Comparison of Prostate Cancer Gene 3 Score, Prostate Health Index and Percentage Free Prostate-specific Antigen for Differentiating Histological Inflammation from Prostate Cancer and Other Non-neoplastic Alterations of the Prostate at Initial Biopsy

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Abstract. *Aim: To determine if prostate cancer gene 3 (PCA3) score, Prostate Health Index (PHI), and percent free prostate-specific antigen (%fPSA) may be used to differentiate prostatitis from prostate cancer (PCa), benign prostatic hyperplasia (BPH) and high-grade prostate intraepithelial neoplasia (HG-PIN) in patients with elevated PSA and negative digital rectal examination (DRE). Patients and Methods: in the present prospective study, 274 patients, undergoing PCA3 score, PHI and %fPSA assessments before initial biopsy, were enrolled. Three multivariate logistic regression models were used to test PCA3 score, PHI and %fPSA as risk factors for prostatitis vs. PCa, vs. BPH, and vs. HG-PIN. All the analyses were performed for the whole patient cohort and for the 'gray zone' of PSA (4-10 ng/ml) cohort (188 individuals). Results: The determinants for prostatitis vs. PCa were PCA3 score, PHI and %fPSA (Odds Ratio [OR]=0.97, 0.96 and 0.94, respectively). Unit increase of PHI was the only risk factor for prostatitis vs. BPH (OR=1.06), and unit increase of PCA3 score for HG-PIN vs. prostatitis (OR=0.98). In the 'gray zone' PSA cohort, the determinants for prostatitis vs. PCa were PCA3 score, PHI and %fPSA (OR=0.96, 0.94 and 0.92, respectively), PCA3 score and PHI for prostatitis vs. BPH (OR=0.96 and 1.08, respectively), and PCA3 score for prostatitis vs. HG-PIN (OR=0.97). Conclusion: The clinical benefit of using PCA3 score and PHI to estimate prostatitis vs. PCa was comparable; even %fPSA had good diagnostic performance, being a faster and cheaper marker. PHI was the only determinant for prostatitis vs. BPH, while PCA3 score for prostatitis vs. HG-PIN.*

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In patients with a negative biopsy performed for elevated prostate-specific antigen (PSA) level, either undetected prostate cancer (PCa) or sub-clinical prostatitis (if histological evidence of inflammation is present) may serve as plausible explanations for a high PSA level.

Acute and chronic histological inflammation, *i.e.* infiltration of the prostate by inflammatory cells, is commonly found among asymptomatic men biopsied for elevated PSA. Previous studies have revealed that 35% to 100% of prostate biopsies, performed for concern about PCa, had some histological evidence of inflammation (1). The prevalence of histological acute inflammation ranges from 22 to 63% (1-3), while that of chronic inflammation varies extremely, from 8% to 99% (2-4), probably due to analytical and methodological differences. Furthermore, proliferative inflammatory atrophy has been proposed as a precursor lesion for prostatic intraepithelial neoplasia (PIN) and PCa (5).

To reduce unnecessary biopsies and to improve upon PSA specificity, past research primarily investigated PSA derivatives, such as percentage of free prostate-specific antigen (%fPSA) PSA velocity/density (6-7). Most of these

derivatives are of some diagnostic value, although without significantly reducing the rate of negative biopsies.

Prostate cancer antigen 3 gene (*PCA3*) is a non-coding, prostate-specific mRNA, highly overexpressed in 95% of PCa cells. An increased PCA3 score corresponds to an increased probability of a positive biopsy; its diagnostic value has been primarily demonstrated in men with a previous negative biopsy and a high PSA level (8-11). In the first published study on the measure of PCA3 score in patients with suspicion of PCa, (serum PSA >3 ng/ml), up to 37% of the 108 tested patients were diagnosed with chronic prostatitis (12). Their median PCA3 score was not different from that of men with normal histology and it was concluded that both kinds of patients could be grouped in a unique population of patients without cancer; similar results were obtained in other studies (13-15).

Recently, a multicentric observational study demonstrated that the Prostate Health Index (PHI) might improve discrimination between men with and without PCa at initial biopsy, potentially lowering the rate of unnecessary biopsies (16). Lazzeri *et al*. reported lower levels of [-2]proPSA (p2PSA), an isoform of PSA in serum, and of its derivatives, namely p2PSA over fPSA (%p2PSA) and PHI in chronic prostatic histological inflammation, compared to those measured in patients with PCa; conversely, no difference was observed between inflammatory pattern and benign prostatic hyperplasia (BPH) (17).

The aim of the present study was to investigate the possible impact of histological prostatic inflammation on PCA3 score, PHI and %fPSA in men undergoing first prostate biopsy for suspected PCa, considering the remarkable differences among the cost involved in determining each of these biomarkers.

Patients and Methods

Urinary PCA3 score, serum PHI and %fPSA were prospectively tested between January 2012 and January 2014 in 274 consecutive patients scheduled for first biopsy due to high PSA and negative digital rectal exam (DRE) in two different Italian institutions (San Luigi Gonzaga Hospital, Orbassano, and Gradenigo Hospital, Torino, Italy). Among them, 31 patients (11.3%) had a history of chronic prostatitis (pelvic/perineal pain or discomfort, dysuria, urinary frequency, ejaculatory symptoms). Exclusion criteria were: patients with acute bacterial prostatitis in the three months before biopsy, men with previous diagnosis of atypical small acinar proliferation (ASAP), or patients being treated with dutasteride/finasteride. Patients with marked blood protein alterations (normal range in plasma=6-8 g/dl), suffering from haemophilia, or previously polytransfused, were excluded from the study because such conditions may alter the concentration of p2PSA, necessary to calculate the PHI. Patients then underwent transrectal ultrasound-guided biopsy according to a standardized extended scheme: at least 12 biopsy cores were taken from the peripheral portion of the prostate gland (apex, midgland, and base). Biopsy specimens were placed in specific single-core specimen containers, processed and evaluated by an experienced uropathologist. PCa was identified and graded according to the International Society of Urological Pathology definitions (18). Histological chronic prostatic inflammation was pathologically defined as moderate to large infiltration of lymphomononuclear cells, sometimes scattered in the prostate stroma, sometimes building nodular (isolated or confluent) aggregates. Histological acute prostatitis was defined as a granulocyte aggression, sometimes necrotizing, of the prostate gland epithelium.

According to the Italian law, no formal internal ethical committee approval was needed (19).

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* mRNA and *PSA* mRNA copies were counted with a luminometer and the *PCA3* score was calculated as *PCA3*/*PSA* mRNA×1000. Urine samples were considered as non-informative for prostate cells if the number of *PSA* mRNA transcripts detected was<10,000. The PCA3 test was considered negative if the PCA3 score was <35, positive if PCA3 score was ≥35.

Prior to prostate biopsy and to any manipulations (*e.g*. DRE, enema), that might cause a transient increase of biomarkers, blood was drawn to measure the total (t)PSA, fPSA, and p2PSA. These values were used to calculate the standardized PHI score, according to the formula: $p2PSA/fPSA \times \sqrt{tPSA}$. The samples were centrifuged within three hours of the blood draw. The samples were then frozen at −80˚C and centrally processed using an Access 2 Immunoassay System, an automated random-access analyser that performs immunoassays on body fluid samples (Beckman Coulter, Brea, CA, USA).

Statistical methods. Patients characteristics were tested by the Fisher's exact test for categorical variables and by the Mann-Whitney and Kruskal-Wallis tests for continuous ones, using the Wilcoxon test for *post hoc* pairwise comparisons. All results for continuous variables are expressed as the median and range. Univariate and multivariate binary logistic regression models were used to test PCA3 score, PHI and %fPSA (continuous, independent variables) as risk factors for the onset of prostatitis *vs*. PCa, prostatitis *vs*. BPH, and prostatitis *vs*. high-grade prostatic intraepithelial neoplasia (HG-PIN) (dependent variables). Finally, multivariate decision curve analysis (DCA) was used to compare the net benefit of using PCA3 score, PHI and %fPSA against the threshold probability for the onset of prostatitis *vs*. PCa, prostatitis *vs*. BPH, and prostatitis *vs*. HG-PIN (20). All the above analyses were perfomed for the whole patient cohort (274 subjects) and for the 'gray zone' PSA (4-10 ng/ml) cohort (188 individuals).

All reported *p*-values were obtained by the two-sided exact method, at the conventional 5% significance level; the Bonferroni method was used for multiple comparison adjustment. Data were analyzed as of May 2014 by R 3.1.0 (R Foundation for Statistical Computing, Vienna-A, http://www.R-project.org).

Results

The characteristics of the study population are summarised in Table I. The median age was 69 (range=48-87); median total PSA, %fPSA, PCA3 and PHI scores were 7.5 ng/ml (range=0.2-51), 12.8% (3%-36%), 31 (2-227) and 42 (10-205), respectively. One hundred and seven men (39.1%) had cancer.

Table I. *Main patient characteristics*.

	All patients (n=274)	Positive biopsy (n=107, 39.1%)	Normal parenchyma and BPH (n=18, 6.6%)	Acute prostatitis (n=31, 11.3%)	Chronic prostatitis (n=97, 35.4%)	HG-PIN (n=21, 7.6%)	*p*-Value*
Age (years)	69 (48-87)	70 (52-75)	68 (50-81)	71 (53-84)	66 (48-87)	70 (56-81)	
Family history of cancer	2 (0.7%)	2	-	-	-	-	
History of chronic prostatitis	31 (11.3%)	6 (19.3%)	5 (16.2%)	7 (22.6%)	6 (19.3%)	7 (22.6)	
Biopsy result	274	107 (39.1%)	18 (6.5%)	31 (11.3%)	97 (35.4%)	21 (7.7%)	
Serum total PSA (ng/ml)	7.5 (0.2-51)	8 (3.9-46)	6.3 (3.8-18.5)	7.1 (3.2-51)	6.5 (1.3-51)	7 (0.2-20.6)	0.457
%fPSA	12.8 (3-36)	14 (4-29.9)	10.8 (3.8-37)	11.5 (3-28)	12 (4-36)	12 (4-31)	0.999
PCA3 score	31 (2-227)	47 (7-227)	25.5 (2-95)	20 (2-167)	23 (2-149)	34 (5-98)	0.035
PHI value	42 (10-205)	52 (20.6-204.6)	30 (10-60)	45 (22-140)	35 (10-132)	42 (10-184)	0.056

%fPSA: percentage free prostate-specific antigen; PCA3: prostate cancer antigen 3; PHI: Prostate Health Index; BPH: benign prostatic hyperplasia; HG-PIN: high-grade prostate intraepithelial neoplasia. *Comparison of the four groups with negative biopsy.

Table II. *Univariate and multivariate binary logistic regression models for all 274 patients and for 188 with 'gray zone' Prostate-Specific Antigen (PSA) (4-10 ng/ml)*.

	Prostatitis *vs*. cancer		Prostatitis *vs*. BPH		Prostatitis *vs*. HG-PIN	
Cohort	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
All 274 patients						
Univariate models						
%fPSA	0.94 (0.90-0.98)	0.009	0.99 (0.92-1.08)	0.876	0.99 (0.93-1.08)	0.898
PCA3	0.96 (0.95-0.97)	<0.001	0.99 (0.98-1.02)	0.613	0.98 (0.96-0.99)	0.008
PHI	0.96 (0.94-0.97)	<0.001	1.06 (1.01-1.12)	0.030	0.98 (0.96-1.00)	0.042
Multivariate models						
%fPSA	0.94 (0.89-0.99)	0.023	1.00 (0.92-1.09)	0.957	0.99 (0.92-1.07)	0.840
PCA3	0.97 (0.95-0.98)	<0.001	0.99 (0.96-1.01)	0.261	0.98 (0.96-1.00)	0.032
PHI	0.96 (0.95-0.98)	<0.001	1.06 (1.01-1.12)	0.021	0.99 (0.97-1.01)	0.254
Accuracy	84.1%		66.0%		69.7%	
Sensitivity	81.3%		64.8%		75.0%	
Specificity	69.2%		55.6%		61.9%	
Gray zone PSA patients						
Univariate models						
%fPSA	0.94 (0.89-0.99)	0.024	1.08 (0.97-1.23)	0.213	1.00 (0.91-1.11)	0.969
PCA3	0.96 (0.94-0.97)	<0.001	0.99 (0.96-1.01)	0.239	0.98 (0.96-1.00)	0.054
PHI	0.93 (0.91-0.96)	<0.001	1.06 (1.00-1.14)	0.083	1.00 (0.96-1.05)	0.999
Multivariate models						
%fPSA	0.92 (0.85-0.99)	0.027	1.12 (0.99-1.30)	0.091	1.00 (0.91-1.12)	0.917
PCA3	0.96 (0.95-0.98)	<0.001	0.96 (0.93-1.00)	0.025	0.97 (0.95-1.00)	0.027
PHI	0.94 (0.91-0.97)	<0.001	1.08 (1.02-1.16)	0.012	1.02 (0.98-1.07)	0.346
Accuracy	89.9%		75.4%		62.3%	
Sensitivity	84.6%		71.4%		78.0%	
Specificity	82.9%		69.3%		57.1%	

%fPSA: Percentage free prostate-specific antigen; PCA3: prostate cancer antigen 3; PHI: Prostate Health Index; BPH: benign prostatic hyperplasia; HG-PIN: high-grade prostate intraepithelial neoplasia; OR: odds ratio; 95% CI: 95% confidence interval.

In this group, an inflammatory pattern was associated in 36.4% (chronic prostatitis in the majority of cases). Among the 167 patients with a negative biopsy (60.9%), 18 had normal parenchyma or BPH (6.6%), 31 acute prostatitis (11.3%), 97 chronic prostatitis (35.4%), and 21 HG-PIN (7.6%), multifocal in four cases. Most frequently, acute and chronic inflammatory pattern was associated with BPH (66.8% and 84.7% respectively), seldom with HG-PIN (5.4% *vs*. 8.3%).

The median PCA3 score was significantly different between men with a negative *versus* those with positive

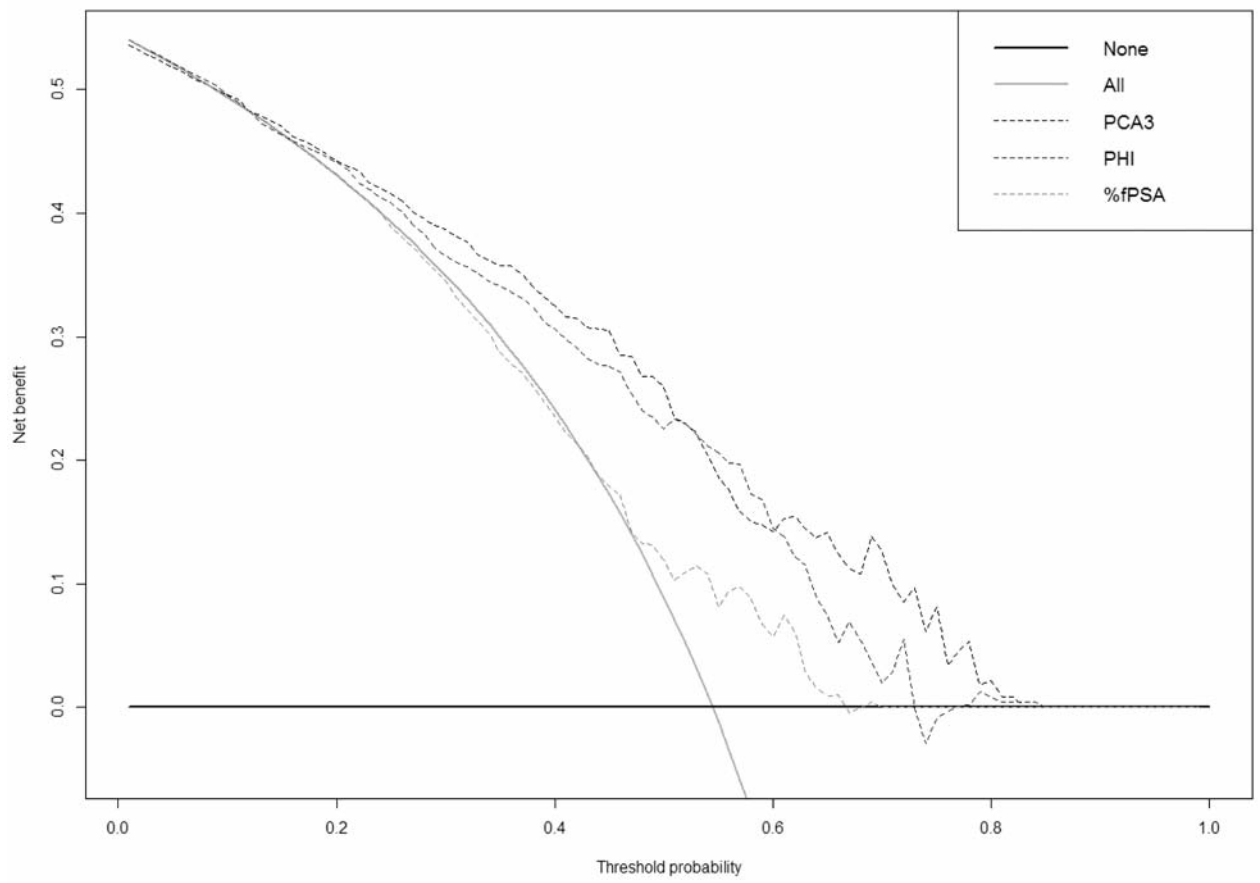

Figure 1. *Decision curve analysis (all patients) for percentage free prostate-specific antigen (%fPSA), prostate cancer antigen 3 gene (PCA3) score and Prostate Health Index (PHI) in the diagnosis of prostatitis vs. cancer.*

biopsy (25 *vs.* 47, $p<0.001$), as for PHI (37.1 *vs.* 52, $p<0.001$) and %fPSA (12% *vs.* 14%, $p=0.001$).

Out of 107 patients with a positive biopsy, 21 had a PCA3 score ≤35 (19.6%); conversely, 6/18 men with a negative biopsy (normal parenchyma or BPH) had a PCA3 score >35 (33.3%), as did 9/31 with acute prostatitis (29%), 28/97 with chronic prostatitis (28.8%), and 9/21 with HG-PIN (42.8%). Twenty out of 107 of patients with a positive biopsy had a PHI ≤40 (18.7%); conversely, 5/18 men with a negative biopsy (normal parenchyma or BPH) had PHI >40 (27.7%), as did 24/31 with acute prostatitis (77.4%), 61/97 with chronic prostatitis (62.8%), and 16/21 with HG-PIN (76.1%).

Among 274 patients, 188 had a PSA between 4 and 10 ng/ml; 70 patients had prostate cancer (37.2%). In this 'gray zone' PSA cohort, the median PCA3 score, PHI and %fPSA were significantly different in men with a negative *versus* those with a positive biopsy: 24 *vs.* 53 ($p<0.001$), 34.8 *vs.* 52 ($p<0.001$), 11.7% *vs.* 13.5% ($p=0.007$), respectively.

Table II reports the univariate and multivariate binary logistic regression models (prostatitis *vs.* cancer, *vs.* BPH, *vs.* HG-PIN) for PCA3 score, PHI and %fPSA in all patients and in the 'gray zone' PSA cohort; accuracy, sensitivity and specificity for each multivariate model are also reported. In multivariate binary logistic model predicting the onset of cancer, all three biomarkers were important risk factors, both for the whole population [PCA3 score and PHI ($p>0.001$), %fPSA ($p=0.021$)] and for those with 'gray zone' PSA [PCA3 score ($p>0.001$), PHI ($p>0.001$), and %fPSA ($p=0.017$)].

For the overall population, the predictors for the onset of prostatitis *vs.* PCa were PCA3 score, PHI and %fPSA; for prostatitis *vs.* BPH, PHI; and for prostatitis *vs.* HG-PIN, PCA3 score.

In the 'gray zone' PSA cohort, the determinants for the onset of prostatitis *vs.* PCa were PCA3 score, PHI and %fPSA; for prostatitis *vs.* BPH, PCA3 score and PHI; and for prostatitis *vs.* HG-PIN, PCA3 score.

Figures 1 (all patients) and 2 ('gray zone' PSA patients) show the DCA for PCA3 score, PHI and %fPSA in the diagnosis of prostatitis *vs.* PCa. When estimating prostatitis *vs.* PCa, the net benefit of using PCA3 and PHI was comparable

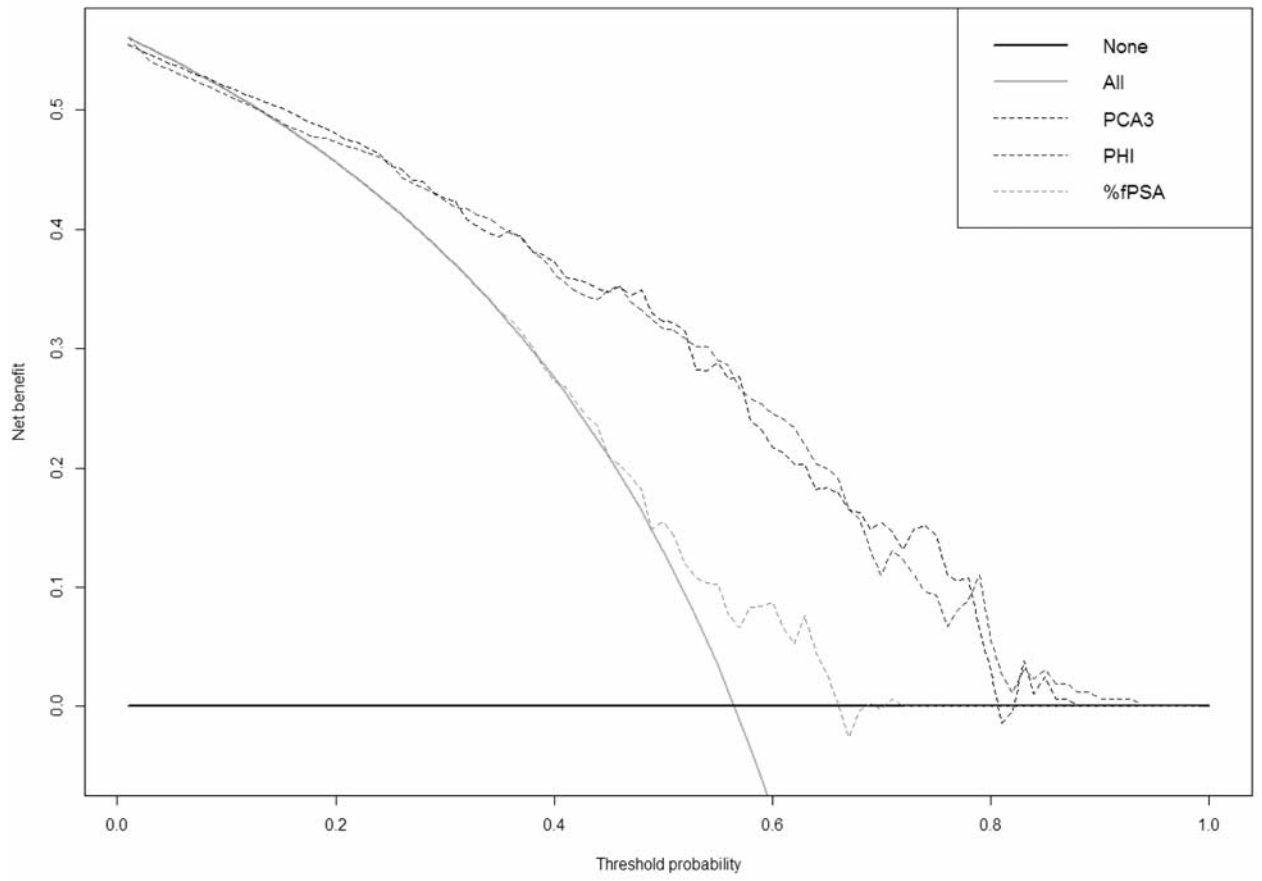

Figure 2. *Decision curve analysis ("gray zone" prostate-specific antigen [PSA] patients only) for percentage free prostate-specific antigen (%fPSA), prostate cancer antigen 3 gene (PCA3) score and Prostate Health Index (PHI) in the diagnosis of prostatitis vs. cancer.*

and extended to a wide range of threshold probabilities (20-80%), while that from using %fPSA was lesser and limited to a small range of threshold probabilities (30-45%).

No net benefit was found when trying to estimate prostatitis *vs*. BPH and *vs*. HG-PIN. A comparable pattern was reported for the whole population and for the gray-zone PSA cohort. Comparing men with acute inflammatory pattern (31 patients) *versus* those with a chronic one (97 patients), there was no difference in median PCA3 score (20 *vs*. 21, p=0.985) or %fPSA (12% *vs*. 12%, p=0.385), conversely there was for PHI (44 *vs*. 34.7, p=0.002). Finally, in patients with PCa associated with histological evidence of prostatitis, the inflammatory pattern (chronic prostatitis in the majority of cases) did not influence the biomarker results.

Discussion

The incidence of asymptomatic prostatitis is much higher than symptomatic prostatitis. This is supported by the fact that inflammatory cells were found in the prostate biopsy, or leukocytes were found in semen analysis from patients without a history of prostatitis (21). Histological evidence of chronic prostatitis is reported in literature in up to 78% of surgical specimens (22); granulomatous types (idiopathic, tuberculosis-related; BCG induced, others) should be considered separately.

Simardi demonstrated that the presence of inflammation in >20% of prostate glands might be responsible for a significant increase of serum PSA levels (23). In a series of 162 cases of surgically-resected hyperplastic prostates, the incidence of inflammation was 98.1% (24). Along with direct trauma (*e.g*. biopsy, cystoscopy), acute and chronic prostatitis are the most common causes of a sudden, marked increase in serum PSA levels (25-26). Mild or moderate inflammation may be sufficient to alter cellular integrity and cause the leakage of PSA into the serum.

Histological inflammation is commonly found among asymptomatic men, biopsied as a result of an elevated PSA level. In the present study, about 77% of patients with negative biopsy had an acute or chronic inflammatory pattern (only 11.3% had a history of chronic prostatitis).

Urologists often manage asymptomatic men with a high serum PSA level by observation after antibiotic treatment. Investigating PSA management patterns by non-urologist providers, Lin *et al*. reported a 30% rate of empirical antibiotic use (25). However, the evidence supporting such practice is limited; antibiotics may alter the course of bacterial prostatitis but the aetiology is non-bacterial in 90% of patients with symptomatic prostatitis and in almost all instances of asymptomatic prostatitis (26). Thus, antibiotics are not likely to affect PSA levels in asymptomatic men with elevated PSA, as reported by Scardino (27).

The cause of chronic prostatic inflammation, as well as its putative role in carcinogenesis, remains unclear: previous studies have found both positive (28) and negative (29) associations between inflammation and the incidence of PCa. The association between inflammation and circulating PSA levels strengthens the biological plausibility of a link between inflammation and cancer, although it also confounds studies investigating this topic (30).

The PCA3 assay test was introduced to select candidates for prostate biopsy. At the cellular level, PCA3 specificity for cancer is very good, due to the gross overexpression of the gene by cancer cells. PCA3 is overexpressed in up to 95% of all prostate carcinomas tested and is expressed 60- to 100-fold less in non-cancerous tissues compared to cancerous tissues (1, 9-10).

Recent studies have demonstrated that serum isoform p2PSA and its derivate PHI could be valid tools for discriminating between men with and those without PCa, avoiding overdiagnosis and overtreatment (16-17). Indeed, %p2PSA and PHI were found to be among the strongest predictors of PCa at initial and repeat biopsy, showing a better accuracy than the currently used tests (tPSA, %fPSA, and PSA density).

In a prospective study aiming to evaluate the effect of chronic prostatitis on PCA3 score, the score was found to be <35 in all patients with a negative biopsy. This suggests that the PCA3 score could be a valuable tool in patients with raised PSA levels and suspicion of chronic prostatitis/BPH, to distinguish those patients who will benefit from prostate biopsy (12). According to Vlaeminck-Guillen *et al*., increased PCA3 score in these patients is unlikely related to chronic prostatitis/BPH, and represents an additional reason for performing a biopsy (15).

In this research, the median PCA3 score, PHI and %fPSA varied significantly in men with a negative *versus* those with a positive biopsy. In patients with PCa associated with histological evidence of prostatitis, the inflammatory pattern (chronic prostatitis in the majority of cases) did not influence on biomarker results. In the histological prostatitis sub-group, PHI significantly varied in men with acute *versus* those with chronic prostatitis, but not in the 'gray zone' PSA cohort; on the contrary, the median PCA3 score and %fPSA were not significantly different.

Lazzeri *et al*. demonstrated that p2PSA, %p2PSA, and PHI values were able to discriminate between chronic histological prostatic inflammation and PCa, but not between chronic prostatitis and BPH in patients with a total PSA of 4-10 ng/ml and a normal DRE (17). Conversely, when comparing prostatitis with BPH, we found that only PHI predicted an inflammatory pattern *vs*. BPH in all patients, while PCA3 score did the same only in the 'gray zone' PSA cohort. We observed no significant differences in total PSA and %fPSA values between these two common benign conditions.

Recently, we observed that histologically documented chronic prostatitis and HG-PIN have a PCA3 score similar to those in patients with BPH or normal parenchyma at biopsy (31). At the same time, the PCA3 score was the only determinant for prostatitis *vs*. HG-PIN, while being significantly lower in men with HG-PIN *vs*. Pca.

The clinical benefit of using PCA3 score, PHI and %fPSA for predicting the onset of prostatitis *vs*. other entities was quite different at DCA.

PHI was the main determinant for prostatitis *vs*. BPH, while PCA3 score for prostatitis *vs*. HG-PIN; the former information seems to be clinically negligible, the latter could strengthen the role of PCA3 score in the oncogenetic pathway.

When estimating prostatitis *vs*. PCa, the net benefit of using PCA3 and PHI was comparable and extended to a wide threshold probability; even %fPSA had good diagnostic performance, being a faster and cheaper marker.

All these patterns were mostly comparable for both PSA cohorts (all patients and 'gray zone').

A second trial, investigating these markers' behaviour at repeat biopsy, is actually ongoing. These results will be decisive to clearly elucidate the biomarkers role in differentiating histological inflammation from PCa and other non-neoplastic lesions.

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