ORIGINAL ARTICLE

Optimizing prostate cancer screening; prospective randomized controlled study of the role of PSA and PCA3 testing in a sequential manner in an opportunistic screening program

J. Rubio-Briones a,1, J. Casanova a, R. Dumont a, L. Rubio b, A. Fernandez-Serra b, I. Casanova-Salas b, J. Domínguez-Escrig a, M. Ramírez-Backhaus a, A. Collado a, A. Gómez-Ferrer a, I. Iborra a, J.L. Monrós a, J.V. Ricós a, E. Solsona a, D. Salas a, F. Martínez d, J.A. Lopez-Guerrero b

a Servicio de Urología, Fundación Instituto Valenciano de Oncología, Valencia, Spain
b Laboratorio de Biología Molecular, Fundación Instituto Valenciano de Oncología, Valencia, Spain
c Departamento de Salud Pública, Conselleria de Sanidad, Generalitat Valenciana, Valencia, Spain
d Departamento de Bioestadística, Universidad de Valencia, Spain

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KEYWORDS
Prostate cancer; Screening; PCA3; Prostate biopsy

Abstract
Objectives: To reduce unnecessary biopsies (Bx) in an opportunistic screening programme of prostate cancer. Material and methods: We performed a prospective evaluation of PCA3 as a second-line biomarker in an opportunistic screening for prostate cancer (PCa). From September 2010 until September 2012, 2,366 men, aged 40–74 years and with >10 years life expectancy, were initially screened with PSA/digital rectal examination (DRE). Men with previous Bx or with recent urine infections were excluded. Men with abnormal DRE and/or PSA >3 ng/ml were submitted for PCA3. All men with PCA3 >35 underwent an initial biopsy (IBx) —12cores—. Men with PCA3 <35 were randomized 1:1 to either IBx or observation. Re-biopsy (16–18 cores) criteria were PSA increase >.5 ng/ml at 4-6months or PSAv >.75 ng/ml/year.
Results: With a median follow-up (FU) of 10.1 months, PCA3 was performed in 321/2366 men (13.5%), 289 at first visit and 32 during FU. All 110 PCA3+ men (34.3%) were biopsied and PCa was identified in 43 men in IBx (39.1%). In the randomized arm, 110 were observed and 101 underwent biopsy, finding 12 PCa (11.9%), showing a statistically significant reduction of PCa detection rate in this cohort (p < .001). Global PCa detection rates were 40.9% and 9.5% for the PCA3+ and PCA3− branches, respectively (p < .001). Area under the curve for PSA and PCA3 were .601 and .74, respectively. This is an ongoing prospective study limited by its short follow-up period and still limited enrolment.

* Corresponding author.
E-mail address: jrubio@fivo.org (J. Rubio-Briones).

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Conclusions: PCA3 as a second-line biomarker within an opportunistic dual screening protocol can potentially avoid 65.7% and 50.1% biopsies at first round and at median FU of 10.1 months, respectively, just missing around 3.2% of high grade PCA.
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Optimización de un programa de cribado oportunista de cáncer de próstata; ensayo aleatorizado prospectivo del papel del PSA y del PCA3 en uso secuencial

Resumen
Objetivos: Reducir el número de biopsias (Bx) innecesarias en un programa de cribado oportunista de cáncer de próstata (CaP).
Material y métodos: Estudio prospectivo y aleatorizado evaluando el PCA3 como biomarcador de segunda línea. De septiembre de 2010 a septiembre de 2012 2.366 hombres con edad en rango 40–74 años, y más de 10 años de expectativa de vida, fueron estudiados mediante PSA y tacto rectal (TR), excluyendo los biopsiados previamente o con infección urinaria reciente. Ante un TR sospechoso y/o PSA > 3 ng/ml se les realizó un PCA3. A todos aquellos con PCA3 ≥ 35 se les realizó una Bx inicial (IBx) −12 cilindros−. Con PCA3 < 35 fueron aleatorizados 1:1 a IBx u observación. Los criterios de rebiopsia (16–18 cilindros) durante el seguimiento fueron un incremento de PSA > 0,5 ng/ml a 6 meses o PSAv > 0,75 ng/ml/año.
Resultados: Con un seguimiento medio de 10,1 meses se testó el PCA3 en 321/2.366 hombres (13,57%), 289 en la primera visita y 32 durante el seguimiento. Entre los 110 hombres con PCA3+ (34,3%) se identificó CaP en 43 en IBx (39,1%). En el brazo aleatorizado 110 se observaron y 101 se biopsiaron, encontrando 12 CaP (11,9%), mostrando una reducción en la detección de CaP estadísticamente significativa en esta cohorte (p < 0,001). Las tasas de detección global de CaP fueron de 40,9 y 9,5% para las ramas PCA3+ y PCA3− respectivamente (p < 0,001). AUC para PSA y PCA3 fueron 0,601 y 0,74. Este es un protocolo abierto en este momento, limitado por su seguimiento insuficiente.
Conclusiones: El PCA3 como biomarcador de segunda línea en un programa de cribado oportunista podría potencialmente evitar un 65,7% de IBx y 50,1% a 10 meses de seguimiento, dejando de diagnosticar 3,2% de CaP de alto grado.
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Background

The results of the European Randomized Study of Screening for Prostate Cancer (ERSPC) after 11 years of follow-up have shown that a lower number of patients required screening and a lower number of patients required life-saving treatment. Compared with previous publications, the study also showed an improvement in cancer-specific survival compared with the control arm. However, this result was at the expense of too many negative biopsies (Bx). In addition to optimizing the age ranges to be included and the chronology of the screening, we all seek to avoid unnecessary biopsies, given the undesirable morbidity (infections, bleeding, urinary obstruction, etc.) and emotional stress that biopsies inflict on healthy men.1,4

PCA3 is an FDA-approved biomarker of prostate cancer (PC) for the indication of repeat biopsies with a cutoff point of 25. After an initial internal validation was conducted on the data provided by the commercial kit and the data published to date with a cutoff point of 35, we began using PCA3 in January 2009, along with other clinical variables to optimize our clinical judgment for indicating an initial biopsy (IBx) or a rebiopsy. Prior to this date, we biopsied all our patients regardless of their PCA3. We achieved the best performance of PCA3 for IBx. In this context, nomograms that consider PCA3 significantly improve the baseline clinical models that lack PCA3 (p < .001), improving their predictive capacity by 4.5–7.1% after including PCA3, leaving only 2% of high-grade PCa undiagnosed.7

Given that the role of PCA3 in the framework of opportunistic screening is yet to be established, we designed a prospective randomized study using PCA3 as a second-line biomarker after PSA and rectal examination (DRE) performed by a urologist. Our primary objective was to assess our preliminary result in terms of the potential reduction in the number of biopsies, without undermining the inherent benefits of opportunistic screening. Our secondary objectives were to evaluate the false negative rates for PCA3 and their prognostic value within opportunistic screening.

Material and methods

Our opportunistic screening program for PCa consists of a prospective randomized study (Fig. 1) with the following inclusion criteria: healthy men aged 40–75 years, with more than 10 years of life expectancy, with no prior Bx, who freely committed to the protocol and signed the informed consent for that purpose (opportunistic screening and nonopportunistic). The study was approved by the Ethics Committee of the Valencian Foundation Institute of Oncology (no. ref. 2010-20).

During the initial visit, a specialist nurse reviewed the general medical history with an emphasis on dietary habits and obtained the PSA, after which a urologist evaluated the
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Figure 1  Study scheme. Abbreviations: IBx, initial biopsy; PSA, prostate-specific antigen; DRE, digital rectal examination.

urological, sexual and family history of PCa and performed the DRE. Men who had already been biopsied or who had a history of prostatitis or urinary infections during the previous year were excluded. Men with normal DRE and PSA results (<3 ng/dL) proceeded to PSA and DRE monitoring at 1, 2, 3 or 4 years, based on whether the PSA level was <0.5, 0.5–1, 1–2 or 2–3 ng/mL, respectively. The rest of the candidates or study group (PSA ≥ 3 ng/mL and/or abnormal DRE results) underwent a second DRE by another urologist, and their PCA3 levels were determined (Progensa™ PCA3 test; Genetics Probe-Hologic, San Diego, USA).

All men with PCA3 levels ≥ 35 were recommended to undergo a 12-core IBx. Participants with PCA3 levels < 35 were blindly randomized 1:1 (using a software application) to IBx or observation (Fig. 1). Participants with positive biopsies for PCa were withdrawn from the study.

The men with negative PCA Bx were scheduled for 4-month follow-ups if their PCA3 levels were ≥ 35 or 6-month follow-ups if their PCA3 levels were <35, due to a recommendation by the center’s ethics committee, and then annually, always performing PSA determinations and DREs during each visit. Rebiopsy (16–18 core) was proposed during the follow-up according to the following criteria: increased PSA levels > 0.5 ng/mL since the last visit, PSAv levels > 0.75 ng/mL/year or persistent or de novo abnormal DRE results.

The initial sample size was established at 1065 men; however, given the initial results and in accordance with the ethics committee and health authorities, we continued recruiting volunteers and the protocol remained open. The data were analyzed with the SPSS and R statistical packets. The frequency comparisons were performed with the $\chi^2$ test (Fisher’s exact test for 2 × 2 contingency tables) for categorical variables. For the continuous variables, the differences between means were studied with Student’s t-test when the associations were acceptable. When the normality was not acceptable, the samples were compared using the Mann–Whitney U test. The areas under the curve (AUC) of the receiver operating characteristic (ROC) were compared with the De Long test. We used a two-sided test, and a p-value ≤ 0.05 was considered statistically significant. The randomization was automatic using a computer program blind to the study.

Results

The screening began in September 2010, and from then until September 30, 2012, 2422 men requested PCa screening (Fig. 2). We included 2366 participants and rejected 56 cases for various reasons (Fig. 2). Their mean age was 57.5 (SD, 6.2) years, with a median of 57 years and a range of 40–74
years. The median follow-up time for the entire group was 10.1 months (range: 1–33 months).

At the initial visit, 289 men (12.2%) entered the study for an abnormal PSA and/or DRE. Another 32 joined during the follow-up, ultimately resulting in 321 (13.6%) men tested for PCA3 in second line. This study group had a mean age of 60.8 years (SD: 5.9; range: 43–74 years), significantly greater than that of the overall group, with a mean age of 57 years (SD: 6.1; range: 40–74 years) (p < .001). The mean PSA level of the study group was 4.63 ng/mL (SD: 2.25) with a median of 4.04 ng/mL (0.37–19.50). A suspicious DRE was detected in 20 men, this being the only factor of inclusion for 15 of the men.

In the study group, the mean and median PCA3 levels were 33.7 (SD: 39) and 23, respectively (range: 1–371). A weak relationship was detected between PSA and PCA3, which was not significant (p = .377). A total of 110 men had PCA3 levels ≥ 35 (34.3%), with these men being a mean of 2 years older than those with PCA3 levels < 35 (p = .006), but without showing differences in their PSA readings (p = .122).

A total of 211 Ibx were performed; 110 of these men were PCA3+ and 101 were PCA3−, all of whom were randomized to this procedure. Table 1 shows the results of the Ibx and the statistically significant relationship between a PCA3+ and the diagnosis of PCa in Ibx (p < .001). The mean and median of the PCA3 levels among the patients with PCa were 71.5 (SD, 67.2) and 54, respectively (range, 11–371), while these statistics for negative Ibx were 33.1 (SD, 27.8) and 28 (range, 1–189), respectively. Fig. 3 shows the ROC curves for PSA and PCA3. The AUC for PSA was 0.601 (95% CI: 0.504–0.689) and 0.748 (95% CI: 0.677–0.819) for PCA3, showing statistically significant differences (p < .008). The cutoff of 35 for PCA3 achieved 78.2% sensitivity and 57.1% specificity. Table 2 shows these statistics for other cutoff points.

The remaining 266 men of the study group with no Ibx or negative Ibx were monitored. We performed protocol biopsies in follow-up in 13, 6 and 27 men in the groups of men
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Table 1. Results of the initial biopsy and follow-up biopsies and Gleason score according to PCA3 branch.

<table>
<thead>
<tr>
<th></th>
<th>PCA3 &lt; 35</th>
<th>PCA3 ≥ 35</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Randomized</td>
<td>Randomized</td>
</tr>
<tr>
<td>IBx (n)</td>
<td>IBx n = 101</td>
<td>IBx n = 110</td>
</tr>
<tr>
<td>PCa detection</td>
<td>12 (11.9%)</td>
<td>43 (39.1%)</td>
</tr>
<tr>
<td>Bx during follow-up (n)</td>
<td>6/89 (6.7%)</td>
<td>13/67 (19.4%)</td>
</tr>
<tr>
<td>PCa detection</td>
<td>0 (7.3%)</td>
<td>0 (7.3%)</td>
</tr>
<tr>
<td>Detection of overall PCa up to the last follow-up</td>
<td>20/201 (9.5%)</td>
<td>45/110 (40.9%)</td>
</tr>
</tbody>
</table>

Abbreviations: Bx, biopsies; PC, prostate cancer; IBx, initial biopsy.

a Nine cases of Gleason 3 + 3 and 3 Gleason 3 + 4.
b Twenty-four cases of Gleason 3 + 3, 13 Gleason 3 + 4, 2 Gleason 4 + 3, 3 Gleason 4 + 4 and 1 Gleason 4 + 5.
c Four cases of Gleason 3 + 3, 1 Gleason 3 + 4, 1 Gleason 4 + 3 and 2 Gleason 4 + 4.
d Two cases of Gleason 3 + 3.

Table 2. Statistics for various PCA3 cutoff points.

<table>
<thead>
<tr>
<th>PCA3</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100.0</td>
<td>26.9</td>
</tr>
<tr>
<td>15</td>
<td>94.5</td>
<td>33.3</td>
</tr>
<tr>
<td>20</td>
<td>87.3</td>
<td>41.0</td>
</tr>
<tr>
<td>25</td>
<td>83.6</td>
<td>47.4</td>
</tr>
<tr>
<td>30</td>
<td>80.0</td>
<td>53.2</td>
</tr>
<tr>
<td>≥ 35</td>
<td>78.2</td>
<td>57.1</td>
</tr>
<tr>
<td>≥ 40</td>
<td>70.9</td>
<td>63.5</td>
</tr>
<tr>
<td>≥ 45</td>
<td>63.6</td>
<td>70.5</td>
</tr>
<tr>
<td>50</td>
<td>56.4</td>
<td>74.4</td>
</tr>
</tbody>
</table>

with PCA3+, PCA3− with negative IBx and PCA3− randomized to follow-up, respectively. In the same 3 groups, we diagnosed 2, none and 8 additional cases of PCa (Fig. 2). When we compared the PCA3+ and PCA3− study group arms, we found PCa detection rates of 40.9% and 9.5%, respectively (Table 2), showing clear and statistically significant differences (p < .001). Up to this review, only 3 cases with primary Gleason 4 had been detected in the PCA3− arm (1.4%).

As previously mentioned, we found statistically significant differences in the rates of PCa detection in IBx among the men with PCA3 levels ≥ 35 vs. PCA3 levels < 35 (39.1 vs. 11.9% [Table 1]). However, if we had only biopsied the men with PCA3+, we would not have diagnosed 11.9% of the latent PCa in the IBx, although none of them were primary Gleason 4. Extrapolating these results to a possible implementation of this protocol, we could avoid a total of 65.7% of IBx (211 men with PCA3− out of a total 321 candidates). With the current mean follow-up of 10.1 months, we had to perform follow-up biopsies for 27 (24.5%) men with PCA3− following our rebiopsy criteria.

Discussion

Although populational and opportunistic screening has not been formally compared, the former has not been accepted by various health authorities due to the overdiaognoses it entails. However, there is no turning back the use of opportunistic screening in primary medicine and in our society, which is a system that has resulted in reduced mortality due to PCa. In the epidemiological registry, sponsored by the Spanish Urological Association in 25 public hospitals, the incidence of PCa in Spain, adjusted for age, was 70.75 cases per 100,000 inhabitants. Of these, 40% were of low risk and the majority were detected by opportunistic screening,9 reliable reflections of the sociodemographic problem that confronts public health.

The latest results of the Finnish, Dutch and Swedish branches of the ESRP (with median follow-ups of 12, 12.8 and 16 years) were recently presented at the UAE-2013 Congress. The results showed a reduction in cancer-specific mortality in its study groups, with rates between 0.44 and 0.67. The number of men necessary for screening was between 208 and 1199, and the number of diagnoses necessary to save a life was between 9 and 25, readings much lower than those published with shorter follow-up periods. The differences between these results could
be justified by the differences in strategies and follow-ups allowed by the ERSPC, which points to the possibility of optimizing the scheduling of PCA screening and the need for developing tools for this screening. Age ranges and baseline PSA are examples of these differences and have been proposed as guidelines for the scheduling of screening during follow-up. Other variables such as family history, the DRE, prostate volume and the presence of prior negative biopsies also form part of the risk calculations for experiencing PCA within a screening program.

The optimal cutoff for PCA3 is still controversial, as is the cutoff for PSA after more than 25 years of use, and might not be the same in follow-up IBx or Bx. However, this has still not been tested clearly and prospectively within the framework of opportunistic screening. PCA3 has been tested as a first-line marker with a cutoff of 10 in a pilot study within the Dutch branch of the ERSPC, comparing it with PSA. However, we believe that such a low cutoff point is the reason there would have been no significant differences in the AUC for detecting PCa between the PCA3 (0.64) and the PSA (0.58) (p=0.143), thus requiring 75% of the IBx men, and therefore, not resolving the problem of excessive number of IBx.

Our approach was to assess whether the combination of PSA (with its acceptable sensitivity) as a first-line marker and PCA3 (with its better specificity) as a second-line marker could provide us with a better selection process for IBx, thereby avoiding unnecessary biopsies. We observed that the detection rates in IBx showed significant differences among men undergoing screening in the traditional manner with PCA3 ≥ 35 vs. PCA3 < 35 (39.1% vs. 11.9%) (Table 1). Assuming that we would have only biopsied those men with PCA3+, we would not have detected 11.9% of latent PCa in the IBx, none with primary Gleason 4 (Fig. 2). Therefore, a potential savings of 65.7% of IBx should be weighed with the rate of false PCA3 negatives with a cutoff of 35. We believe, however, that our results require us to continue analyzing the regimen we are proposing.

It is interesting to recognize that more PCa has not been diagnosed in the group of repeat biopsies with PCA3+. However, with a 10-month follow-up, we can see how 24.5% of the men in the group subjected to observation required an IBx (27/110) invoking our rebiopsy criteria, which we considered strict but necessary when faced with a healthy man who wants to know if he has PCa. Therefore, if they had not been randomized and assuming this rate in the entire PCA3– arm, we would have saved 50.1% of Bx at 10.1 months of follow-up.

We recognize various weaknesses in our study. The first is the apparently insufficient recruitment period (24 months) and especially the mean follow-up (10.1 months). However, we believed it necessary to analyze our preliminary results to justify or discard the protocol. We now believe that a longer follow-up would enable us to compare detection rates, biopsy avoidance rates and cost-effectiveness studies of the protocol with traditional screening schemes, calculators of risk and nomograms, tools that improve the isolated use of PSA as a decision element for Bx. We found 3 cases of potentially lethal PCa (primary Gleason 4) with this short follow-up. Therefore, the big question to be answered with longer follow-ups is whether the savings in initial biopsies would delay the detection of these 3.2% Gleason PCa ≥ 7 not diagnosed in the PCA3— branch of the protocol, and to what extent it could worsen the cancer-specific and overall survival rates of these patients. This rate is slightly greater than the 1% of undiagnosed Gleason PC ≥ 7 when the PCA3 is added for better clinical judgment in a scenario of repeat Bx with PSA levels between 2.5 and 10 ng/mL.

We also recognize that a man with a suspicious DRE could be randomized to observation if his PCA3 level is less than 35. Despite this situation not having occurred so far and knowing its high positive predictive value, when the case does present itself we will offer a biopsy outside of the protocol if the patient does not wish to wait for a reassessment at 6 months when, if the suspicious DRE persists, a follow-up Bx would be performed per protocol.

We recognize that repeating the PSA measurements within a prudent time before the performance of the PCA3 would probably have reduced its indication. We therefore did not include men with urinary tract infections or prostatitis in the year prior to their consultation. With a longer follow-up, we can assess pharmacokinetic parameters of PSA for the indication of PCA3, as well as other biomarkers in multimodal panels, as other authors have done. We agree with these authors that this is the road towards improving the AUC for the detection of PCa.

**Conclusions**

Our initial data show that the use of PCA3 at a cutoff of 35 as a second-line biomarker within an opportunistic screening program could entail a potential savings in initial biopsies of 65.7% in the first visit and 50.1% at 10.1 months of follow-up, leaving approximately 3% of Gleason PCa ≥ 7 undetected. In this context, we should accept a rate of false negatives for PCA3 of approximately 12% and its possible diagnostic delay, knowing that the majority is low-grade PCa. We need a longer follow-up to understand its true value as a diagnostic and prognostic tool for our protocol and thereby weigh the rate of biopsy savings and its cost.

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**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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