JBUON 2019; 24(5): 2107-2113
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Impact of total PSA and percent free PSA in the differentiation of prostate disease: a retrospective comparative study implicating neoplastic and non-neoplastic entities

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Summary

Purpose: *To evaluate the potential of prostate cancer detection on the basis of prostate-specific antigen (PSA)-level and percent free PSA (% fPSA) according to the outcome of prostate needle biopsy.*

Methods: *This was a retrospective study of 1040 patients that underwent a prostate biopsy in the Urologic Clinic of the University Hospital of Ioannina, Greece. The patients underwent needle biopsy after abnormal finding in digital rectal examination (DRE). Tissue samples were extracted using a 12-core TRUS-GB. The patients were divided into four groups according to the biopsy outcome. Total serum and free PSA were measured.*

Results: *The mean PSA concentration of cancer versus non-cancer groups was significantly higher (p<0.05). The positive predictive value (PPV) of PSA for serum concentration >10 ng/ml was 47% while the negative predictive value (NPV) in patients with PSA levels <4 ng/ml was 81%. The diagnostic accuracy of % fPSA for patients with PSA level between 4-10 ng/ml was 0.651 (95% CI, 0.549-0.754) (p<0.05). A statistically significant difference in mean PSA concentration was recorded between prostate cancers classified as grade 2 (3+4=7) and 3 (4+3=7) and grade 4 (8) and 5 (9-10) (p<0.05).*

Conclusions: *Though informative and suggestive, PSA and % fPSA are not definitive for cancer or non-cancer determination. The differentiation of PSA level between tumours classified as grade 2 (3+4=7) and grade 3 (4+3=7) could support the determination of treatment by backing pathologist's interpretation of the histological diagnosis.*

Key words: *biomarkers, biopsy, free to total PSA ratio, prostate specific antigen, prostatic neoplasms, prostatic intraepithelial neoplasia*

Introduction

Worldwide prostate cancer accounts for 15% of cancers diagnosed in 2012. The incidence of prostate cancer differs significantly between countries and is higher in countries where the practice of cancer screening with PSA is common (such as Western and Northern Europe and Northern America) [1]. According to the most recent estimates of cancer statistics in Europe, the highest incidence

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Received: 27/02/2019; Accepted: 30/03/2019

rate of prostate cancer is in Norway (193.2) with a lower mortality rate (32.0), whereas in Greece incidence and mortality rates are 34.2 and 17.7, respectively [2]. Annual mortality rates of prostate cancer in Greece increased from 1975 to 2003, from about 12% to 20% [3] despite the introduction of PSA screening in 1996 [4].

The use of PSA for prostate cancer screening was approved in 1994 by the US Food and Drug Administration [5]. Repeated PSA levels >4 ng/ml or abnormal digital rectal examination (DRE) examination indicate the necessity of a transrectal ultrasonography-guided needle biopsy (TRUS-GB) for diagnosis [6]. The presence of prostate malignancy is not the only cause of elevation in serum PSA level. Inflammation of the prostate (prostatitis) and benign prostatic hyperplasia (BPH) also raise serum PSA level [7]. Prostatic intraepithelial neoplasia, a pre-cancerous condition, has no effect on the concentration of serum PSA [8]. In a healthy man, the serum PSA level is below 1 ng/ml. When pathological condition develops that causes damage to the prostate tissue, PSA is diffused to the extracellular space and reaches the systematic circulation [9]. In serum, PSA is found in two major forms: bound to alpha-1 antichymothrypsin and free (inactive form) [10]. Thus, the measurement of total serum PSA refers to both forms. The measurement of free form of PSA is used to calculate %fPSA, a diagnostic index for the detection of prostate cancer, especially in the "grey zone" ranging from 4 to 10 ng/ml [11].

Recently the International Society of Urological Pathology (ISUP) proposed a new grading system for prostate cancer [12] that was accepted for the 2016 edition of WHO Pathology and Genetics: Tumours of the Urinary System and Male Genital Organs [13]. The new system was designed to resolve the deficiencies encountered by the Gleason system.

In Greece, PSA and %fPSA are the two most commonly used diagnostic tools in clinical practice for prostate cancer screening. Recently the ISUP grading system was adopted and implemented. In view of the above, we conducted a study to investigate the relationship of serum PSA level and %fPSA with the: a) histological results of 12-core TRUS-GB and b) prostate cancer biopsies categorized according to the ISUP grading system.

Methods

We performed a retrospective study of 1,040 patients that visited the Urology Clinic of the University Hospital of Ioannina from January 2014 to August 2017. The patients underwent needle biopsy after abnormal findings in DRE. Tissue samples were extracted using a 12-core TRUS-GB. The patients were divided into four groups according to the biopsy outcome and specifically: a) patients diagnosed with prostate cancer, b) patients diagnosed with low-grade prostatic intraepithelial neoplasia (LGPIN), c) patients diagnosed with high-grade prostatic intraepithelial neoplasia (HGPIN) and finally d) patients with negative biopsy who were further classified into four categories: absent (no inflammation), mild, moderate and severe prostatitis. Total serum and free PSA levels were measured at the Medical Laboratory of the University Hospital of Ioannina with an automated chemiluminescent Access Hybritech PSA and free PSA assay (immunoassay system UniCel DxI 800, Beckman Coulter).

Each patient underwent 12-core biopsy performed by a trainee or an experienced urologist. Tissue specimens were collected, stained and analyzed by the Department of Pathology. Biopsies were graded according to the Contemporary Prostate Cancer Grading System [14]. Age, PSA, %fPSA and prostate cancer grade score were compared. The PSA levels were divided into ranges of <4.0 ng/ml, 4.0-10.0 ng/ml and >10.0 ng/ml. The cutoff value for the %fPSA ratio to distinguish prostate carcinoma was set at 19%.

Statistics

Data distribution showed that none of the recorded variables (PSA concentration and %fPSA) met the criteria of normal distribution and non-parametric tests were used in the analysis (Mann-Whitney U test and Kruskal-Wallis test). The diagnostic value of PSA and %fPSA ratio was evaluated by receiver operating characteristic (ROC) curve analysis. Results with $p<0.05$ were

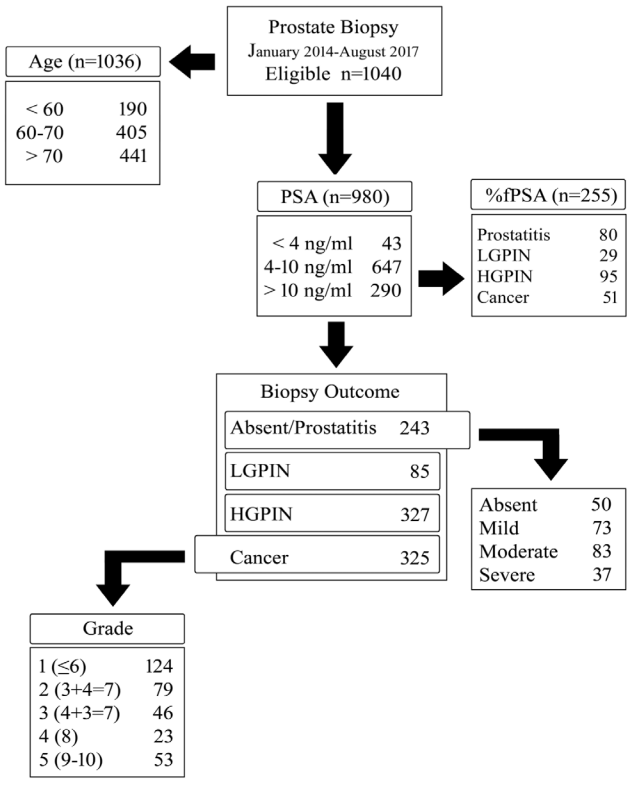

Figure 1. Outline of the study population.

considered statistically significant. Analysis of data was conducted using SPPS version 20.0 (SPSS Inc., Chicago, USA).

Results

The outline of the study population is presented in Figure 1. From the 1,040 medical records, PSA measurement was found in 980 files. The mean age of the men was 69±9 years (range 43-95). Forty-three patients had a PSA lower than 4 ng/ml and 937 greater. Prostate cancer was diagnosed in 325 patients (33%). Two hundred and fifty-five cancer patients had a serum-free PSA measurement in their medical records that allowed us to estimate %fPSA. Most of the patients had PSA concentration between 4 and 10 ng/ml (647 patients - 66%). The mean serum PSA level was 13.2±25.2 ng/ml (range 0.90-450). Analysis of the data showed that cancer patients had significantly higher mean PSA levels compared to non-cancer patients ($p<0.05$) (Table 1). The histological diagnosis was not correlated with the changes in the mean value of %fPSA. Patients with prostate cancer had lower but not statistically significant different values of %fPSA compared to non-cancer patients (Table 1).

Tumours classified as grade 3, 4 and 5 accounted for 38% of all cancers identified in the patient population. The mean PSA level was escalated and it was correlated with prostate cancer grade ($p<0.05$) (Table 2). Analysis of the data also revealed a statistically significant difference between the mean PSA value of prostate cancers classified as grade 2 (3+4=7) and 3 (4+3=7) and grade 4 (8) and 5 (9-10) ($p<0.05$). Unfortunately, only 51 out of the 325 cancer patients had a %fPSA recorded in their medical records. In this rather small sample of patients, the PPV of %fPSA for cancer detection>grade 3 was 23% and the NPV 80% (Table 2).

Patients with total PSA in the range of 4.0-10.0 ng/ml and %fPSA<10% had 25-33% risk of prostate cancer depending on age (50-59 and ≥70 years old, respectively). Those who had %fPSA between 11-18% and were over 70 years had the greatest risk for prostate cancer (39%) while those who were between 50 to 59 years had the lowest (6%) (Table 3). The NPV for PSA level<4 ng/ml was 81% and the PPV for PSA level>10 ng/ml was 47%. Percent free PSA<19% as a threshold of malignancy produced a PPV of 22% and NPV of 83%. These values were slightly higher in patients with a PSA level of 4.0-10.0 ng/ml (23% and 86%, respectively). The

Table 1. Characteristics of patients according to the histological diagnosis and a) PSA level, b) %fPSA

PSA (ng/ml)	*Biopsy*	*Age (years)*			*PSA (ng/ml)*			*%fPSA*		
		No.	*Mean*	*Median*	*No.*	*Mean*	*Median*	*No.*	*Mean*	*Median*
0.90-450	Negative[a]	242	67±8	68	243	8.5±5.1	7.5	80	18.5±8	17.5
	LGPIN	85	66±8	65	85	8.2±3.1	8.0	29	18.6±8	17.0
	HGPIN	326	68±9	68	327	10.4±12.5	7.8	95	19.4±8	18.0
	Prostate Cancer	324	72±9	73	325	21.1±40.6	9.3	51	15.8±7	15.0
		977	69±9	69	980	13.2±25.2	8.0	255	18.3±8	17.0
<4	Negative[a]	17	64±11	61	17	2.7±1.0	2.7	9	20.1±16	15.0
	LGPIN	4	63±10	65	4	3.0±1.1	3.3	0	-	-
	HGPIN	14	66±10	68	14	3.2±1.0	3.5	5	19.2±8	17.0
	Prostate Cancer	8	70±4	70	8	2.9±0.7	3.1	3	17.7±9	14.0
		43	65±10	66	43	2.9±1.0	3.2	17	19.4±13	16.0
4-10	Negative[a]	171	66±7	67	172	7.0±1.6	6.8	57	18.8±7	18.0
	LGPIN	64	66±7	65	64	7.2±1.5	7.2	22	18.6±8	18.0
	HGPIN	231	67±7	67	232	7.0±1.7	7.0	65	19.4±9	19.0
	Prostate Cancer	179	69±7	69	179	7.1±1.7	7.0	35	15.1±6	15.0
		645	67±8	68	647	7.1±1.7	7.0	179	18.3±8	18.0
>10	Negative[a]	54	70±7	70	54	15.2±6.7	12.8	13	16.2±6	16.0
	LGPIN	17	66±7	65	17	13.2±1.9	13.0	7	18.6±9	16.0
	HGPIN	81	73±8	73	81	21.4±21.5	15.0	26	17.8±9	16.5
	Prostate Cancer	137	75±9	76	138	40.4±56.9	17.1	13	17.5±8	17.0
		289	73±9	74	290	28.8±42.4	15.0	59	17.5±8	16.0

[a]Absent (no inflammation), mild, moderate and severe prostatitis. PSA: prostate-specific antigen; %fPSA: percent free PSA; LGPIN: low-grade prostatic intraepithelial neoplasia; HGPIN: high-grade prostatic intraepithelial neoplasia

Table 2. PSA level and %fPSA of tumors classified according to the ISUP Contemporary Prostate Cancer Grading System

	Grade				
	1 (≤6)	*2 (3+4=7)*	*3 (4+3=7)**	*4 (8)*	*5 (9-10)**
PSA (ng/ml)					
Mean	12.5±21.7	13.2±15.2	16.6±18.7#	22.4±31.1	56.7±80.7
Median	8	8	11.5	12	25
IQR	4.5	7.9	10.3	12.6	67.6
Age (years)	68.9±9	72.8±9	72.9±9	69.6±9	77.0±8
%fPSA					
Mean	16.3±6.0	15.5±5.0	15.0±8.0	18.0	14.3±12.0
Median	16.0	14.0	12.0	-	13.5
IQR	9	8	13	-	23
Age (years)	68.4±7	75.3±7	70.0±9	61	73.5±4
%fPSA (n)					
< 0.19 (36)	55% (20)	22% (8)	14% (5)	3% (1)	6% (2)
> 0.19 (15)	67% (10)	13% (2)	7% (1)	0% (0)	13% (2)

*indicates significant difference (p<0.05) among the current and the exact previous (lower) grade. PSA: prostate-specific antigen; %fPSA: percent free PSA; IQR: Interquartile range

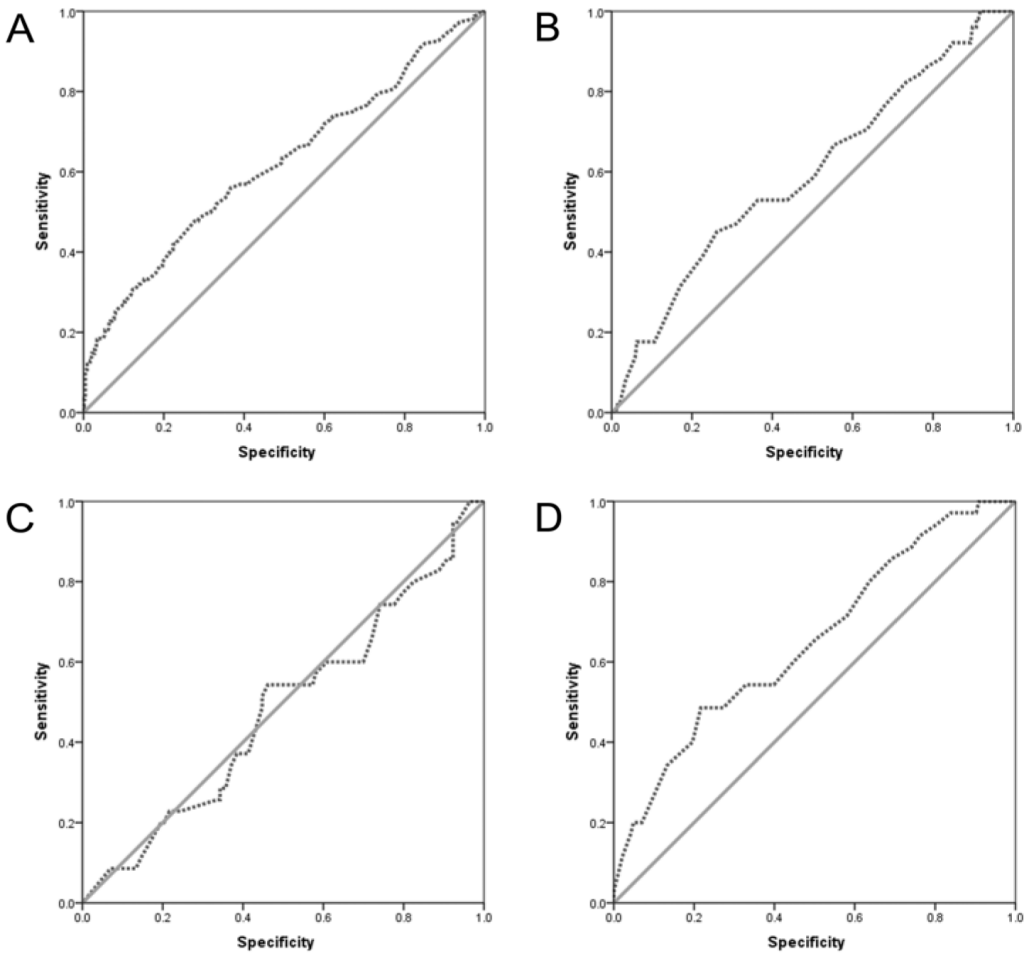

Figure 2. Receiver operating curves of PSA in predicting prostate cancer for patients with PSA 0.90-450 ng/ml **(A)** and PSA 4.0-10.0 ng/ml **(C)**. Receiver operating curves of %fPSA in predicting prostate cancer for patients with PSA 0.90-450 ng/ml **(B)** and PSA 4.0-10.0 ng/ml **(D)**.

diagnostic value of PSA versus %fPSA (PSA range 0.9-450 ng/ml) was higher (0.619 vs 0.597) (Figure 2A and 2B). Compared to PSA the discriminative power of %fPSA was stronger in patients with PSA level of 4.0-10.0 ng/ml. ROC analysis indicated an AUC of 0.651 (95% CI, 0.549-0.754) for %fPSA and 0.482 (95% CI, 0.374-0.590) for PSA (p=0.742) (Figure 2C and 2D). The dispersion of the histological diagnosis according to the PSA level and %fPSA value is presented in Figure 3. The PSA grey zone (4-10 ng/ml) is indicated with dashed vertical lines while the cut-off value of %fPSA (19%) with a dashed horizontal line. Although most of the cancer biopsies were below the %fPSA threshold it was shown that neither the combination of PSA with %fPSA could be indicative of higher malignancy risk (Figure 3).

Table 3. Probability (%) of finding prostate cancer on a TRUS-GB by age in years

%fPSA	*Age group (years)*		
	50-59	*60-69*	*≥70*
≤ 10	25%	33%	25%
11-18	6%	19%	39%
19-25	17%	21%	18%
> 25	NA	NA	6%

NA: not available (no patients between the age of 50 to 69 with a %fPSA measurement). %fPSA: percent free PSA

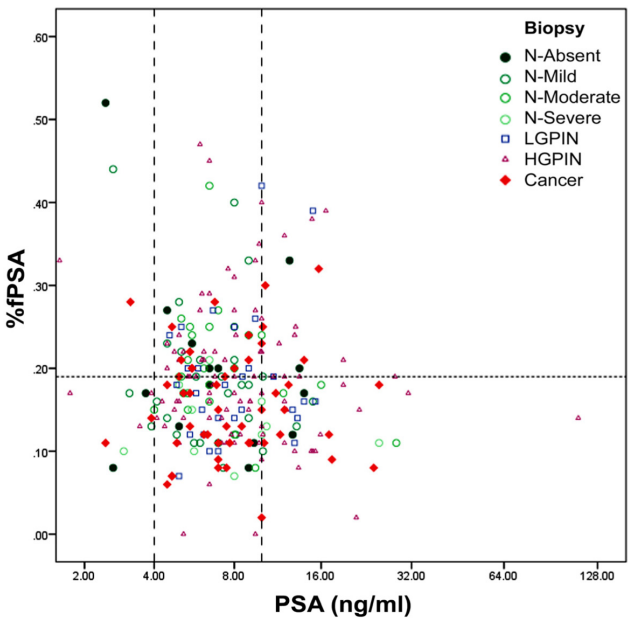

Figure 3. Allocation of histological diagnosis according to PSA level and % fPSA values. PSA: prostate-specific antigen; %fPSA: free:total PSA; LGPIN: low grade prostatic intraepithelial neoplasia; HGPIN: high grade prostatic intraepithelial neoplasia.

Discussion

PSA was introduced as a prostate cancer marker almost 25 years ago. In Greece, serum PSA examination in men suspicious for prostate pathology is commonly used in clinical practice. On the other hand, measurement of free PSA and estimation of %fPSA is applied to a lesser extent. In this study, we analyzed more than a thousand medical records of men living in Western Greece that underwent a prostate biopsy in order to examine the role of total PSA and %fPSA in the differentiation of prostate disease by histological diagnosis.

The AUC analysis (PSA range 0.9-450 ng/ml) showed a higher value for serum PSA (0.619) than for %fPSA (0.597). For patients with PSA range between 4 to 10 ng/ml the AUC was higher for %fPSA (0.482 vs 0.651). Higher results for PSA were recorded in five previous studies by Stephan et al (0.550, PSA range 1.6 to 8.0 ng/ml) [15], Loeb et al (0.527, PSA range 1.6 to 7.8 ng/ml) [16], Fillela et al (0.508, PSA range 2 to 10 ng/ml) [17], Lazzeri et al (0.500, PSA range 2 to 10 ng/ml) [18] and Guazzoni et al (0.530, PSA range 2 to 10 ng/ml) [19]. On the contrary, four of the previous mentioned studies have reported lower AUC for %fPSA than we had in the present study (0.590, 0.490, 0.640 and 0.580) [15,16,18,19] and one higher (0.700) [17]. Compared with our results, we have reported the lowest AUC for PSA (0.482) and the second highest for %fPSA (0.651). Still, our data agree with all the previous studies; AUC values lower than 0.7 are indicative of the poor diagnostic ability of PSA and %fPSA for prostate cancer screening. To further evaluate the screening success of %fPSA in the men with PSA levels between 4 and 10 ng/ml (grey zone) we calculated a PPV of 23% and an NPV of 86%. In Turkey, a study reported a PPV of 29% for %fPSA (cut-off value was set at 20%) in men with PSA level within the grey zone [20] while in indigenous West African men the PPV was 34% (for the same PSA range and cut-off value) [21].

According to Alexander et al elevated serum PSA concentration is not associated with HGPIN [8]. Percent free PSA in men with HGPIN is higher than in men with prostate cancer and similar to men with benign prostate hyperplasia [22]. Regardless of PSA concentration African American men with a history of HGPIN have increased risk for prostate cancer [23]. Our results also confirmed that prostate intraepithelial neoplasia (high and low) had no effect on PSA level or %fPSA. We found a statistically significant difference between the mean PSA levels of cancer biopsies classified as grade 2 (3+4=7) and 3 (4+3=7) and grade 4 (8) and 5 (9-10). To the best of our knowledge, this is the

first study to report a difference in PSA level between grade 2 (3+4=7) and 3 (4+3=7) (Gleason score 7). This finding supports the motion for the use of the new Grading System over the Gleason score since grade 2 (3+4=7) vs 3 (4+3=7) and grade 4 (8) vs 5 (9-10) have very different prognoses. In our population, men with grade 2 tumours seemed to produce less PSA compared to men with grade 3 tumours. The different categorizations (≤6, 3+4=7, 4+3=7, 8, and 9–10) of tumour grade [24] have a significant prognostic impact and there is a profound difference in survival time between grade 2 (3+4=7) and 3 (4+3=7) [25]. Yang et al showed that mortality rates are lower within Gleason grade categories 6 and 7(3+4) compared to men with Gleason grade 7(4+3), or higher than 8 [26]. Classification of tumour grade is a very important step as it helps determine the proper treatment according to specific guidelines. The differentiation in PSA levels, especially between grade 2 and 3, could attribute a more accurate interpretation of biopsies by a pathologist and contribute to the reduction of interobserver variability.

In conclusion, our findings showed that both PSA and %fPSA were weakly correlated with prostate malignancy and thus were not definitive for cancer or non-cancer determination. Prostate cancer biopsies classified as grade 3 (4+3=7) presented higher PSA levels than those classified as grade 2 (3+4=7). Although the diagnostic value of PSA for prostate cancer screening is limited, its ability to detect aggressive tumours (>grade 3) should be exploited.

Conflict of interests

The authors declare no conflict of interests.

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