Biopsy and Radical Prostatectomy Pathological Patterns Influence Prostate Cancer Gene 3 (PCA3) Score

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Abstract. *Aim: To evaluate the relationship between Prostate cancer gene 3 (PCA3) score and prostate cancer as assessed by Gleason Score (GS) and pathological stage in a series of Italian patients, with elevated Prostate specific antigen (PSA) undergoing radical prostatectomy (RP). Patients and Methods: A total of 222 patients underwent RP for clinically localized prostate cancer; total PSA, free-PSA (%fPSA) and PCA3 score were collected and the possible associations among PCA3 and histological grade/pathological stage at biopsy and RP were investigated. Results: Median PCA3 scores by GS at radical prostatectomy were 51 vs. 67 (GS <7 vs. GS ≥7, p=0.007), while scores at the biopsy were 56 vs. 67 (GS <7 vs. GS ≥7, p=0.007), and in pT2 vs. pT3 patients they were 54 vs. 80 (p=0.001). Positive digital rectal examination (DRE) (odds ratio (OR)=5.47, p=0.026), pT3 pathological stage (OR=3.68, p=0.006) and PCA3≥35 (OR=2.04, p=0.030) were the main risk factors for the presence of an aggressive disease (GS≥7 at RP). Conclusion: PCA3 score could play an interesting role in predicting significant disease: positive DRE (OR=5.47, p=0.026), pT3 pathological stage (OR=3.68, p=0.006) and PCA3≥35 (OR=2.04, p=0.030) were the main independent risk factors for GS≥7 at RP.*

Prostate cancer exhibits a considerable biological variability which hampers accurate prediction of disease aggressiveness by current prognostic markers.

Many men with low-risk cancer are still treated actively and exposed to potential complications, such as incontinence and erectile dysfunction (1). In these men, active surveillance may be more appropriate (2-5). New predictors are urgently awaited to improve cancer classification, thus facilitating decision-making and patient counseling.

Prostate cancer gene 3 (*PCA3*), first described by Bussemakers *et al.* in 1999 (6), is a noncoding, prostate-specific mRNA that is highly overexpressed in 95% of prostate cancer cells, with a median 66-fold up-regulation compared with adjacent non-neoplastic prostatic cells (7-9). An increased PCA3 score corresponds to an increased probability of a positive biopsy and its diagnostic value has been primarily demonstrated in men with a previous negative biopsy and elevated Prostate specific antigen (*PSA*) levels (10).

Ideally, PCA3 might not only be helpful for diagnostic purposes, but also for prognostic estimation for its possible capability to predict cancer aggressiveness (2, 11-13). Unfortunately, when focusing on the latter topics, the results are still conflicting. Some studies revealed a clear association between PCA3 and Gleason Score (GS) (2,12), while others did not (14-16).

Different hypotheses could explain these contradictory findings. For instance, a higher PCA3 score could be associated with a more aggressive carcinoma as increasing cell de-differentiation may ease shedding of tumor cells into prostatic ducts during digital rectal examination (*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 (17).

The aim of this study was to evaluate the relationship between PCA3 score and prostate cancer significance as assessed by GS and pathological stage in a series of Italian patients with elevated PSA undergoing radical prostatectomy (*RP*).

Patients and Methods

Between January 2010 and April 2012, a series of 222 patients underwent RP for clinically-localized prostate cancer in two different Italian Institutions (Gradenigo Hospital, Torino and San

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Luigi Gonzaga Hospital, Orbassano). These 222 patients belong to a larger population of men tested for urinary PCA3 before undergoing prostate biopsy (18). Seventy-three patients out of these patients were scheduled for a initial prostate biopsy due to serum PSA ≥2.5 ng/ml associated with suspicious DRE in 25 cases, while the other 149 had either one (n=126) or two (n=23) previous negative biopsies and were scheduled for repeat biopsy due to persistent PSA elevation.

Specific exclusion criteria were 5-alpha-reductase inhibitor therapy and/or prior transurethral resection or open adenomectomy.

Blood and urine specimens were collected before positive biopsy; total PSA, Percentage Free PSA (*%fPSA*) and PCA3 score were determined for each patient.

At least 10-14 standardized peripheral zone biopsy cores were taken at first biopsy, and 14-18 peripheral and transition zone biopsy cores were performed at repeat biopsy by experienced urologists. All biopsies were performed within the two study centers. Biopsy-indolent prostate cancer was defined according to the Epstein biopsy criteria as T1c, PSA Density (*PSAD*) <0.15 ng/ml, biopsy GS ≤6 and percentage of positive cores of 33% (19).

All patients were then treated by retropubic or laparoscopic RP, 177 accompanied by standard pelvic lymph node dissection, due to their class of risk. The median time from biopsy to RP was 2 months (range=1-4 months). Tumour volume was unavailable for the vast majority of men. Pathological staging was performed according to the sixth edition of the TNM classification of malignant tumors (20). Histological grading was assessed according to the 2005 revised Gleason grading system by an experienced pathologist specialised in uropathology (21).

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

Analytical methods. All PCA3 tests were carried out using PROGENSA PCA3 assay (Gen Probe Inc., San Diego, CA, USA) according to the manufacturer's specific instructions. Briefly, *PCA3* and *PSA* mRNAs were extracted from exfoliated prostate cells in urine samples after DRE, then amplified and finally hybridized using DNA probes tagged with a chemiluminescent substance. The hybridized number of *PCA3* and *PSA* mRNA copies were counted with a luminometer and the ratio of the two (PCA3 score) was calculated as *PCA3* mRNA/*PSA* mRNA *1000. Urine samples were considered as non-informative for prostate cells if the number of PSA mRNA transcripts detected was fewer than 10,000. The PCA3 score test was considered positive if the PCA3 score was 35 or more, its usual cut-off (6-8).

Statistical methods. Patients' characteristics were tested using the Fisher's exact test for categorical variables and the Mann-Whitney and Kruskal-Wallis tests for continuous ones. All results for continuous variables are expressed as the median (range). The diagnostic accuracy of PCA3, total PSA and %fPSA in predicting an aggressive disease (GS at RP ≥7) was assessed by a receiver operating characteristic (ROC) analysis; since it was impossible to identify clear cut-offs, in the subsequent analyses for PCA3 and %free PSA, the classical values of 35 and 10% respectively, were used, while for PSA, the median value of 8.5 ng/ml was used, with only four patients with PSA ≤4 ng/ml. The GS at RP ≥7 was then used as a dependent variable in different univariate and multivariate binary logistic regression models, testing age at diagnosis (>67 *vs.* ≤67 years, median value), DRE (positive *vs.* negative), clinical stage (pT3 *vs.* pT2), PCA3 score (≥35 *vs.* <35), PSA values (≥8.5 *vs.* <8.5 ng/ml) and %free PSA score (≥10% *vs.* <10%) as independent risk factors for aggressiveness. All reported p-values were obtained by the two-sided exact method, at the conventional 5% significance level. Data were analyzed as of March 2013 by SPSS 21.0.0 (IBM Corp., Armonk, USA, http://www-01.ibm.com/software/analytics/spss/products/statistics/) and R 2.15.2 (R Foundation for Statistical Computing, Vienna-A, http://www.R-project.org).

Table I. *Patients' characteristics*.

	Median(range)/n(%)
Age (years)	67 (48-77)
Digital rectal examination	
Negative	197 (88.7%)
Positive	25 (11.3%)
Serum total PSA (ng/ml)	8.5 (3.6-23)
PSA	
<4 ng/ml	3 (1.4%)
4-10 ng/ml	143 (64.4%)
>10 ng/ml	76 (34.2%)
% free PSA	15 (3-28)
% free PSA	
<10	42 (18.9%)
10-20	132 (59.4%)
>20	48 (21.7%)
PCA3 score	59 (8-263)
PCA3 score	
<15	9 (4.1%)
15-20	11 (4.9%)
21-35	28 (12.6%)
36-50	52 (23.4%)
51-100	67 (30.2%)
>100	55 (24.8%)
Clinical stage	
T1c	142 (63.9%)
T2-T2c	55 (24.7%)
T3a-T3b	25 (11.2%)
N0	191 (86%)
Nx	31 (14%)
Pathological stage	
pT2	175 (78.8%)
pT3	47 (21.2%)
pN0	171 (77.0%)
pN1	6 (2.7%)
pNx	45 (20.3%)

Results

The main patients' clinical and pathological characteristics are summarised in Table I. The median age was 67 (range 48-77) years. Most patients (88.7%) had a negative DRE. All 222 patients had adequate levels of *PCA3* and *PSA* mRNAs to calculate the PCA3 score before surgery.

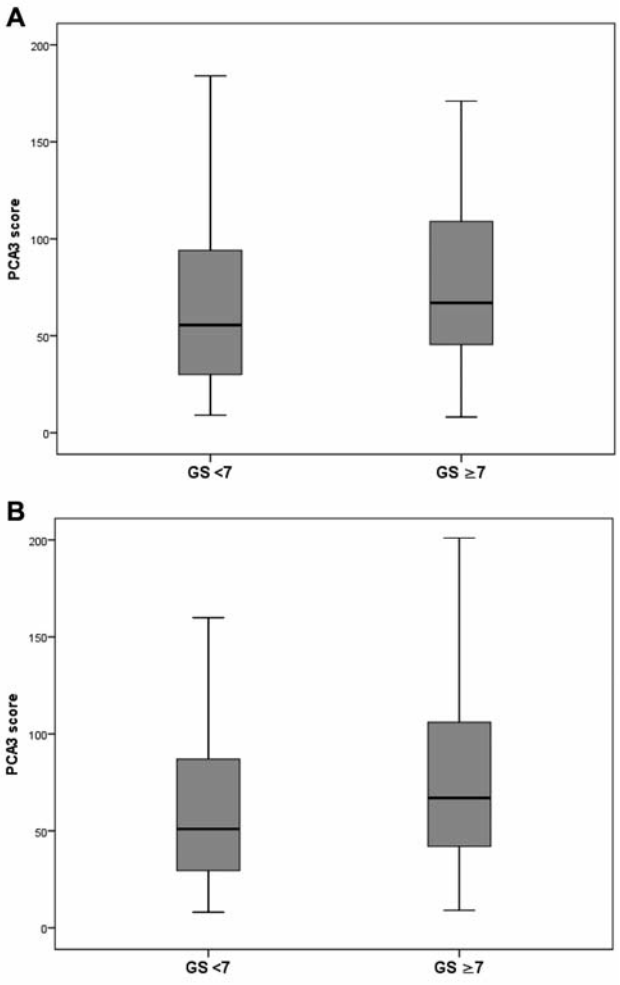

Figure 1. *PCA3 score by GS at biopsy (A) and at radical prostatectomy (B). In the box-plot, the bottom and top of the box are the first and third quartiles, the band inside the box is the second quartile (the median), while the whiskers indicate the minimum and maximum of all of the data.*

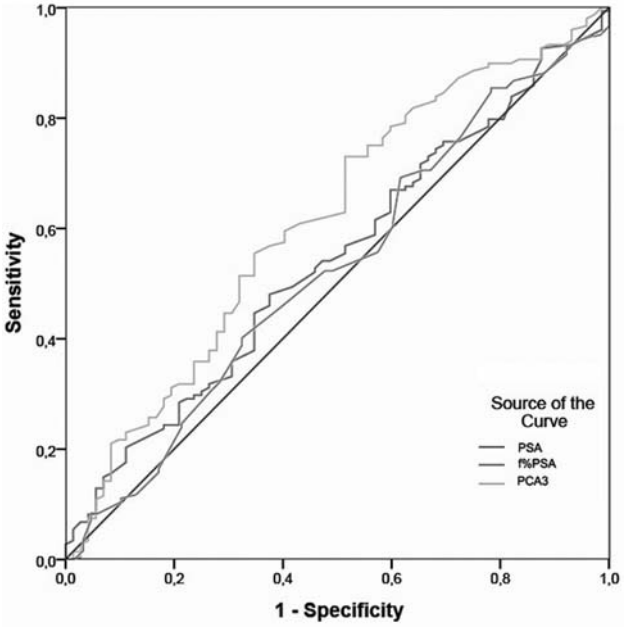

Figure 2. *The diagnostic accuracy of PCA3 score, total PSA and %fPSA in predicting an aggressive prostate cancer using the receiver operating characteristic curve.*

The median (range) PSA, %fPSA and PCA3 score were 8.5 (3.6-23) ng/ml, 15% (3-28%) and 59 (8-263), respectively. Forty-eight patients (21.6%) had a PCA3 score of ≤35.

No linear correlations were found between PCA3 score and PSA (p=0.312) nor between PCA3 score and %fPSA (p=0.174).

Most patients (88.6%) had clinically organ-confined disease (T1c or T2). Disease in almost 79% was pT2, while that in the others were pT3, and 6 patients had positive lymph nodes. Due to their low risk class, 45 patients (20.3%) had not undergone standard pelvic lymph node dissection.

GS <7 at biopsy and at RP was 58.5% *vs*. 32.4%, respectively (p<0.001); no GS <6 was observed. Biopsy GS was confirmed at RP in 125 men (56.3%), was up-graded in 85 (38.3%) and down-graded in another 12 patients (5.4%).

The median PCA3 scores were statistically significantly lower in men with ≤33% *vs*. >33% positive biopsy cores (PCA3 score 44.2 *vs*. 72.7, p<0.001) and in patients with biopsy-indolent (defined as: clinical stage T1c, PSA density <0.15, GS biopsy ≤6, percentage of positive cores ≤33%) *vs*. biopsy-significant prostate cancer (PCA3 score 31.2 *vs*. 66.3, p<0.001).

At biopsy, 130 patients with a GS <7 had a median PCA3 score of 56 *vs*. 67 among the 92 patients with GS ≥7 (p=0.007) (Figure 1A). Similarly for the prostatectomy GS, comparing 72 patients with a biopsy GS <7 to 150 patients with a GS ≥7, the difference between PCA3 scores (51 *vs*. 67) was again highly significant (p=0.007) (Figure 1B).

The median PCA3 score increased in the group with up-graded GS (n=85): 71 *vs*. 52, p=0.046; conversely, no differences were found for median PSA and %fPSA values between the not-upgraded (n=137) *vs*. up-graded (n=85) GS groups: 8.30 *vs*. 8.80 ng/ml for PSA, p=0.873; 15.0% *vs*. 15.0% for %fPSA, p=0.486.

As for the relationship between PCA3 score and pathological stage, in the pT2 group (n=175) the median (range) PCA3 score was 54 (8-263), while it was 80 (9-254) in the pT3 one (n=47), with an extremely significant difference (p=0.001). Even considering the very low cluster of patients, median PCA3 scores were markedly different comparing the six patients with pN1 disease to the all remaining patients with pN0 (189 *vs*. 59, p=0.025).

Table II. *Univariate and multivariate binary logistic regression models.*

	Univariate			Multivariate		
Risk factors	OR	95% CI	*p-Value*	OR	95% CI	*p-Value*
Age ≥67 years	1.00	0.57-1.76	0.988			
Positive DRE	6.34	1.45-27.68	0.014	5.47	1.23-24.43	0.026
Pathological stage (pT3 vs. pT2)	4.14	1.67-10.28	0.002	3.68	1.46-9.30	0.006
PCA3 ≥35	2.40	1.29-4.47	0.006	2.04	1.07-3.89	0.030
PSA ≥8.5 ng/ml	1.28	0.73-2.25	0.390			
%fPSA ≥10	1.27	0.61-2.66	0.524			

Dependent variable: aggressive disease (GS ≥7 at RP).

Figure 2 shows the ROC analysis for predicting a status of aggressive disease (GS at RP ≥7) by different biomarkers: the PCA3 score had a statistically significant diagnostic accuracy (AUC=0.614; p=0.006), in contrast to total PSA (AUC=0.544; p=0.295) and %fPSA (AUC=0.585; p=0.070).

Finally, the logistic regression model was used to identify the possible predictors for aggressive cancer (Table II). The multivariate model confirmed the results of the univariate ones well: positive DRE (odds ratio OR=5.47, p=0.026), pT3 pathological stage (OR=3.68, p=0.006) and PCA3 score ≥35 (OR=2.04, p=0.030) were the main independent risk factors for GS≥7 at RP. In this logistic model, sensitivity, specificity, and positive and negative predictive values were 88%, 41%, 37%, and 89%, respectively. Conversely, PSA ≥8.5 ng/ml (OR=1.28, p=0.390) and %fPSA ≥10% (OR=1.27, p=0.524) could not be used as predictors of aggressive disease.

Discussion

At present, up to two-thirds of patients undergoing a prostate biopsy have negative histology, since the address to biopsy is based on serum PSA assessment, a sensitive but mostly non-specific test (22). To reduce unnecessary biopsies, by improving PSA specificity, research in the past has primarily looked at PSA derivates such as %fPSA, and PSA velocity or density (23, 24). Most of these derivates are of some diagnostic use, but none are effective enough to solve the problem; indeed, some of them remain controversial altogether.

The PCA3 score appears to be a promising new marker as its mRNA is clearly overexpressed in prostate cancer tissue compared to non-malignant prostatic tissue. Meanwhile, several studies have confirmed the usefulness of the PCA3 test for the detection of prostate cancer and the possible reduction of needless biopsies (18, 22, 25).

Previous findings strongly indicate the superiority of PCA3 score over PSA for predictive value and specificity, but with a slightly lower sensitivity; these results are particularly encouraging for patients having a initial negative biopsy, for whom using a PCA3-based assay would avoid a pointless repeat biopsy (25, 26).

Our experience confirmed this evidence, indeed the test performed very differently in patients having or not having had previous biopsies; in the latter, the PCA3 ROC curve gave significantly better results at all PSA intervals (18).

Given that PCA3 is highly overexpressed in cancer tissue and improves the prediction of biopsy outcome, several studies have focused on its potential ability to predict tumour stage and aggressiveness before definitive therapy (2, 11-17, 26).

The assumption that higher PCA3 scores are associated with more aggressive cancer is based on the hypothesis that with increasing de-differentiation, neoplastic cells become more invasive and can therefore more easily be shed into the ductal system of the prostatic gland after DRE, and that larger tumours simply have more surface area over which to shed PCA3 (16, 26).

The preoperative anticipation of histological prognostic features at RP would affect the therapeutic approaches to localised prostate cancer, such as the decision for active surveillance and preservation of neurovascular bundles, and stratify patient risk for surgical margins (27, 28). Unfortunately, focusing on this latter topic the results of the predictive prognostic accuracy of PCA3 are still conflicting.

One of the first reports addressing PCA3 as prognostic marker was published by Nakanishi *et al.* (2), showing that the PCA3 score statistically significantly correlated with tumour volume (p=0.008) and with prostatectomy GS (<7 *vs.* ≥7, p=0.005) in 96 patients. Using a PCA3 score cut-off of 25, for predicting small volume tumours in combination with low grade (GS <7), sensitivity and specificity were 70% and 73.3%, respectively. However, they found no statistical difference between pT2 and pT3 tumors (p=0.852).

An analysis of the reduction by dutasteride of prostate cancer events (REDUCE) study, including 1,140 men, with a PSA level of 2.5-10 ng/ml, undergoing repeat biopsies, validated the PCA3 test for predicting repeat biopsy outcome (29). The trial showed that the median PCA3 score was significantly higher in patients with a biopsy GS ≥7 than

those with GS <7 (49.5 *vs*. 31.8, p=0.002). Another recent study showed that the PCA3 score was associated with GS (p<0.001) and tumour volume (p=0.003) (30).

In a multi-center European study, van Poppel *et al*. investigated the relationship between PCA3 and different cancer features in 159 men undergoing RP (12); the authors reported a statistical difference (p=0.007) between patients with GS <7 compared to those with GS ≥7 (mean PCA3 score, 63.2 *vs*. 65.5).

In contrast to these studies, Hessels *et al*. and van Gils *et al*. were unable to detect any relationship among PCA3 score and the classical prognostic parameters, such as prostatectomy GS, pathological stage, and tumour volume (14, 16). Nor did Whitman *et al*., find a significant association of PCA3 with pathologic GS (11).

Augustin *et al*. assessed the tumour volume in RP specimens with different primary and secondary Gleason patterns in order to gain more detailed information about the relationship between PCA3 and tumour volume (31). In this sub-group analysis, neither the primary nor the secondary Gleason pattern-specific tumour volumes were significantly associated with PCA3; a marginal difference (p=0.081) was found between patients with a prostatectomy GS <7 compared to those with a GS ≥7 (mean PCA3 score, 60.7 *vs*. 76.4). Similarly, in a recent analysis of 160 men by Durand *et al*., a PCA3 score >35 did not appear to be an independent risk factor for GS ≥7 from prostatectomy specimens (p=0.5) (13), therefore, not confirming the hypothesis that undifferentiated tumour cells would exfoliate and cause an increase in PCA3.

Conversely from previous studies, the present research found that the median PCA3 score was lower in men undergoing a RP with GS <7 comparing to those with GS ≥7 (51 *vs*. 67, p=0.007); a similar trend was also reported at biopsy, whereas the median PCA3 scores by GS were 56 *vs*. 67 (p=0.007). Positive DRE, pT3 pathological stage and PCA3 ≥35 were the main risk factors for GS≥7 at RP, while PSA and %fPSA had no role as predictors of aggressive disease. Moreover, estimating the role of PCA3 even as a continuous variable (and not only as a binary one, see Table II), an unit increase of PCA3 score would enhance the risk of aggressive cancer at staging by 0.7%, adjusting for any other risk factor.

Of note, simply to discriminate aggressive from non-aggressive cancers, most previous trials used a PCA3 score different from the classical cut-off of 35 (32).

Several limitations of this study must be acknowledged: among them, tumour volume was not available for the vast majority of men (being then impossible to define pathologically-insignificant prostate cancer according to the Epstein criteria); the minimal cluster of pN1 patients is too low to investigate its potential association with PCA3; finally, the comparison of the GS at biopsy and at RP showed a notable GS upgrading from biopsy to RP specimens (38.3%).

It has been reported that nearly a third of patients with prostate cancer will have a significant GS up-grade between biopsy and RP (33). This may have important consequences for treatment decision-making, in particular for selecting men with clinically insignificant cancer in whom active surveillance may be proposed. Pathology results in men who were initially followed with active surveillance showed organ-confined disease and favourable Gleason grading in the majority of cases; however, the proportion of unfavourable outcomes could not be neglected (4, 15, 30, 31). Therefore, it remains an important focus for active surveillance protocols to improve the selection of patients at the time of inclusion in order to minimise reclassification of risk during follow-up.

Until reliable biomarkers to predict disease become definitively available, strict follow-up of men on active surveillance with repeated PSA and multiple regular repeat biopsies must be warranted to preserve the chance for curative treatment. In this context, the PCA3 score could play a new interesting role, following validated nomograms; at the same time, due to the inconclusive results so far obtained, more prospective controlled trials are needed on larger patient cohorts.

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