Pathological Patterns of Prostate Biopsy in Men with Fluctuations of Prostate Cancer Gene 3 Score: A Preliminary Report

STEFANO DE LUCA[1], ROBERTO PASSERA[2], SUSANNA CAPPIA[3], ENRICO BOLLITO[3], DONATO FRANCO RANDONE[4] and FRANCESCO PORPIGLIA[1]

[1]Urology and [3]Pathology, San Luigi Gonzaga Hospital and University of Torino, Orbassano, Italy; [2]Nuclear Medicine, San Giovanni Battista Hospital and University of Torino, Torino, Italy; [4]Urology, Gradenigo Hospital, Torino, Italy

Abstract. *Background: To evaluate pathological patterns of prostate biopsy in men with changes in risk class by prostate cancer gene 3 (PCA3) score and with elevated serum prostate-specific antigen (PSA) or positive digital rectal examination (DRE), undergoing a repeat biopsy. Patients and Methods: A total of 108 males of two Italian Institutions who had undergone at least two PCA3 score assessments with changed PCA3 risk class were selected. Comparison of PCA3 score in patients with negative re-biopsy [normal parenchyma, benign prostatic hyperplasia (BPH), chronic prostatitis, high-grade prostate intraepithelial neoplasia (HG-PIN), atypical small acinar prostate (ASAP)] or positive re-biopsy was performed. Results: The up- and down-grading rates for PCA3 score were 71.3% (n=77) and 28.7% (n=31), respectively. Among the 77 up-graded patients, the median change in PCA3 score was 24 (range=4-69), while among the 31 down-graded ones, the median change was 17 (2 to 55). The PCA3 score in 24 out of 29 (82.7%) patients with prostate cancer (PCa) was up-graded. No association was found for correlation of PCA3 score change with age >65 years (p=0.975), family history of prostate cancer (p=0.796), positive DRE (p=0.179), use of 5-alpha-reductase inhibitors (p=0.793) and BPH/prostatitis/HG-PIN/ASAP diagnosis (p=0.428). Conclusion: PCA3 score can be considered a marker that is stable over time in most cases; notably, up to 20% of patients have a clinically relevant change of risk class. The rate of PCa was higher in patients whose PCA3 score was up-graded, even if no robust cut-off for PCA3 score fluctuation was identified.*

Correspondence to: Roberto Passera, PharmD Ph.D., Division of Nuclear Medicine, San Giovanni Battista Hospital and University of Torino, Corso AM Dogliotti 14, 10126 Torino, Italy. Tel: +39 0116336171, Fax: +39 0116335019, e-mail: rpassera@cittadellasalute.to.it

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Prostate cancer gene 3 (*PCA3*) is a non-coding, prostate-specific mRNA (a transcript of a pseudogene) of unknown function. It is highly overexpressed (about 70- to 100-fold) in PCa cells with respect to normal or inflamed prostate tissue (1). Several studies have confirmed the usefulness of the PCA3 test for the detection of prostate cancer (PCa) and the possible reduction of needless biopsies (2-6). In contrast to prostate-specific antigen (PSA), the PCA3 score ([PCA3 mRNA/PSA mRNA]*1,000) is not expected to be influenced by benign prostatic hyperplasia (BPH) and prostatitis, nor by prostatic volume and patient age (1, 7-8).

Since the first use of PCA3 diagnostics, the number of patients with a PCA3 score of 2 or more has been increasing. Being based on a genetic marker, the PCA3 score would be expected to be stable on repeated measures over time.

Very few data in literature have reported a 20-30% fluctuation in repeated measures PCA3 score, but these covered only a limited 3- to 4-week time period (1, 3). Nevertheless, it would be expected that the risk class associated with the PCA3 score would be maintained. In a recent study, we demonstrated that even if the PCA3 risk class was unchanged in the majority of patients, there was a non-negligible sub-group (around 18%) of patients with an unpredictable fluctuation in repeated PCA3 measures; in particular, two-thirds of them had a PCA3 score crossing up from ≤35 to >35 (9).

Large differences in repeated measures of PCA3 score would question its role in the decision-making process for re-biopsy and in active surveillance protocols. The genesis of this phenomenon is still unknown. These changes in class risk might be due to laboratory inter/intra-variability or to PCa-presumed biological modifications.

Table I. *Main clinical and biochemical characteristics of the study cohort at positive and negative re-biopsy.*

		Re-biopsy result		
Characteristic	Total patients (n=108)	Positive (n=29)	Negative (n=79)	*p*-Value
Median age, years (range)	66 (51-80)	69 (52-80)	66 (51-80)	0.001
Cancer familiality, n (%)	6 (5.6)	2 (6.8)	4 (5.0)	0.753
DRE: positive/negative, n (%)	6/102 (5.6)	2/27 (6.9)	4/75 (5.1)	0.658
Median PSA at re-biopsy, ng/ml (range)	7.8 (3.4-28)	7.3 (3.1-28)	8.0 (2.8-27)	0.942
Median %fPSA at re-biopsy (range)	14 (2-32)	14 (3-32)	14 (2-32)	0.445
PCA3 score at re-biopsy	44 (3-88)	46 (5-88)	44 (3-87)	0.139

DRE: Digital rectal examination; PSA: prostate-specific antigen; %fPSA: free-PSA.

Table II. *Prostate cancer antigen 3 (PCA3) score changes with possible risk class changes for all patients and in patients with positive/negative re-biopsy.*

	PCA3 score up-graded low- to high-risk				PCA3 score down-graded high- to low-risk			
		Median score (range)				Median score (range)		
	Upgrading from low-to-high risk N (%)	First	Second	Change	Downgrading from high-to-low risk N (%)	First	Second	Change
Cohort	77 (71.3)	24 (8-47)	48 (36-88)	24 (4-69)	31 (28.7)	48 (36-71)	22 (3-35)	17 (2/55)
Positive re-biopsy	24 (82.7)	24 (10-47)	46 (36-88)	22 (6-69)	5 (17.3)	42 (36-51)	33 (20-34)	8 (8/18)
Negative re-Bx patients (%):	53 (67.1)				26 (32.9)			
Normal parenchyma+BPH	13	26 (11-34	43 (39-62)	22 (7-48)	15	50 (37-69)	22 (3-35)	–18 (–2/–43)
Chronic prostatitis	13	21 (8-34)	49 (36-57)	30 (15-45	3	37 (37-43)	27 (21-28)	–16 (–9/–16)
HG-PIN	22	24 (12-35)	48 (37-74)	25 (4-49	8	49 (45-71)	17 (7-29)	–34 (–16/–55)
ASAP	5	31 (10-32)	52 (41-63)	22 (20-47)	-	-	-	-

The aim of the present study was to evaluate the pathological patterns of prostate biopsy in men with changed risk class by PCA3 score in individual patients with elevated PSA or positive DRE, undergoing a re-biopsy.

Patients and Methods

Patients. Between October 2008 and June 2014, a series of 437 men from two Italian Institutions (San Luigi Gonzaga Hospital, Orbassano and Gradenigo Hospital, Torino), underwent at least two PCA3 score assessments in the same laboratory. All of them had one previous negative biopsy (that was performed due to PSA >4 ng/ml or positive DRE) and were scheduled for re-biopsy due to persistent PSA elevation. PCA3 score testing depended on the individual urologist's clinical judgement.

Men with high-grade prostate intraepithelial neoplasia (HG-PIN) (n=34, 31.5%) at first biopsy were included in the study. Patients using 5-alpha-reductase inhibitors (n=22, 20.4%) were also enrolled to verify the possible impact of finasteride/dutasteride administration either on fluctuation of PCA3 score or modification of PCA3 score risk class.

Specific exclusion criteria were prior transurethral resection or open adenomectomy and men with ASAP at first biopsy, given the different related risks (10-11).

Out of the 437 men, 329 (75.3%) maintained their PCA3 score risk category: 189 of them had PCA3 score ≤35, while 140 had PCA3 score >35. Only the remaining 108 patients (24.7%) whose PCA3 score risk class changed were enrolled in this survey.

First-catch urine samples were collected and processed as described below. At least 16-18 peripheral and transition zone cores were performed at re-biopsy by experienced urologists; all biopsies were performed within the two study centers.

Pathological staging was performed according to the seventh edition of the TNM Classification of Malignant Tumors (12). Histological grading was assessed according to the 2005 revised Gleason grading system by an experienced pathologist specialized in uropathology (13).

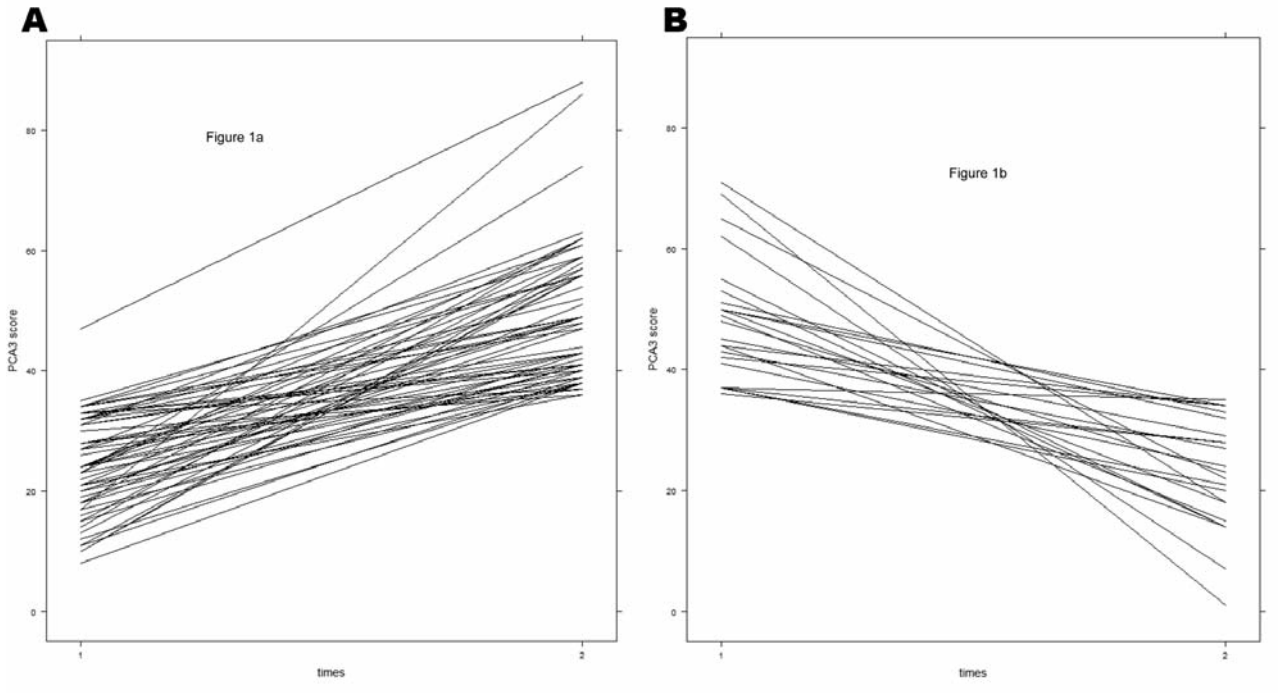

Figure 1. *Repeated measures of the prostate cancer antigen 3 (PCA3) score for patients whose PCA3 score was up-graded (a) and down-graded (b).*

Due to the retrospective observational nature of this research and according to Italian law (Agenzia Italiana del Farmaco-AIFA, Guidelines for observational studies, March 20 2008), no formal ethical committee approval was needed.

Analytical methods. All PCA3 tests were carried-out using the PROGENSA PCA3 assay (Gen-Probe Inc., San Diego, CA, USA). Briefly, *PCA3* and *PSA* mRNAs were extracted from exfoliated prostate cells in urine samples after prostate massage, then amplified and finally hybridized using DNA probes, tagged with a chemiluminescent substance. The hybridized number of *PCA3* mRNA and *PSA* mRNA copies were counted by a luminometer and the PCA3 score was then calculated. Urine samples were considered as non-informative for prostate cells if the number of *PSA* mRNA transcripts detected was ≤10,000. The PCA3 score test was considered negative if ≤35, while positive if >35.

Statistical methods. Patients' characteristics were analyzed by Fisher's exact test for categorical variables, while for continuous ones, the Mann-Whitney and Kruskal-Wallis (for independent measures) or the Wilcoxon and Friedman tests (for repeated measures) were used. All results for continuous variables are expressed as the median and range. The diagnostic accuracy of PCA3 score fluctuations in predicting PCa at re-biopsy was assessed by a receiver operating characteristic (ROC) analysis. All reported *p*-values were obtained by the two-sided exact method, at the conventional 5% significance level. Data were analyzed as of November 2014 by R 3.1.1 (R Foundation for Statistical Computing, Vienna-A, http://www.R-project.org).

Results

The main patient characteristics of the whole study cohort and at re-biopsy are reported in Table I. The median age was 66 (range=51-80) years; most patients (94.4%) had a negative DRE; a family history of cancer was reported in six patients (5.6%).

The median first and second PSA were 6.5 (range=2.8-28) and 7.8 (range=3.4-28) ng/ml, respectively; the median free-PSA (%fPSA) was 14% (range=2-32) at re-biopsy, but was unavailable at first biopsy for the vast majority of the cohort.

The median first and second PCA3 scores were 29 (8-71) and 44 (3-88), respectively. The median time between the two PCA3 score assessments was 18.7 (range=9-67) months; only in five cases (4.6%) was it less than 12 months. The median time between the second PCA3 score and re-biopsy was 1.5 (range=0.5-2.2) months.

There was no significant difference in median PSA level among the men with normal parenchyma/BPH, chronic prostatitis, ASAP or HG-PIN at re-biopsy (7.9, 7.8, 9.2 and 8.0 ng/ml, respectively; p=0.604). A comparable pattern was found at re-biopsy for median %fPSA (17, 12, 10 and 15%; p=0.176); conversely, the median PCA3 score at re-biopsy was significantly different (35, 41, 52 and 42; p=0.042).

Twenty-nine out of the 108 men (26.9%) had a positive re-biopsy; their first biopsy result was HG-PIN (n=15),

chronic prostatits (n=6) and normal/BPH (n=8). The median PSA and %fPSA values in men with a negative *vs*. positive re-biopsy were similar (p=0.445); as was the PCA3 score (44 *vs*. 46, p=0.139).

The median PCA3 score was 43 (range=28-71) in the 15 patients with a Gleason score (GS) <7, while it was 59 (range=32-88) among the 14 patients with a GS ≥7 (p=0.007).

The median PCA3 score was significantly lower in men with ≤33% *vs*. >33% positive biopsy cores (42 *vs*. 69, p<0.001) and in patients with 'indolent biopsy' PCa (defined as: clinical stage T1c, PSA density <0.15, GS biopsy ≤6, positive cores ≤33%) *vs*. 'significant biopsy' PCa (40 *vs*. 61, p<0.001).

Fluctuations in PCA3 score and possible risk class changes for all patients and in patients according to the results of re-biopsy are reported in Table II.

The median first PCA3 score for up-graded/down-graded patients were 24 (8-47) and 48 (36-71), respectively; the median second PCA3 score for up-graded/down-graded patients were 48 (36-88) and 22 (1-35), respectively.

The upgrading and downgrading rates for PCA3 score were 71.3% (77 pts) and 28.7% (31 pts), respectively. Among the 77 upgrading patients, the median PCA3 score up-grade was 24 (4-69), while among the 31 downgrading ones, the median PCA3 score down-grade was -17 (-2/-55).

Twenty-four patients out of 29 (82.7%) PCa patients up-graded their PCA3 score. Their median first and second PCA3 scores were 24 (10-47) and 46 (36-88), while the median up-grade was 22 (6-69).

PCA3 score in the remaining five patients with PCa was down-graded. Their median first and second PCA3 scores were 42 and 33, while the median down-grade was -8.

Notably, two out of five patients developed PCa (GS <7) despite a remarkable downgrading of their PCA3 score (from 51 to 33 and from 37 to 20, respectively); in both cases, their first biopsy revealed an HG-PIN, while their PSA values almost doubled from the first to the second biopsy. For the three remaining patients with PCa, the PCA3 score was down-graded by 8; their first biopsy showed two cases of HG-PIN and one of chronic prostatitis.

In total, 79 (73.1%) patients had a negative re-biopsy. Out of these, 30 (37.9%) and 5 (6.3%) had a diagnosis of HG-PIN (multifocal in four patients) and ASAP, respectively; 28 (35.4%) patients had normal parenchyma/BPH and 16 (20.2%) had a diagnosis of chronic prostatitis. Their median PCA3 score changes are reported in Table II.

Spaghetti plots for patients with up-graded and down-graded PCA3 scores are shown in Figure 1.

No robust cut-off for PCA3 score fluctuation was identified as being able to predict PCa at re-biopsy by a ROC analysis. No association was found between change in PCA3 score and age >65 years (p=0.975), family history of prostate cancer (p=0.796), positive DRE (p=0.179), use of 5-alpha-reductase inhibitors (p=0.793) and BPH/prostatitis/HG-PIN/ASAP diagnosis (p=0.428).

Discussion

PCA3 was identified by Bussemaker *et al*. in 1999 under the name DD3, using digital display screening for prostate cancer-specific RNAs (3). The PCA3 score appeared to be a promising genetic test as *PCA3* mRNA is clearly over-expressed in PCa tissue compared to non-malignant prostatic tissue. Because PCA3 is also expressed in non-cancer cells, its content in clinical specimens must be normalized to the amount of prostate-derived RNA. This is achieved by using the ratio of *PCA3*/*PSA* mRNA as the diagnostic indicator; *PSA* mRNA yield is also used to verify that the amount of RNA present is sufficient to yield an accurate result.

Assays are available to accurately measure *PCA3* mRNA and *PSA* mRNA; the PCA3 score derived from these measures has good sensitivity and specificity for predicting a positive re-biopsy (1-8, 14). A recent meta-analysis suggests that urinary PCA3 may serve as a diagnostic indicator, with specificity 0.71, and may represent a useful marker in PCa diagnosis (15).

For DNA assays, it is probably true that a score should be stable over time for every patient. However, when measuring PCA3 and RNA concentration, there is likely some variation over time, especially if the extent of cancer changes. Some authors reported a 20-30% fluctuation in PCA3 score on repeated measures (3-4), but covering only a limited 3- to 4-week time period (1, 3). Nevertheless, we would at least expect a maintenance of risk class.

In a recent study, we evaluated the PCA3 score fluctuations in 360 men who had undergone at least two PCA3 score assessments (9). The median time between the two PCA3 assessments was 16 (range=3-54) months. We demonstrated that about 80% of patients maintained their risk class category (using a PCA3 score cutoff of 35); among the remaining patients, the rates of down- and up-grading of the PCA3 score were about 30% and 70%, respectively (9).

The current results confirm the same proportion (28.7 *vs*. 71.3% respectively).

Some studies demonstrated that there are no significant differences in PCA3 score fluctuations, depending on DRE methods (standard *vs*. extended DRE) (16).

Preliminary data suggest that a random, short-term, physiological variation does not significantly affect an individual PCA3 score (17-18).

In the current, highly selected cohort (having a double PCA3 score assessment with risk class change, and a rebiopsy after the second PCA3 assesment), we demonstrated that PCA3 score was up-graded in around 83% of patients with PCa. A possible explanation for this could be related to carcinogenesis itself: an oncogene modulation

mechanism could influence PCA3 expression. In this regard, a prospective study demonstrated that the PCA3 score was significantly higher in the HG-PIN group than in a PCa-negative group (19). HG-PIN is the only accepted precursor of prostatic adenocarcinoma, according to several animal and human models (20). It is characterized by progressive abnormalities of phenotype and genotype, intermediate between benign prostatic epithelium and cancer. Carcinoma develops in most patients with HG-PIN within 10 years (21). It should be noted that in the current cohort, the HG-PIN rate was higher in the subgroup with up-graded risk class by PCA3 score, comparing to that down-graded (Table II).

According to some authors, a higher PCA3 score in the HG-PIN group than in PCa-negative patients probably reflects early molecular changes in a presumably premalignant lesion (19). These data agree with previous reports, showing that the PCA3 score had poor discriminative performance between HG-PIN and Pca (22-23).

In our experience, the increasing rate of HG-PIN among patients with up-graded PCA3 score risk class could confirm the role of mutations in the mechanism of carcinogenesis being responsible for the increasing PCa rate. The large PCA3 score increase (≥60) for five patients with PCa at rebiopsy (four with HG-PIN at first biopsy), might support this hypothesis. At the same time, PCA3 score risk class was down-graded in 17% of those with PCa, however, their GS was 7, and the number of positive cores was ≤33% ('indolent biopsy' PCa).

In a recent study, in agreement with other studies (24-27), we showed that PCA3 score could play an interesting role, being one of the main independent risk factors for GS ≥7 at radical prostatectomy (odds ratio [OR]=2.04) (28). This finding was confirmed in the present study: the median PCA3 score was significantly lower in men with GS <7 *vs*. ≥7 and in those with ≤33% *vs*. >33% positive biopsy cores, and in patients with 'indolent biopsy' PCa *vs*. 'significant biopsy' PCa.

With regard to the possible capability of this biomarker to predict cancer aggressiveness, focusing on the latter topics, the results are still conflicting. Some studies revealed a clear association between PCA3 score and GS (23-25), while others did not (29-31).

Different hypotheses could explain these contradictory findings. For instance, a higher PCA3 score could be associated with more aggressive PCa, as increasing cell

De-differentiation may ease shedding into prostatic ducts, during DRE. On the other hand, aggressive tumors become more solid and lose their glandular differentiation and lumens, which may hamper cell shedding into the urine (32).

The two principal limitations of this study are: Firstly, being a retrospective observational study, it was not designed to systematically address the issue of PCA3 score variability and there was an ascertainment bias (the enrolled patients were extracted from a large cohort of 2,851 patients undergoing repeated PCA3 score measures and biopsy). Secondly, the decision and the timing of PCA3 scoring depended on the individual urologist and not on a pre-established schedule.

The PCA3 score can be considered a stable marker over time in most cases; notably, there is a group of patients (up to 20%) having a clinically relevant change in risk class.

Further investigations are required to determine what the driving force for fluctuation of PCA3 score is. From this research, the open questions for the urologist are: When should the PCA3 score be re-assessed? How these patients be managed in the decision-making process for re-biopsy? Taking into account these possible changes in risk class, is the role of the PCA3 score in active surveillance protocols questionable?

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