

Behavior of the PCA3 gene in the urine of men with high grade prostatic intraepithelial neoplasia

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Abstract

Objective An ideal marker for the early detection of prostate cancer (PCa) should also differentiate between men with isolated high grade prostatic intraepithelial neoplasia (HGPIN) and those with PCa. Prostate Cancer Gene 3 (PCA3) is a highly specific PCa gene and its score, in relation to the PSA gene in post-prostate massage urine (PMU-PCA3), seems to be useful in ruling out PCa, especially after a negative prostate biopsy. Because PCA3 is also expressed in the HGPIN lesion, the aim of this study was to determine the efficacy of PMU-PCA3 scores for ruling out PCa in men with previous HGPIN.

Patients and methods The PMU-PCA3 score was assessed by quantitative PCR (multiplex research assay) in 244 men subjected to prostate biopsy: 64 men with an isolated HGPIN (no cancer detected after two or more repeated biopsies), 83 men with PCa and 97 men with benign pathology findings (BP: no PCa, HGPIN or ASAP).

Results The median PMU-PCA3 score was 1.56 in men with BP, 2.01 in men with HGPIN ($p = 0.128$) and 9.06 in

men with PCa ($p = 0.008$). The AUC in the ROC analysis was 0.705 in the subset of men with BP and PCa, while it decreased to 0.629 when only men with isolated HGPIN and PCa were included in the analysis. Fixing the sensitivity of the PMU-PCA3 score at 90%, its specificity was 79% in men with BP and 69% in men with isolated HGPIN.

Conclusions The efficacy of the PMU-PCA3 score to rule out PCa in men with HGPIN is lower than in men with BP.

Keywords PCA3 · Prostate cancer · High grade prostate intraepithelial neoplasia

Introduction

An ideal marker for the early detection of prostate cancer (PCa) should also be able to differentiate between men with an isolated high grade prostatic intraepithelial neoplasia (HGPIN) and those with associated PCa [1]. Serum prostatic-specific antigen (PSA) levels have been widely used for diagnostic purposes for more than 25 years. However, unnecessary biopsies are frequently performed as a consequence of the high rate of false-positive results of PSA [2]. The low specificity of PSA has been the main reason for the development of intensive research projects to find new markers for PCa during the last decade.

Prostate Cancer Gen 3 (PCA3) was described by Bussemakers et al. [3] in 1999 as a PCa-specific mRNA that belongs to the class of non-coding RNAs whose biological role remains unknown. PCA3 was soon regarded as a potential diagnostic tool for the detection of PCa cells in tissue biopsies and bodily fluids [4]. The expression ratio between PCA3 mRNA and PSA mRNA, as the control transcript, in post-prostate massage urine (PMU-PCA3 score) was proposed as a useful tool, in addition to the

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current methods utilized for the early detection of PCa [5–7]. Recently, the APTIMA[®] assay (Gen-Probe, San Diego, CA, USA; PROGENSA[™] for European countries) resolved the problem of urine mRNA conservation and also provided a standardized technique [8]. After establishing the usefulness of the PMU-PCA3 score in ruling out PCa in men undergoing repeat biopsies [9], the basis for its usage was established through recent studies [10–13].

The biopsy finding of an HGPIN is a frequent indication for repeating the prostate biopsy [1]. However, the utility of the PMU-PCA3 score for ruling out PCa in men with HGPIN has not been analyzed. Today we know that PCA3 is expressed in the HGPIN lesions surrounding PCa [14] and that the PMU-PCA3 score seems to be higher in men with HGPIN [12]. Therefore, discovering the behavior of the PMU-PCA3 score in men with HGPIN seems to be a priority. In this study, we analyzed whether the PMU-PCA3 score was able to rule out PCa in men with HGPIN.

Materials and methods

Study design and patients

This is a case–control study where the PMU-PCA3 score was determined before prostate biopsy in 244 men. The study group was represented by 64 men with isolated HGPIN. The condition of isolated HGPIN was defined as no PCa detection after one to four repeat biopsies (1 in 32 men, 2 in 18 men, 3 in 11 men and 4 in 3 men). The control group was represented by 180 men: 83 men with PCa and 97 men with benign pathology findings (BP: no PCa, HGPIN or ASAP). Ten of the 83 men with PCa had previous HGPIN. The men with HGPIN or ASAP in the first biopsy were not included in this study. The median age of the overall group was 64 years (39–85), and the median serum PSA level was 6.4 ng/mL (1.5–189). Prostate massage was performed by systematically applying severe digital pressure to the prostate from the base to the apex and from the lateral to the median line of each lobe. Biopsies were performed using an end-fire ultrasound transducer (Falcon 2101, B-K Medical Inc.) and an automatic 18 gauge needle (Bard Inc.). The minimum number of cores removed in every procedure was 10, plus 1–8 additional cores removed according to the prostate volume and age. Written informed consent was obtained from all the study participants. The protocol was approved by the Institutional Ethical Research Committee.

Sample preparation and procedure

Samples of PMU (~50 mL first capture) were collected in urine collection cups, kept on ice, transported to the labora-

tory and processed within 30 min. The urine samples were centrifuged at $2,500\times g$ for 10 min at 4°C, and then the pellets were washed twice with cold PBS 1×. Finally, the pellets were stored with 1:5 RNA Later (Ambion, Foster City, CA) at –80°C until RNA extraction. Urine RNA was extracted with the QIAamp[®] Viral RNA Mini Kit (Qiagen, Hilden, Germany). Single-stranded cDNA synthesis was carried out using the SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and stored at –80°C until pre-amplification with the TaqMan Preamp Master Mix Kit (Applied Biosystems, Foster City, CA).

Quantitative PCR analysis

PCA3 and the control transcript PSA, all from the TaqMan[®] Gene Expression Assay (Applied Biosystems, Foster City, CA), had to be analyzed by quantitative real time PCR (qPCR). Reactions were carried out in triplicate on an ABI-Prism-7900 qPCR machine, and only those results with a standard deviation <0.38 value were accepted (as recommended by the manufacturer). Threshold levels were set into the exponential phase of the qPCR. The data analysis was carried out using the ABI-Prism-7900SDS Software V2.3 (Applied Biosystems, Foster City, CA) with the same baseline and threshold set for each plate to generate threshold cycle (C_t) values for all of the genes in each sample. Since only a relatively small number of prostate cells are to be found in urine, we performed a cDNA preamplification step before the qPCR. The score was calculated as $ctPCA3/ctPSA \times 1000$ [8].

Statistical analysis

All PMU-PCA3 scores were carried out on log-transformed data, which were applied to stabilize the variances. Quantitative variables were expressed in medians and semi-interquartile range (SIR) and qualitative variables in rates. Univariate analysis was carried out using the Mann–Whitney U and the Kruskal–Wallis test to compare quantitative variables according to two or more groups. Receiver-operating-characteristic (ROC) curves and the area under the curve (AUC) were used to assess the efficacy of the PMU-PCA3 score and to compare groups. Statistical analyses were performed using the statistical package SPSS v.15.

Results

The medians (SIR) of the PMU-PCA3 scores were 1.56 (3.62) in men with BP, 2.01 (11.7) in men with IHGPIN and 9.06 (22.35) in men with PCa. A significant difference was observed among the three scores, $p < 0.001$. A significant difference was observed between the PMU-PCA3

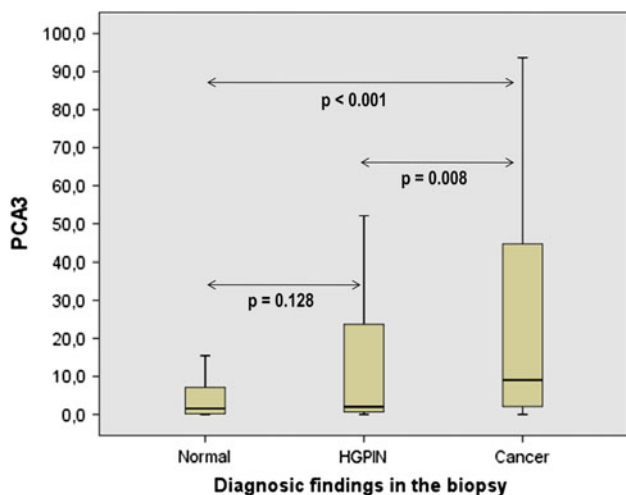


Fig. 1 PMU-PCA3 score according to the biopsy diagnosis. *p* values represented the comparative analysis between every two groups

score of men with IHGPIN and PCa, *p* = 0.008; however, no differences were observed between the PMU-PCA3 score of men with HGPIN and BP, *p* = 0.128 (Fig. 1).

ROC curves for the two different populations were generated. The AUC was 0.629 in the subset of men with IHG-PIN and PCa, while it was 0.705 in the subset of men with BP (without HGPIN) and PCa (Fig. 2). Setting the sensitivity for the PMU-PCA3 score at 90%, the specificity was 79% in men with BP, while it decreased to 67% in men with IHGPIN.

Discussion

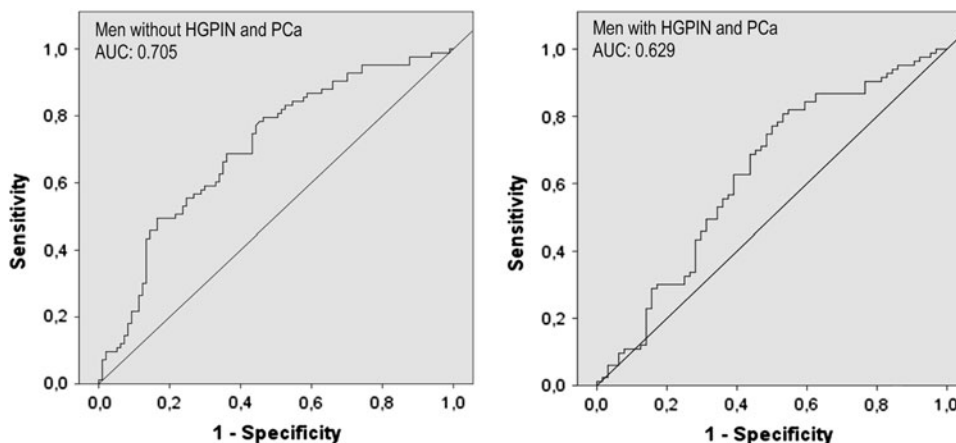
The finding of an HGPIN lesion in a prostate biopsy is a frequent cause for repetition of the procedure. Although some predictors of PCa in men with HGPIN have been analyzed, the urological community still wonders if and when a new biopsy may be required. We have recently described

how, in contrast to isolated HGPIN lesions, the PTOV1 protein is over-expressed in HGPIN lesions associated with PCa. Therefore, we have suggested that PTOV1 immunostaining in HGPIN lesions could identify those men requiring a repeat biopsy, due to the high probability of associated PCa [1]. At present, we are involved in a validation study to confirm these results.

The PMU-PCA3 score has emerged as a tool for selecting those men with negative prostate biopsy, who require a repeat procedure. However, no study has been conducted to analyze the behavior of the PMU-PCA3 score in men with HGPIN. Today we know that PCA3 is expressed in the HGPIN lesions surrounding PCa. Popa et al. [14] recently conducted a study by means of in situ hybridization in radical prostatectomy specimens, in which PCA3 was also expressed in HGPIN lesions. Unfortunately, nothing is known about the PCA3 expression in isolated HGPIN lesions. Therefore, the efficacy of the PMU-PCA3 score in ruling out PCa in men with HGPIN should be defined. Till date, all the PMU-PCA3 score studies have considered a diagnosis of HGPIN as a negative result for PCa. Unfortunately, the recent biopsy nomogram, based on the worldwide data of 809 men subjected to prostate biopsy in a multi-institutional study using the Aptima platform to determine the PMU-PCA3 score, did not include HGPIN as a predictive variable [10].

We decided to review the data from our genomic and proteomic research project conducted on PMU, in order to better determine the behavior of the PMU-PCA3 score in men with HGPIN. We designed a case–control study with a study group represented by those men with a high probability of having an isolated HGPIN, since PCa had not been detected after one or more repeat biopsies. As a control group, we selected men with PCa (true positives) and men with benign pathology findings (true negatives). We observed that the PMU-PCA3 score of men with PCa was significantly higher than that observed in men with BP or IHGPIN, while the PMU-PCA3 score was only slightly

Fig. 2 Efficacy of PMU-PCA3 score to predict PCa by ROC analysis in the subset of men with and without previously diagnosed HGPIN



higher in men with IHGPIN than it was in those men with BP. After the above observation, we wondered whether the efficacy of the PMU-PCA3 score for ruling out PCa in men with IHGPIN was similar to that observed in men with benign pathology. The efficacy of the PMU-PCA3 score was significantly lower in the subset of men having a previous HGPIN. The specificity of the PMU-PCA3 score decreased from 79 to 67% at 90% sensitivity. Therefore, we believe that previous HGPIN should be taken into account, in order to establish the usefulness of the PMU-PCA3 score as a tool for avoiding repeat biopsies. Perhaps a specific cut-off level for the PMU-PCA3 score should be established for men with previous HGPIN, or perhaps a previous HGPIN should be taken into account as a predictive variable in the biopsy nomogram.

Our study had two main limitations. First, negative repeat biopsies cannot exclude a small probability of PCa in men with previous HGPIN. Second, the technique used to assess the PMU-PCA3 score in this study was not the same as the one used in the Aptima platform. For our PMU genomic project, our laboratory has developed a multiplex PCR technique for the analysis of PMU-multiple gene scores. Nevertheless, the present study has demonstrated the presence of cells expressing PCA3 in the PMU of men with isolated HGPIN.

In summary, the main message of this study is that the finding of an HGPIN in a prostate biopsy should be taken into consideration, in order to establish the clinical usefulness of the PMU-PCA3 score as a tool for avoiding unnecessary repeat biopsies.

Conclusions

The efficacy of the PMU-PCA3 score in ruling out PCa in men with previous HGPIN seems to be lower than that observed in men with a previous negative biopsy. This finding should be taken into account, in order to establish appropriate thresholds for the PMU-PCA3 score. Moreover, biopsy nomograms, based on the PCA3 score, should take previous HGPIN into consideration as a predictive variable.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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