21 (31)	6 (17)	12 (25)	39 (26)
>20	32 (48)	24 (67)	12 (25)	68 (45)
Biopsy Gleason score				
<7	/	/	21 (44)	/
≥7	/	/	27 (56)	/
Clinically significant	/	/	42 (87)	/
Clinically insignificant	/	/	6 (13)	/

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Table 2
Descriptive statistics of the analyzed variables.

	No PCa	HGPIN	PCa	All				
Variable	Median [range]	Median [range]	Median [range]	Median [range]	Kruskall Wallis p	No Pca vs HGPIN	No Pca vs PCa	HGPIN vs PCa
Age (years)	64.0 [50–87]	66.5 [48–80]	66.0 [48–81]	64.5 [48–87]	0.054	n.s.	n.s.	n.s.
Prostate volume (cc)	50 [20–200]	45 [25–80]	47 [15–84]	48 [15–200]	0.421	n.s.	n.s.	n.s.
PSA (ng/ml)	6.79 [2–20]	5.97 [3–17]	7.88 [4–20]	6.85 [2–20]	0.019	n.s.	n.s.	0.005
fPSA (ng/ml)	1.25 [0.46–4.02]	1.37 [0.5–2.64]	1.15 [0.27–4.43]	1.21 [0.27–4.43]	0.537	n.s.	n.s.	n.s.
p2PSA (pg/ml)	17.46 [7.54–76.93]	18.55 [6.01–46.33]	20.73 [3.75–86.3]	18.88 [3.75–86.3]	0.155	n.s.	n.s.	n.s.
% p2PSA	1.45 [0.43–4.17]	1.64 [0.93–3.44]	1.86 [0.26–5.05]	1.63 [0.26–5.05]	<0.001	n.s.	<0..001	<0.001
% fPSA	19.36 [7.6–50]	22.23 [5.5–45]	15.41 [4.8–38.9]	19.20 [4.8–50]	0.001	n.s.	0.004	0.001
Phi	36.21 [14.02–142.77]	36.82 [19.05–106.04]	53.38 [5.05–162.89]	40.66 [5.05–162.89]	<0.001	n.s.	<0.001	<0.001
PCA3	28.0 [2–257]	54.5 [7–254]	57.0 [3–339]	47.0 [2–339]	<0.001	<0.001	<0.001	n.s.

HGPIN (median values: 1.86 vs 1.64 and 53.38 vs 36.82 respectively, $p<0.001$) (Fig. 1A–B). %fPSA values were significantly lower in PCa patients compared with non-malignant conditions and with HGPIN (median values: 15.4 vs 19.36 and 15.4 vs 22.23, $p=0.004$ and $p<0.001$, respectively) (Fig. 1C). PCA3 score values were significantly higher in malignant compared with PCa-negative group (median values: 57 vs 28, $p<0.001$) and in HGPIN vs benign conditions (median values: 54.5 vs 28, $p<0.001$) (Fig. 1D). Differently from phi, PCA3 score of PCa-positive patients was not significantly different from HGPIN diagnosed group.

ROC curve analysis (Fig. 2) showed that %p2PSA, phi and PCA3 are good indicators of malignancy (AUCs = 0.73, 0.77 and 0.71, respectively).

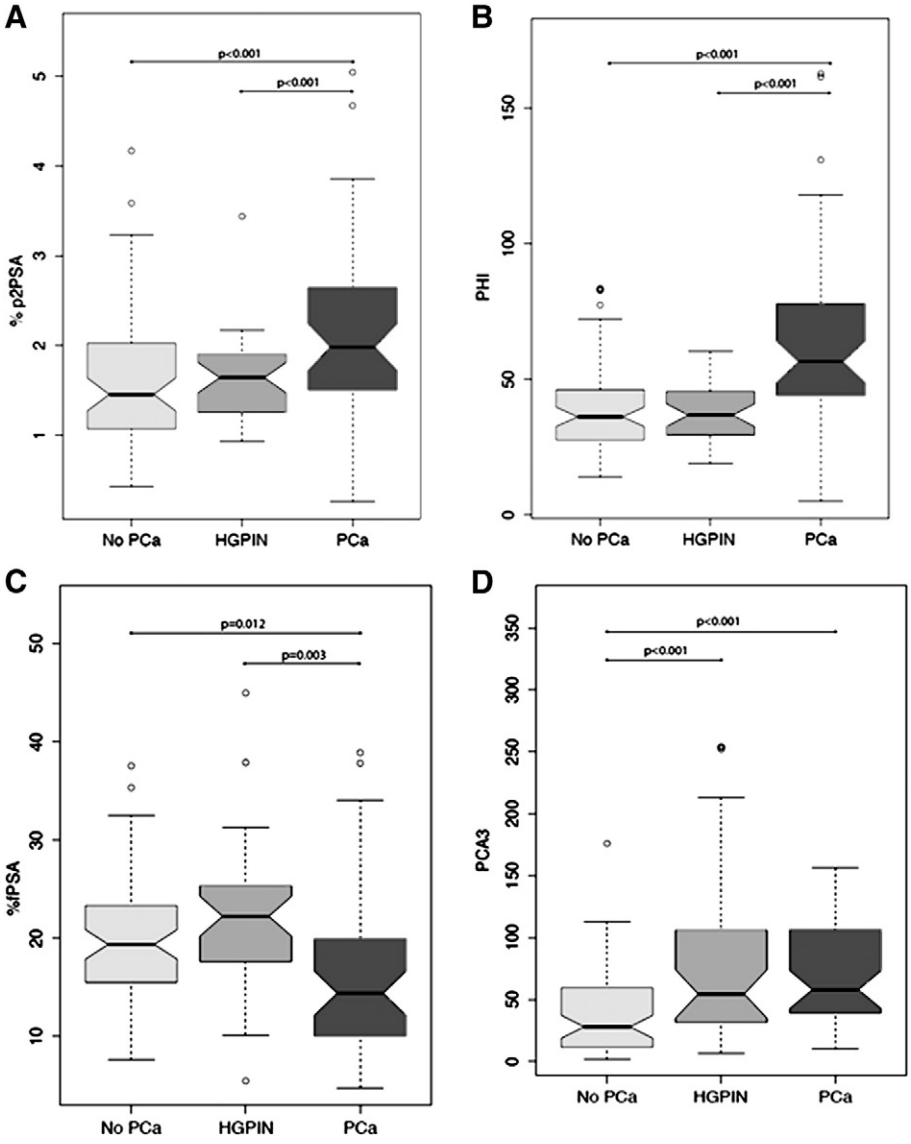

Fig. 1. (A–D). Median values of p2PSA, Phi, %fPSA and PCA3 score in study population: comparison among PCa-negative, pre-neoplastic condition and PCa-positive patients.

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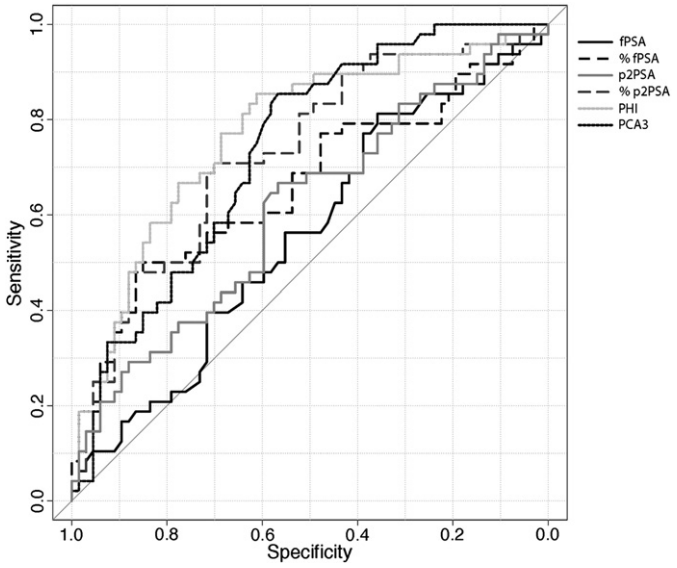

Fig. 2. ROC curve analysis comparing discriminating ability between benign and malignant condition of fPSA, p2PSA, %p2PSA, %fPSA, phi and PCA3 with respective AUCs.

Phi had the highest AUC, but the difference between phi and PCA3 was not statistically significant ($p = 0.368$). On the contrary, significant differences in AUCs were found between phi and both fPSA and p2PSA ($p = 0.006$ and $p < 0.001$ respectively) and between PCA3 and fPSA (p 0.036). Also %p2PSA showed a better discriminatory performance with respect to both fPSA ($p = 0.011$) and p2PSA ($p = 0.009$) (Fig. 2). As shown in Table 3 the optimal cut-point for phi was 38.7, corresponding to 85% sensitivity and 61% specificity, whereas the optimal cut-point for PCA3 was 32.5, corresponding to 81% sensitivity and 57% specificity. To achieve 90% specificity, cut-point was 63.5 for phi and 86 for PCA3, but at the cost of a lower sensitivity which decreased to 36% and 32%, respectively. A specificity of 80%, was associated with a cut-point of 50.2 for phi and 73 for PCA3, corresponding to a sensitivity of 60% and 43%, respectively.

Multivariable analysis produced no significant model to improve the performance of the single biomarker (data not shown).

4. Discussion

The controversy on PCa screening [27] provided an ideal environment in which to develop tools that may assist in predicting who may have PCa. Such predictive model would have implications when counseling patients whether or not to have a biopsy in the first instance.

%p2PSA and phi represent early-evaluated tests for PCa detection in patients with tPSA between 2 and 10 ng/ml [28], whereas PCA3 score has been well recognized as good indicator of cancer on repeat biopsy [11,12,29].

Some authors compared the performance of PCA3 with those of PSA as first-line diagnostic test. Ploussard et al. reported that high PCA3 score was a good indicator of positive re-biopsy, especially in the group of patients with f/t PSA ratio > 10% [30]. Moreover, the authors noted that PCA3, contrary to PSA and f/t PSA ratio, was not associated with prostate volume, age or stage, making PCA3 a potentially good complementary marker to PSA and its molecular forms.

To date, a head-to-head comparison between phi and PCA3 has yet to be reported. Such comparison could be very interesting in order to establish which the best test as predictor of biopsy outcome is and whether the combination of the two biomarkers improves PCa detection.

In the current study we examined the diagnostic performance of fPSA, p2PSA, phi and PCA3 in the tPSA range 2–20 ng/ml. We found that %p2PSA, phi and PCA3 were significantly higher and %fPSA was significantly lower in cancer than in noncancer. Furthermore, statistical analysis indicated that phi values, as %p2PSA and %fPSA, unlike PCA3 score, were significantly different in HGPIN conditions and cancer. PCA3 score was significantly higher in HGPIN than in PCa-negative group. Interestingly, most part of the analyzed tumors was clinically significant. ROC curve analysis showed that %p2PSA, phi and PCA3 outperformed fPSA, %fPSA and p2PSA. Of note, the AUCs of these biomarkers were not significantly different, indicating comparable ability to discriminate benign from malignant condition.

A relevant finding of this study was that men with HGPIN show comparable PCA3 scores as men with PCa, differently from phi. These data agree with previous reports showing that the discriminative performance of PCA3 score is lower between HGPIN and PCa [11,31]. Moreover, we found that PCA3 scores were significantly higher in HGPIN than in PCa-negative group. This finding probably reflects early molecular changes in a presumptive premalignant lesion [32]. In addition, HGPIN are strong risk factor for PCa in re-biopsy [2,3], thus some of our patients diagnosed as HGPIN may actually have PCa. According to previous report, this result supports that low PCA3 score is a good indicator of benign lesion [33]. To our knowledge, this is the first report indicating the association between phi and HGPIN biopsy outcome. Interestingly, our results are consistent with recent study showing that the intensity of [−2]pro-PSA staining at immunochemistry progressively increased in benign, HGPIN and neoplastic specimens [34].

The choice of evaluating HGPIN was made as it represents the only other category of biopsy outcome that may be realistically considered as pre-malignant condition, according to virtually all available evidence [35,36].

Despite the strength of a prospective observational study including patients with available data of both phi and PCA3 and with centrally pathological evaluation, our study examined a small number of cases. Therefore larger studies are needed to address several important questions such as a) the comparison of the single biomarker and the usefulness of the "combined" one; b) the identification of valuable cut-off values to be used in clinical practice; c) the ability of phi and PCA3 alone or in combination to predict clinically significant PCa.

Table 3
Optimal cut-point of the analyzed variables.

	Optimal			90% specificity		80% specificity	
	Cutpoint	Sensitivity [95% C.I.]	Specificity [95% C.I.]	Cutpoint	Sensitivity [95% C.I.]	Cutpoint	Sensitivity [95% C.I.]
fPSA	≤1.6	0.81 [0.7–0.91]	0.36 [0.24–0.46]	≤0.6	0.13 [0.04–0.28]	≤0.8	0.21 [0.11–0.43]
p2PSA	≥18.37	0.66 [0.53–0.79]	0.57 [0.45–0.69]	≥36.0	0.23 [0.11–0.38]	≥28.4	0.32 [0.17–0.49]
% p2PS	≥1.7	0.70 [0.57–0.83]	0.70 [0.58–0.81]	≥2.5	0.38 [0.17–0.6]	≥2.1	0.51 [0.36–0.72]
% fPSA	≤13.7	0.49 [0.34–0.64]	0.85 [0.76–0.93]	≤11.4	0.34 [0.15–0.57]	≤14.4	0.49 [0.32–0.64]
phi	≥38.7	0.85 [0.74–0.94]	0.61 [0.49–0.72]	≥63.5	0.36 [0.13–0.62]	≥50.2	0.60 [0.38–0.79]
PCA3	≥32.50	0.81 [0.68–0.91]	0.57 [0.45–0.69]	≥86.0	0.32 [0.04–0.49]	≥73.0	0.43 [0.26–0.64]

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In summary, %p2PSA, fPSA, phi and PCA3 may be useful predictors of PCa in first biopsy. In addition, PCA3 seems to discriminate HGPIN from non cancer condition.

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