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Original article

PCA3 sensitivity and specificity for prostate cancer detection in patients with abnormal PSA and/or suspicious digital rectal examination. First Latin American experience

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Abstract

Introduction: Prostate Cancer Gene 3 (PCA3) is a recently described and highly specific urinary marker for prostate cancer (CaP). Its introduction in clinical practice to supplement low specificity of prostate specific antigen (PSA) can improve CaP diagnosis and follow-up. However, before its introduction, it is necessary to validate the method of PCA3 detection in distinct geographic populations.

Objectives: Our aim was to describe for the first time in Latin America, the application of the PROGENSA PCA3 assay for PCA3 detection in urine in Chilean men and its utility for CaP diagnosis in men with an indication of prostate biopsy.

Materials and methods: Sixty-four Chilean patients (mean age, 64 years) with indication of prostate biopsy because of elevated PSA and/or suspicious digital rectal examination (DRE) were prospectively recruited. PCA3 scores were assessed from urine samples obtained after DRE, before biopsy, and compared with PSA levels and biopsy outcome.

Results: The median PSA value and mean PCA3 score were 5.8 ng/ml and 31.7, respectively. Using a cutoff PCA3 score of 35, the sensitivity and specificity for detecting CaP were 52% and 87%, respectively. The receiver operating characteristic (ROC) curve analysis showed an area under the curve of 0.77 for PCA3 and 0.57 for PSA, for the same group of patients. In patients with previous negative biopsy, PCA3 specificity increased by 2.2%.

Conclusions: This is the first report in Latin America on the use of PCA3 in diagnosing CaP. Our results are comparable to those reported in other populations in the literature, demonstrating the reproducibility of the test. PCA3 score was highly specific and we specially recommend its use in patients with persistent elevated PSA and prior negative biopsies. © 2013 Elsevier Inc. All rights reserved.

Keywords: Prostate cancer; PCA3; Diagnostic; PSA; Key messages; Early prostate cancer detection

1. Introduction

Prostate cancer (CaP) is a serious health problem in Western countries, representing one of the leading causes of death in men over 50 years. The main prognostic factor, in terms of survival, is tumor extension at diagnosis [1]. Screening methods currently used have improved early detection of CaP with a controversial impact in reducing its related morbidity and mortality [2].

Prostate specific antigen (PSA) is a serum marker widely used in screening for CaP [3]. However, several controversies have arisen about its use for CaP diagnosis, mainly related to the low specificity of PSA for CaP detection. Patients with increased PSA (>4 ng/ml) should undergo a prostatic biopsy to provide the diagnosis between the different prostatic diseases that increase PSA levels [4]. In addition, PSA does not correlate with biopsy findings and Gleason score [5]. In men over 50 years old, PSA is increased approximately in 15% of the patients. From these, only 3% are diagnosed with CaP [5]. Macefield et al. described that around a 75% of men with increased PSA have benign biopsy findings, implying that most of the patients undergo this procedure unnecessarily [6].

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During the last years, several diagnostic and prognostic factors of CaP have been included in "risk calculators" to improve the management of patients with suspicion of CaP. These include recently described CaP markers that need to be evaluated and validated in different populations previous to their introduction to clinical practice, mainly because of potential demographic differences [7,8].

In 1999, Bussemakers et al. [9] described that the prostate cancer gene 3 (PCA3) was highly expressed in CaP tissue, but not in normal prostate and other tissues. Later, Hessels et al. [10] described a method for the detection of the transcript of PCA3 in urine with high specificity for CaP detection [11]. The values for PCA3 specificity range from 80% to 90% depending on the PCA3 value used as cutoff [12]. In addition, contrary to PSA, PCA3 correlates with tumor volume and histologic features [13,14], and is related to biopsy outcome [14,15]. Wolf et al. [16] described that approximately 10% of patients with increased PSA and negative initial biopsy results were positive to CaP on repeated biopsies. In this type of patients, high PCA3 scores are related with high probability of a positive repeated biopsy [14]. PCA3 increases significantly the specificity of CaP detection, but cannot replace PSA as screening method.

Although PCA3 measurement in urine has been used successfully in other countries, to our knowledge, no experience regarding to PCA3 detection has been reported previously in Latin America, and no data are available to support its introduction in our clinical practice.

In the present work, we evaluated for the first time the PROGENSA PCA3 assay (Gen-Probe, San Diego, CA) as a method for PCA3 detection in urine, and its utility for CaP detection in Chilean men with an indication of prostate biopsy because of elevated PSA and/or suspicious digital rectal examination (DRE).

2. Materials and methods

2.1. Patients

Sixty-four patients with an indication of transrectal (TR) prostate biopsy, either for elevated PSA and/or suspicious DRE, were prospectively enrolled at 2 institutions (Las Condes Clinic and Clinical Hospital of University of Chile) between November 2009 and January 2010. PSA values were obtained from the clinical laboratory of each institution.

2.2. Ethical considerations

This study was approved by the Ethics Committee of the Las Condes Clinic and Clinical Hospital of the University of Chile. All patients included signed an informed consent to participate in the study.

2.3. Prostate biopsy protocol

Biopsies consisting of at least 12 cores (at least 2 cores per sextant) were performed by urologists by TR ultrasound. The pathologists at each institution who examined the biopsy cores were blinded to PCA3 score results.

2.4. Samples

From each patient, first-stream urine (20–30 ml) was collected after an extended DRE, (3 sweeps on each side of the prostate). Whole urine specimens were processed within 4 hours by mixing with an equal volume of detergent based stabilization buffer, which lyses the cells and stabilizes the RNA. Processed specimens were stored at −70°C until tested.

2.5. PCA3 detection

PCA3 scores are determined by using the PROGENSA PCA3 assay (Gen-Probe, San Diego, CA). Briefly, prostate cells are recovered from urine and lysed. PCA3 and PSA mRNAs are captured by hybridization to magnetic particles via target-specific oligonucleotides, then amplified by a transcription-based nucleic acid amplification method, that utilizes a unique set of primers for each target, and combines 2 enzymes: a reverse transcriptase that generates cDNA from target mRNAs and an RNA polymerase that produces multiple copies of RNA amplicon from the DNA copy template. Later, amplification products are detected using target specific acridinium ester-labeled probes with a luminometer. Finally, PCA3 score is calculated as the ratio of PCA3 mRNA copies/PSA mRNA copies, multiplied by 1,000 [17]. We used a score of 35 as a cutoff [18], however different cutoffs were established to compare its effect in diagnostic parameters of the technique.

2.6. Statistical analysis

Receiver operating characteristic (ROC) curves were built for both PCA3 score and PSA. A P value < 0.05 was considered statistically significant.

3. Results

We prospectively enrolled 64 patients between November 2009 and January 2010. With regard to demographic characteristics, all patients had a similar ethnic origin (Latin American) and corresponded to a high socioeconomic class. The average age of patients was 62 years. The median PSA level of all patients included in this study was 5.7 ng/ml, with a range of 0.8–138 ng/ml. The mean value for PCA3 score in our cohort was 31.7, with a range of 1.35–240. Fifteen patients had undergone a previous prostate biopsy that turned out to be negative for cancer (Table 1).

Table 1
Characteristics of patients included in the study

	Mean/median	Range; SD
Age (years)	62.1/61.5	44–83; 8.4
PSA (ng/ml)	10.4/5.7	0.8–138; 18.4
PCA3 score	31.7/17	1.35–240.5; 43.8
Prostate volume, ml (TRU)	40.9/37	21–62.5; 14.2
	Yes, no (%)	No, no (%)
DRE abnormal	6 (9.4)	58 (91.6)
Previous negative biopsies	15 (23.4)	49 (76.6)

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

Results were analyzed according to the outcome of TR prostate biopsy (Table 2). We found no differences between groups with respect to age, however, PSA level and PCA3 score showed differences that were significantly higher in patients with CaP.

When patients were grouped according to Gleason score obtained on TR biopsy, there seemed to be a positive correlation between the degree of dedifferentiation of cancer and the PSA level as well as the PCA3 score (Table 3). However, in patients with undifferentiated cancers (Gleason 9), PCA3 scores were lower than more differentiated cancer.

Considering a cutoff value of 4 ng/ml for PSA ($n = 52$ patients), we calculated a sensitivity of 83% and a specificity of 21% with a positive predictive value (PPV) and negative predictive value (NPV) of 40% and 64%, respectively (Table 4). Considering a PCA3 score of 35, the sensitivity and specificity of PCA3 score for detecting CaP were 52% and 87%, with a PPV of 72% and an NPV of 74%. The ROC curve analysis displayed an area under the curve (AUC) of 0.77 for PCA3 and of 0.57 for PSA in the same group of patients ($P = 0.004$) (Fig. 1).

The same analysis was performed by separating the group of patients according to history of having undergone a previous biopsy (Table 5). Although in the group of patients with previous biopsies the sensitivity of PCA3 decreases, the specificity increased by 2.2%.

Table 2
Characteristics of patients depending on biopsy result

	Men with negative CaP biopsy	Men with positive CaP biopsy	*P* value
Number	49	25	
Age: mean	61.5	63	0.26
Range; SD	45–76; 7.4	44–83; 9.8	
PSA (ng/ml): mean/median	6.8/5.7	15.7/5.9	0.02
Range; SD	0.8–14.8; 3.5	3.4–138; 28.7	
PCA3 (ng/ml): mean/median	15.5/11.4	56.2/37	0.001
Range; SD	1.3–55; 14.1	2–240; 60.7	
Number of patients with previous negative biopsies	6 (23.1%)	9 (24%)	

Table 3
PSA levels and PCA3 score in patients grouped by Gleason score

Gleason Score	Frequency (%)	PSA ng/ml mean (range; SD)	PCA3 score mean (range; SD)
6	13 (52%)	6.65 (4–12; 2.7)	52 [3–13,54]
7	5 (20%)	4.33 (3–6; 1.0)	32 (9–55; 16.2)
8	5 (20%)	26.28 (4–68; 25)	99 (10–241; 100.2)
9	2 (8%)	76.65 (15–138; 86.7)	39 (24–53; 20.1)

We also performed an analysis considering different PCA3 scores as cutoff points. Table 6 shows the change in the percentage of patients with cancer depending on PCA3 scores. In this sense, PCA3 scores can predict the biopsy outcome. In patients with PCA3 scores lower than 5 ($n = 8$) only 1 patient had cancer (12%) whereas in the group of patients with PCA3 scores higher than 100, we observed that all patients had cancer (100%).

4. Discussion

Since the introduction of PSA as screening method for CaP, an important advance was achieved in terms of early detection of this disease. However, its use was related to an increase in the number of unnecessary prostate biopsies performed to diagnose CaP [4].

During the last years, new diagnostic markers came under study to complement and improve PSA specificity [19]. PCA3, one of these new markers, is highly specific of CaP and was first described in 1999, when Bussemakers et al. [9] compared mRNA expression patterns from tumor and normal samples. PCA3 mRNA was overexpressed in 53 from 56 tumor samples, and was undetectable in normal, BPH, and the other 18 tissues [9]. The development of the technique for its detection in urine has increased its utility as a powerful tool for CaP diagnosis [10].

Although earlier reports found a clear association between PCA3 score and Gleason score [15,20], we did not obtain a linear correlation in the present study. We believe that this discrepancy may be due to the small number of patients included in this study, and the large standard deviations obtained for the PCA3 score, which may decrease with the inclusion of a greater number of patients.

Our results are similar to those who reported previously that PCA3 scores are related with the biopsy outcome. Van Gil et al. described that in men who went on to have a

Table 4
Sensitivity, specificity, and predictive values for PSA and PCA3

	PSA >4 ng/ml	PCA3 score >35
Sensitivity (%)	83	52
Specificity (%)	21	87
Positive predictive value (%)	40	72
Negative predictive value (%)	64	74

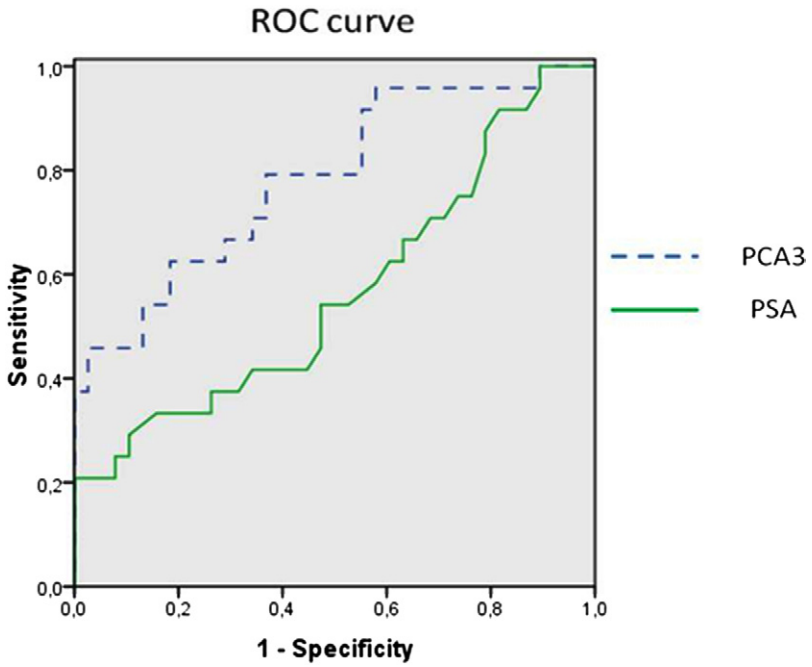

Fig. 1. ROC curve for prognostic power of PSA and PCA3 score. ROC curve of the area under the curve (AUC) for prognostic power of PSA >4 ng/ml (green) and PCA3 score >35 (blue) in detecting prostate cancer; $n = 64$.

negative biopsy the median PCA3 score was 24, whehreas for patients who went on to have a positive biopsy result the median score was 90 (range 0–4.088) [21]. Similarly, Hessels et al. described that patients with CaP showed higher PCA3 scores than those with a negative prostate biopsy (56 vs. 16; $P < 0.001$) [10]. In the present study, we saw that patients with CaP had PSA levels and PCA3 scores significantly higher ($P = 0.02$ and 0.001, respectively) that patients for whom the biopsy was negative for cancer. However, we observed that in patients with Gleason 9, PCA3 score was lower than more differentiated cancer, which could be related to the loss of the prostatic phenotype of the cells.

As reported previously by other studies [15,17,18,21] we observed that PCA3 score sensitivity (52%) was lower than PSA (80%), indicating that PSA is superior to PCA3 as a screening method. However, determination of PCA3 score increases significantly the specificity of CaP detection due to PCA3 score specificity (87%), which was higher than PSA specificity (18%) (Table 4).

The utility of PCA3 as a diagnostic tool for CaP detection differs between studies. In the literature, the sensitivity values range between 46.9% and 82.3%, whereas specificity ranged from 56.3% to 89% [4]. These differences are related mainly to the PSA level used as cutoff for biopsy indication and, therefore, the number of patients included in the different experimental groups. Compared with our study, Hessels et al. observed higher levels of sensitivity (67%) for PCA3 using lower levels of PSA for biopsy indication [10].

To evaluate the performance of PCA3 for diagnosing CaP, we made the ROC curve analysis in comparison with PSA. Our results are similar to those reported in the literature. For PCA3 we obtained an AUC of 0.77, higher than AUC for PSA > 4 ng/ml (0.57). Previously, van Gils et al. obtained similar values in a cohort of 534 men with biopsy indication of an elevated PSA between 3 and 15 ng/ml [21]. They described an AUC for PCA3 detection in urine of 0.66 compared with 0.57 for serum PSA. Similarly, Laxman et al. also described an AUC for PCA3 of 0.662 [22].

Table 5
Sensitivity, specificity, and predictive values for PSA and PCA3 depending on biopsy history

	Men with previous negative biopsy ($n = 15$)		Men without previous biopsies ($n = 48$)	
	Sensitivity	Specificity	Sensitivity	Specificity
PSA >4 ng/ml	100%	22.2%	79.8%	23.3%
PCA3 score >35	33%	88.9%	57.9%	86.7%

Table 6
Percentage detection of cancer with different cutoffs for PCA3

PCA3 Score	*n*	Positive CaP biopsy	
		n	%
<5	8	1	12
15	30	6	20
35	46	14	30
75	59	20	34
>100	6	6	100

There are different opinions regarding what cut-off of PCA3 values should be used. Roobol et al. described a specificity of 90% when they increased the cutoff to 100 [23]. Although we did not carry out a study of sensitivity and specificity with different cutoff points for the PCA3 score, it is important to note that when we set higher cutoffs for PCA3, the percentage of detection increased. The detection of cancer rises from 12% (using a cutoff of 5), up to 100% (PCA3 levels >100.

In this sense, Marks et al. established different cutoffs reporting that for PCA3 scores lower than 5, only 12% of men had CaP on repeated biopsy, whereas for PCA3 scores >100, the risk of positive biopsy was 50% [18].

Marks et al. described the superiority of PCA3 over PSA in predicting the outcome of biopsy in men with repeated biopsy [18]. In this subset of patients, they obtained an AUC of 0.68 for PCA3 score compared with 0.52 for PSA for predicting CaP in the biopsy. We found similar interesting results in patients who had elevated PSA levels and have undergone 1 or more previous negative biopsies. Around one-half to two-thirds of the patients who undergo prostate biopsy have undergone at least 1 previous negative biopsy but experience persistent elevated PSA afterward [19]. This group of patients will obtain the most benefit from PCA3 introduction in clinical practice since a large number of unnecessary biopsies might be avoided. In our study, the specificity of PCA3 score was close to 90% for these patients, substantially improving diagnostic accuracy and predicting with great certainty the outcome of the biopsy. Our results agree with other groups [14,15,19] and confirm the utility of PCA3 to predict biopsy outcome in patients with repeated biopsies.

5. Conclusions

PSA and DRE are no longer the only factors used to predict the risk of CaP. The possibility exists to include other factors before performing a biopsy, thereby increasing CaP prediction. The inclusion of new factors, such as PCA3, need to be validated in different populations before implementation because of potential demographic and genetic differences. Results from PCA3 measurement in urine samples from Chilean patients agree with previous international reports. Therefore, our experience confirms the utility of PCA3 as specific marker for CaP. Based on the findings of the present study, we can recommend its use in the specific subset of patients who experience a persistent elevated PSA after earlier negative biopsies.

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