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PSA-based parameters and their diagnostic performances in patients with prostate cancer and benign prostatic hyperplasia

[Prostat kanserli ve benign prostat hiperplazili hastalarda PSA bazlı parametreler ve tanısal performansları]

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ABSTRACT

Objective: The aim of this study is to evaluate the diagnostic performance of PSA-based parameters in prostate cancer (PCa) and benign prostatic hyperplasia (BPH) and to determine the relation between serum PSA and histopathological grade in PCa. **Methods:** This retrospective study includes data of 320 patients with PCa (n: 155) and BPH patients (n: 165). Serum PSA levels and Gleason scores of patients were determined by examining the records of Clinical Biochemistry and Pathology Laboratory. We classified the patients according to total PSA (tPSA) levels to determine diagnostic performance of PSA-based parameters at different cut-off levels. Serum tPSA, free PSA (fPSA) and complexed PSA (cPSA) were analyzed with chemiluminometric method. **Results:** There were significant differences between BPH and PCa patients in tPSA, fPSA, cPSA and f/tPSA values ($p<0.05$) in whole group (WG). There were significant differences between BPH and PCa patients in cPSA and f/tPSA in group with tPSA<4 ng/mL (LG); in f/tPSA values in group with tPSA 4-10 ng/mL (intermediate group, IG). According to histopathological classification, all of the parameters except f/tPSA were significantly different between groups in PCa ($p<0.001$). Significant positive correlations were found between Gleason scores and tPSA ($r=0.577$), fPSA ($r=0.491$) and cPSA ($r=0.562$) ($p<0.001$). **Conclusion:** We suggest the use of f/tPSA to improve the differentiation of BPH and PCa in IG. The best cut-off points for tPSA, fPSA, cPSA and f/tPSA were 4.0, 2.21, 3.16 ng/mL and 0.17 respectively. Based on the results of ROC analysis, a cut-off value of 0.17 for f/tPSA and 3.16 ng/mL for cPSA may be acceptable.

Key Words: Prostate cancer, BPH, PSA, diagnostic performance

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı, Prostat kanseri (PCa) ve benign prostat hiperplazisinde (BPH) PSA bazlı parametrelerin tanısal performanslarını değerlendirmek ve PCa' lı hastalarda serum PSA değerleri ile histopatolojik grade arasındaki ilişkiyi belirlemektir. **Yöntemler:** Bu retrospektif çalışmaya PCa (n: 155) ve BPH' lı (n: 165) toplam 320 hastanın sonucu dahil edildi. Hastaların serum PSA düzeyleri ile Gleason skorları Klinik Biyokimya ve Klinik Patoloji Laboratuvarı kayıtları incelenerek belirlendi. PSA-bazlı parametrelerin farklı cut-off değerlerindeki tanısal performanslarını belirlemek için, hastalar total PSA (tPSA) değerlerine göre sınıflandırıldı. Serum tPSA, free PSA (fPSA) ve kompleks PSA (cPSA) düzeyleri kemilüminesans yöntem ile ölçüldü. **Bulgular:** Hastaların tümü değerlendirildiğinde, PCa ve BPH gruplarının tPSA, fPSA, cPSA ve f/tPSA değerleri arasında anlamlı farklılık tespit edildi ($p<0.05$). Ek olarak, tPSA<4 ng/mL olan grupta (LG) cPSA ve f/tPSA' nın, tPSA' sı 4-10 ng/mL olan grupta (IG) f/tPSA değerlerinin anlamlı olarak farklı olduğu görüldü ($p<0.05$). PCa' da histopatolojik sınıflandırma yapıldığında, gruplar arasında f/tPSA dışında bütün parametrelerde anlamlı farklılık olduğu gözlendi ($p<0.001$). Gleason skorları ile tPSA ($r=0.577$), fPSA ($r=0.491$) ve cPSA ($r=0.562$) değerleri arasında anlamlı pozitif korelasyon tespit edildi ($p<0.001$). **Sonuç:** Sınır gruptaki PCa ve BPH hastalarının ayırıcı tanısında f/tPSA oranının kullanılması önerilebilir. ROC eğrisi analizinden tPSA, fPSA, cPSA ve f/tPSA için elde edilen en iyi kestirim değerleri sırasıyla 4.0, 2.21, 3.16 ng/mL ve 0.17 idi. Bu bulgulara dayanarak PCa ile BPH' nın ayırıcı tanısında kestirim değerleri olarak f/tPSA için 0.17, cPSA için 3.16 ng/mL kabul edilebilir.

Anahtar Kelimeler: Prostat kanseri, BPH, PSA, tanısal performans.

Çıkar Çatışması: Yazarlar hiçbir çıkar çatışması bulunmadığını beyan eder.

Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer death among men. The lifetime risk of developing PCa for men is 1 in 6. Since most risk factors for this disease cannot be modified, efforts to reduce mortality related to PCa have focused on early recognition and treatment [1].

Prostate-specific antigen (PSA), serin protease produced by ductal and acinal epithelial cells of normal, hyperplastic, and malignant tissue of the prostate [2]. Because of its high specificity for prostate tissue, PSA is the preferred serum marker for PCa. Although, PSA is specific for prostate tissue not for PCa. For that reason it can also be found in abnormal concentrations in the benign changes of the prostate such as BPH and other non-neoplastic prostatic lesions. The usefulness of PSA as an early marker of prostate cancer by itself is questionable, owing to the overlap in PSA values seen in patients with BPH and in those with organ-confined prostate cancer [3].

When the conventional tPSA cut-off of 4 ng/mL is used as the discrimination limit between cancer and non-malignant prostatic diseases, the false-positive rate is 65 % because increased serum PSA concentrations are also found in benign prostatic hyperplasia (BPH) and inflammatory prostatic diseases. However, the differentiation between BPH and PCa can be improved by determination of the serum PSA isoforms (total, complex, free PSA) [4].

Serum PSA exists in various molecular forms: approximately 70-90% of total PSA (tPSA) is bound to α1-antichymotrypsin, and smaller amounts are bound to α1-antitrypsin and protein C. Serum free PSA (fPSA) accounts for 10-30% of tPSA. A lower ratio of fPSA to tPSA (f/tPSA) in patients with PCa has been found in several studies and this ratio appears to be a helpful tool for distinguishing PCa and BPH. Recently, an assay for PSA binding to serum proteins except α2 macroglobulin -complexed PSA (cPSA)- was developed. However, there was not any difference between c/tPSA and f/tPSA [5, 6].

It's known that, the gold standard method for diagnosis of PCa is histopathological evaluation. In PCa evaluation there are a few systems used for estimation of tumor cells differentiation i.e. histopathological grade of tumor. Gleason's system is nowadays one of the most used grade systems in PCa. The base of Gleason's system [7] is represented by five histological figures, by using small microscopic magnification, encompass analysis of gland architectonics, the degree of glandular differentiation as well as stromal invasion, but not the degree of nuclear anaplasia.

We performed this study with following aims: *(a)* to evaluate the diagnostic performance of tPSA, cPSA, fPSA and f/tPSA in PCa and BPH and *(b)* to determine the relation between serum PSA and histopathological grade in PCa.

Materials and Methods

We retrospectively evaluated 165 patients with histologically confirmed BPH (mean age, 70.0±8.6 year), and 155 patients with PCa (mean age, 73.4±7.3 year) who submitted to Training and Research Hospital, Kayseri, Turkey, between March 2007 and May 2011 from Clinical Pathology Laboratory records. Serum levels of tPSA, fPSA, cPSA and f/tPSA ratios of patients were determined by examining the records of Clinical Biochemistry Laboratory and the Gleason scores of patients with PCa were obtained from the records of Clinical Pathology Laboratory.

We classified the patients according to their tPSA levels to determine diagnostic performance of tPSA, cPSA, fPSA and f/tPSA in PCa and BPH at different cut-off levels; tPSA levels lower than 4 ng/mL defined as low tPSA group (LG), tPSA levels between 4 and 10 ng/mL defined as intermediate group (IG), and tPSA levels higher than 10 ng/mL defined as high tPSA group (HG). Of 320 men (whole group; WG), 138 had tPSA levels lower than 4 ng/mL, 90 had tPSA levels between 4 and 10 ng/mL, and 92 had tPSA levels higher than 10 ng/mL.

Serum tPSA, fPSA and cPSA were measured with the fully automated Advia Centaur (Bayer Health Care, Tarrytown, NY, USA) according to instructions of the manufacturers. This test uses two-site sandwich immunoassay using chemiluminometric technology.

The Gleason scores of PCa patients were recorded and histological grades were classified as low for Gleason scores of 1-4 (well differentiated), medium for scores of 5-7 (moderately differentiated), or high for scores of 8-10 (poorly differentiated).

Statistical analysis

Data were analysed using the statistical software package SPSS 15.0 for Windows (SPSS, Chicago, IL). Intergroup comparisons were made by the independent sample *t*-test. To compare the parameters according to histopathological differentiation the one way ANOVA test was used. Spearman's correlation test was used to explore correlations between histopathological results and other parameters. Receiver operating characteristic (ROC) curves were generated using SPSS for Windows. $p<0.05$ was considered as statistically significant.

Results

This study included 165 patients with histologically confirmed as BPH, and 155 patients as PCa. Table 1 shows ages and serum tPSA, fPSA, cPSA, f/tPSA values of patients with PCa and BPH. All parameters were significantly different between BPH and PCa patients in WG. When we grouped patients according to their tPSA levels, there were significant differences in cPSA and f/tPSA values in the LG; in f/tPSA values in the IG; and in tPSA, fPSA, cPSA, f/tPSA values in the HG between BPH and PCa patients.

Gleason scores of PCa group were low-grade in 33 patients, medium-grade in 74 patients, and high-grade in 48 patients. When the patients were grouped according to their histopathological grades, all of the parameters except f/tPSA ratio were significantly different between groups (Table 2). Positive correlations were found between Gleason scores and tPSA, fPSA and cPSA (r= 0.577, 0.491, 0.562 respectively and $p<0.001$). Correlation coefficient of Gleason scores versus other parameters are shown in Table 2.

The diagnostic validity criteria sensitivity and specificity of tPSA, fPSA, cPSA, and f/tPSA at different decision limits of the ROC curves are shown in Table 3. The best cut-off points obtained from the ROC curve analysis for tPSA, fPSA, cPSA and f/tPSA were 4.0, 2.21, 3.16 ng/mL and 0.17 respectively. AUCs of tPSA, fPSA, cPSA and f/tPSA for all patients were 0.85, 0.74, 0.86 and 0.79 respectively. When we performed ROC analysis only for IG, AUC values of tPSA, fPSA, cPSA and f/tPSA changed to 0.52, 0.69, 0.57 and 0.73 respectively. The ROC curves of f/tPSA and cPSA for WG are shown in Fig. 1.

Discussion

PSA is produced exclusively by the epithelial cells lining the prostatic acini and ducts of prostatic tissue and increased evidence indicates that PSA-based diagnostic parameters and f/tPSA, in addition to tPSA, can improve the sensitivity and specificity of PCa detection [8].

In our patient population, serum tPSA, fPSA, cPSA levels and f/tPSA of PCa and BPH were significantly different in WG and HG; however only f/tPSA was significantly different (p=0.004) in IG. This means, only f/tPSA ratio had a discrimination power for differentiation between PCa and BPH. In addition, f/tPSA ratios were significantly different between BPH and PCa in LG (p=0.029). In accordance with our study, in some studies, f/tPSA ratio appears to be helpful for distinguishing BPH and PCa and, it's suggested to be used to decrease unnecessary biopsies in IG [9, 10]. But, in contrast, Serdar et al. reported that f/tPSA was not an important predictor in IG [8].

Brawer et al. found that cPSA alone was a better discriminator between BPH and PCa than tPSA or the f/tPSA in the range between 4 and 10 ng/mL [11]. According to the suggestions of this author, the determination of cPSA could replace the measurements of the two analytes tPSA and fPSA [11]. But, cPSA strongly correlates with tPSA, a large overlapping range of cPSA concentrations consequently exists between PCa and BPH patients within in IG of tPSA concentrations up to 10 ng/mL.

Clinically applicable reference values for this marker are from 0-4 ng/mL, but they don't point out the absence of carcinoma always. tPSA values higher than 10 ng/mL are interpreted as PCa. Intermediary PSA values, i.e., value interval from 4-10 ng/mL, could be present in patients with BPH, prostatitis, intraepithelial neoplasia as well as PCa [12].

Table 1. tPSA, fPSA cPSA and f/t PSA levels in prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients.

Parameter	WG (mean ± SD) (n=321)			LG (mean ± SD)			IG (mean ± SD)			HG (mean ± SD)		
	PCa (n= 155)	BPH (n=165)	p	PCa (n= 22)	BPH (n= 116)	p	PCa (n= 53)	BPH (n= 37)	p	PCa (n= 80)	BPH (n= 12)	p
Age (year)	73.4 ± 7.3	70.1 ± 8.6	<0.001	70.1 ± 8.0	69.5 ± 8.2	0.74	73.1 ± 7.4	69.8 ± 10.0	0.10	74.6 ± 7.0	76.1 ± 5.5	0.39
tPSA (ng/mL)	34.2 ± 46.7	3.7 ± 3.7	<0.001	2.3 ± 0.9	1.9 ± 1.0	0.09	6.2 ± 1.9	6.1 ± 1.4	0.07	61.6 ± 51.8	14.6 ± 4.6	<0.001
fPSA (ng/mL)	5.4 ± 10.7	1.1 ± 1.3	<0.001	0.5 ± 0.2	0.6 ± 0.2	0.14	1.3 ± 1.0	1.6 ± 0.7	0.05	9.32 ± 13.8	4.01 ± 2.8	0.003
cPSA (ng/mL)	29.6 ± 40.2	2.7 ± 3.0	<0.001	1.8 ± 0.9	1.3 ± 0.8	0.024	4.9 ± 1.6	4.4 ± 1.2	0.10	52.3 ± 43.5	10.6 ± 5.2	<0.001
f/t PSA	0.17 ± 0.1	0.3 ± 0.1	<0.001	0.2 ± 0.1	0.3 ± 0.1	0.029	0.2 ± 0.1	0.3 ± 0.1	0.004	0.14 ± 0.09	0.3 ± 0.2	0.045

WG: Whole group
LG: Low tPSA group, patients with tPSA levels <4 ng/mL
IG: Intermediate group, patients with tPSA levels 4-10 ng/mL
HG: High tPSA group, patients with tPSA levels >10 ng/mL

Table 2. PSA levels in prostate cancer (PCa) patients with different clinical grade (medians and ranges)

Parameter	Low Grade (mean ± SD) (n=33)	Medium Grade (mean ± SD) (n=74)	High Grade (mean ± SD) (n=48)	*p*	r
Age (year)	69.9 ±7.3	75.3 ± 6.6	72.9 ± 7.7	<0.001	0.104
tPSA (ng/mL)	6.8 ± 9.8	25.4 ± 35.0	66.8 ± 58.0	<0.001	0.577[a]
fPSA (ng/mL)	1.05 ± 1.0	4.0 ± 5.9	10.5 ± 16.7	<0.001	0.491[a]
cPSA (ng/mL)	5.7 ± 9.0	23.1 ± 32.2	56.2 ± 49.5	<0.001	0.562[a]
f/t PSA	0.19 ± 0.1	0.18 ± 0.13	0.15 ± 0.1	0.317	- 0.128[b]

p: Group differences computed by one way ANOVA test.
r: Correlation coefficient of Gleason scores versus other parameters
[a]: $p < 0.001$
[b]: $p=0.11$

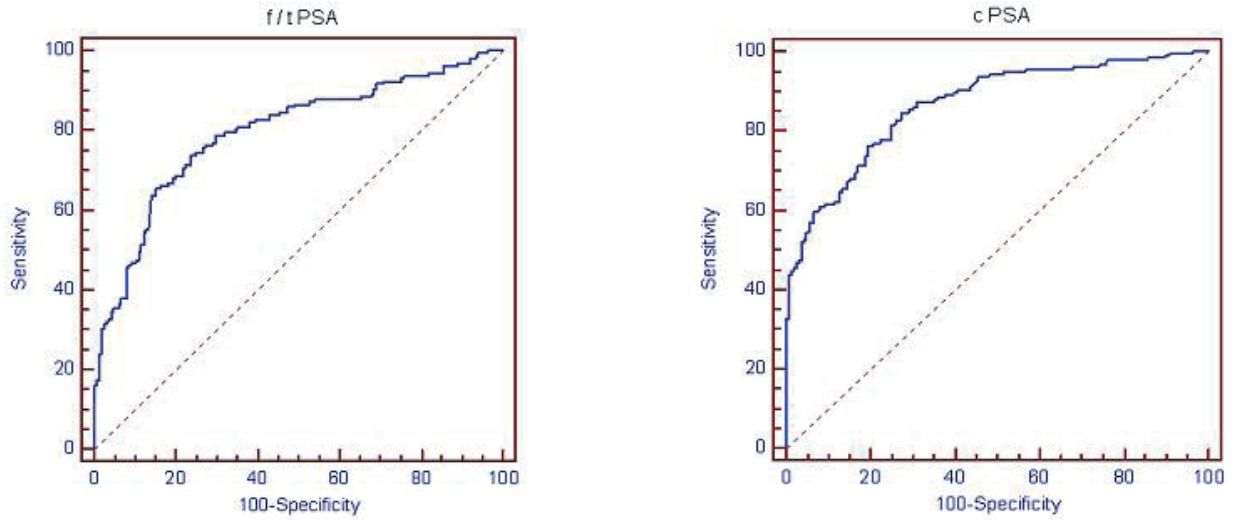

Figure 1. ROC curves of cPSA and f/t PSA in WG

The results of our study showed that tPSA values in patients with PCa are widely ranged, in the interval of reference, intermediary and high values. In our patient population, approximately one third of examined PCa patients (n:53) had serum tPSA levels in the interval of intermediary values, where it was necessary to distinguish whether it was PCa or benign disease, which was only possible to determine by biopsy of prostate. This is one of examples of limited use of tPSA test.

Several authors have reported that cPSA shows better results than tPSA and f/tPSA in the early diagnosis of PCa [11,13,14]. Filella et al. presented that the usefulness of the f/tPSA improves the diagnostic accuracy obtained with tPSA or with cPSA in the differential diagnosis of PCa and BPH [15]. Lein et al. have reported opposing results [16]. Although tPSA levels lower than 4 ng/mL were accepted as the value of secreted from normal prostate tissue, in the present study, 22 patients with PCa had tPSA in the interval of reference. In these patients cPSA and f/tPSA values were statistically different from BPH. Thus, in the differential diagnosis between BPH and PCa, we suggest using of f/tPSA and cPSA. When both tests are used simultaneously, improvement of the differences between patients with BPH and PCa can be obtained, as shown in the study. In accordance with our study, Mutlu et al. reported that, cPSA had higher discriminatory power of in diagnosis of PCa for clinically relevant 2.5-4 ng/mL tPSA range [17].

As mentioned in the literature, in neoplastic processes the increase of serum PSA depends on differentiation of tumor cells. The less differentiated prostate tumors can cause lower PSA concentrations in comparison to those well differentiated [18]. Histopathological grades of these 22 PCa patients were low in 11 and medium grade in 11 patients in this study. Low tPSA values in these patients can be explained by early diagnosis.

We found significant changes in all of the parameters except f/tPSA (p=0.317) in relation to histological score. Also serum tPSA, fPSA, and cPSA levels were positively correlated with grades (r= 0.577, 0.491, 0.562 respectively and p<0.001), except f/tPSA (p=0.11). In accordance with our study, Živković showed that there was a positive correlation between tPSA and Gleason's scores [19]. Similarly, Esen et al. who found positive correlation between PSA levels and Gleason's scores reported that PSA level was reliable indicator of progression of PCa [20]. Meanwhile, some studies [21-23] reported that serum tPSA is not in positive correlation with Gleason's score, which could be explained by the fact that less differentiated tumors sometimes produce less PSA. This could be explained by the loss of phenotype expression of PSA, which follows dedifferentiation of tumor cells [21-23].

In some studies, serum f/tPSA ratio was reported to show significant associations with Gleason score in patients with PCa [24,25]. Pannek et al. reported that f/tPSA ratio was a significant predictor of pathological stage. Serum f/tPSA ≥ 0.15 was a good predictor of organ-confined PCa when used with favorable needle biopsy findings [26]. Catalona et al. found that higher f/tPSA ratios (>0.15), tended to indicate less aggressive disease [27]. But, in our study, we found no correlation between f/t PSA ratio and Gleason's score (r= -0.128, p=0.317).

ROC analysis of our data suggest that use of cut-off value of 0.17 for f/tPSA will be optimum for clinical use to differentiation between PCa and BPH as, sensitivity of 65% and specificity of 84% can be achieved. The best cut-off points obtained from the ROC curve analysis for tPSA, fPSA, and cPSA were 4.0, 2.21, and 3.16 ng/mL respectively (sensitivity and specificity values were given in Table 3). Threshold with the highest diagnostic sensitivity and spesificity were chosen as the best cut-off points. When the highest sensitivities were chosen (3.0, 1.03, 3.16 and 0.21 ng/mL for tPSA, fPSA, cPSA and f/tPSA respectively), deficits based on false positive results can be eliminated.

Comparing the AUC values of tPSA, fPSA, cPSA and f/tPSA for all patients, we observed that tPSA and cPSA have higher AUC (0.85, 0.86 respectively) than f/tPSA (0.79). Our results confirm the data of numerous studies [10,15,28] that the f/tPSA is statistically different between patients with PCa and BPH. Because the diagnostic validity of f/tPSA is not superior to tPSA and cPSA in discrimination of PCa and BPH, we also performed ROC analysis for IG. We observed that, f/tPSA is the most satisfactory parameter compared with the other parameters to distinguish PCa and BPH patients with tPSA 4-10 ng/mL (AUC: 0.73).

Jung et al. performed ROC analysis in PCa and BPH patients with tPSA 2-10 ng/mL, and they found cut-off points for tPSA, cPSA and f/tPSA, as 2.71 ng/mL, 2.60 ng/mL and 0.17 respectively [6]. Serdar et al. reported

Table 3. Diagnostic validity of tPSA, fPSA, cPSA, and f/tPSA to distinguish prostate cancer (PCa) and benign prostate hyperplasia (BPH) patients for whole group

Parameters	Cut-off values	Sensitivity, (%)	Spesificity, (%)
tPSA (ng/mL)	3.0[a]	90	58
	4.0[b,e]	86	70
	4.93[c]	75	75
	7.9[d]	60	90
	10.0[e]	51	92
fPSA (ng/mL)	0.39[a]	90	30
	1.03[c]	65	65
	2.13[d]	50	90
	2.21[b]	50	90.9
cPSA (ng/mL)	2.25[a]	90	59
	3.16[b]	84	72
	3.72[c]	77	77
	5.74[d]	61	90
f/t PSA	0.15[d]	47	90
	0.17[b]	65	84
	0.20[e]	69	78
	0.21[c]	74	74
	0.25[e]	82	61
	0.33[a]	90	31

[a] Threshold with diagnostic sensitivity of 90%.
[b] Threshold with the highest diagnostic sensitivity and spesificity.
[c] Threshold at the point with similar sensitivity and spesificity.
[d] Threshold with diagnostic spesificity of 90%.
[e] Threshold at the conventional reference limits.

that the AUC for f/tPSA and tPSA were 0.69 and 0.65 for all patients [8]. Brawer et al. found that the AUC was 75% for f/tPSA, versus 65% for tPSA [29]. The different results of various studies may be associated with differences in the selection of the patient populations, and problems with the accurate determination of fPSA and tPSA [8].

Problematic results in f/tPSA measurements have been reported due to analytical factors. Nixon et al. showed that the results of different assays are not interchangeable [30]. Clinicians should be aware that different f/tPSA cut-off values need to be used, depending on the particular fPSA and tPSA assays, and that all assays do not have the same diagnostic performance. Different laboratories have different cut-off values, because of analytical problems [8]. Thus, clinicians should be aware cut-off values of their own laboratories.

When we evaluated our patient population, as a result of this study, the differentiation of BPH and PCa in the IG could be improved by f/tPSA, whereas tPSA alone does not have any additional discriminatory power. Also, cPSA can be used for patients with tPSA around 4 ng/mL. When both tests are used simultaneously, maximization of the differences between patients with PCa and BPH can be obtained. Also, tPSA, fPSA, cPSA, f/tPSA values may help us to predict the clinical grade. Based on the results of ROC analysis, for differentiation of BPH and PCa a cut-off value of 0.17 for f/tPSA and 3.16 ng/mL for cPSA may be accepted.

Conflict of Interest

The authors declare no conflict of interest.

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