

Performance of total prostate specific antigen and free prostate specific antigen ratio for screening prostate cancer in a Turkish population

BURCU BARUTÇUOĞLU¹, A. ERKİN BOZDEMİR¹, YEŞİM ERTAN², CEYDA KABAROĞLU¹, SADIK TAMSEL³, MİNE HEKİMGİL², ÇAĞ ÇAL⁴, DİLEK ÖZMEN¹, OYA BAYINDIR¹

Ege University School of Medicine, Departments of ¹Clinical Biochemistry, ²Pathology, ³Radiology and ⁴Urology, İzmir-Turkey

ABSTRACT

The aim of the study was to report the diagnostic performance of total prostate-specific antigen (tPSA) and free prostate-specific antigen ratio (%fPSA). The diagnostic performances of tPSA and %fPSA were evaluated in 364 men by ROC curves, sensitivity, specificity and diagnostic odds ratio calculations. One hundred and sixty-two patients were diagnosed as prostate cancer (PCa) and 202 as normal. The median tPSA levels and %fPSA ratios differed significantly, 11.16 ng/mL and 11 versus 6.51 ng/mL and 20 in PCa and normal groups, respectively. At tPSA levels between 4-10 ng/mL; area under curve (AUC)%fPSA was greater than AUCtPSA. 16 as a cut off value of %fPSA was the optimum choice revealing a sensitivity of 0.703, a specificity of 0.656 and diagnostic odds ratio of 4.254(95% CI 2.157- 8.39). %fPSA ratio is an expedient screening marker in PCa. In our laboratory, at tPSA levels between 4-10 ng/mL, %fPSA ratio of 16 offers a preferable diagnostic performance. [Turk J Cancer 2009;39(1):18-25]

KEY WORDS: Prostate cancer, total prostate specific antigen, free prostate specific antigen ratio

INTRODUCTION

Prostate cancer (PCa) is one of the commonest cancers in men. It is the second leading cause of cancer deaths in American men, after lung cancer and, in 2007, an estimated 218,890 new cases were diagnosed and 27,050 men died of prostate cancer (1). Age-adjusted incidence of prostate cancer has been increasing over the last 50 years and peaked in the early 1990s, associated mostly with increased early detection due to the introduction of prostate-specific antigen (tPSA) in the late 1980s (2). According to the guidelines of American Urological Association (AUA) and American Cancer Society (ACS), the principal screening tests for the detection of asymptomatic prostate cancer are digital rectal examination (DRE) and serum tPSA level (3). Transrectal ultrasound (TRUS) plays a role, only in the investigation of patients with abnormal DRE or tPSA when guided biopsies are required.

Although the use of a baseline tPSA cut-off value 4 ng/mL for prostate cancer screening is well established in clinical practice, some centers may choose to use 3 ng/mL or 2.5 ng/mL as a threshold for prostate biopsy (4,5). However, in a recent cohort study, about 20.5% of men with initial PSA levels <4.0 ng/mL were diagnosed with

PCa at the end of a 7-year period (6). Likewise, in different studies it had been shown that tPSA of 4-10 ng/mL would end up with approximately 20%-35% risk of PCa detected at biopsy (7-9).

In order to improve the specificity of tPSA for borderline levels (4-10 ng/mL, also defined as “gray zone”), percent free PSA (% fPSA) measurement is being used as an adjunct in PCa screening (10). % fPSA is the measurement of the non-protein-bound PSA as a percentage of the tPSA level. It has the potential to improve the ability to distinguish between malignant and benign prostate disease, as the % fPSA is lower in men who have PCa than in men who do not. Nevertheless, there is a lack of consensus regarding the optimal % fPSA cut-off for screening in clinical practice (11).

The aim of this retrospective study was to analyze the test performance of % fPSA ratio at various tPSA levels in Turkish men whom were referred to prostate biopsies and to revise our current %fPSA cut-off value reported in our laboratory.

MATERIALS AND METHODS

The laboratory results of 395 patients suspected of having prostate cancer, which ultrasound-guided needle biopsies were consecutively sent to the pathology section in the years of 2004-2005, were included in the study. All the biopsy materials were examined and evaluated by the same two pathologists. 26 patients who had TRUS-guided needle biopsies for the second time and 5 patients diagnosed as prostatic intraepithelial neoplasia were excluded and 364 patients remained. For all subjects, patient history revealed no use of finasteride/dutasteride before prostate biopsy.

Prostate biopsies were performed according to a pre-determined protocol. A standard peripheral-zone six sector biopsy was used with a prostate volume ≤ 30 mL. In men with prostate volumes >30 mL a further two cores were obtained peripherally at the apical area. Prostate biopsy results were stratified as low, intermediate or high risk, according to the National Comprehensive Cancer Network (NCCN) recommendations. Accordingly, a Gleason score of less than 7 is accepted as low, 7 as intermediate and 8-10 as high risk (12).

After retrieving biopsy data, in the 364 patients laboratory information system was used to investigate tPSA and fPSA levels requested before the biopsy.

For all patients, TRUS examination was performed by the same person using a Sonoline Elegra scanner (Siemens Ultrasound Division, Mountain View, CA) equipped with a 6.5 MHz endorectal probe.

tPSA and fPSA requisitions were performed on an automatic immunoassay analyzer (E170 Roche Diagnostics, Mannheim, Germany) via antigen-antibody complex techniques in the clinical biochemistry laboratory.

The cut-off values used for tPSA and % fPSA were 4 ng/mL and 15%, respectively. For % fPSA ratio 15, Catalonia et al. (11) found 65% sensitivity and 70% specificity in their Multicenter Clinical Trial.

Statistical analysis

To evaluate tPSA and % fPSA population distributions Kolmogorov-Smirnov test was used and distributions were not Gaussian. Therefore, for descriptive statistics median (1st – 3rd quartile) values were used, unless otherwise stated. Mann-Whitney U test was performed to compare variables in patients with and without prostate cancer. Spearman's rank correlation was used if needed. Kruskal Wallis-test was performed for comparisons between patients with low, intermediate and high risk according to Gleason score stratification. Receiver operating characteristics (ROC) curves were carried out for summarizing the performances of tPSA and % fPSA in addition, area under curve (AUC), diagnostic sensitivity, specificity and diagnostic odds ratio (DOR) were calculated. Also, the size of the calculated AUC were compared. For statistical significance p values <0.05 were accepted. Data management was carried out with the statistical program SPSS version 11 (Statistical Package for the Social Sciences Inc. Chicago IL, USA) for Windows.

RESULTS

Of the 364 patients 162 (44.5%) were diagnosed as PCa and 202 (55.5%) patients had normal biopsy results.

The mean \pm SD and the median (1st-3rd quartile) ages at biopsy were 65.7 \pm 9.3 and 65.0 (59-72) years, respectively

in 364 patients. 2 patients were <45 years old, 12 patients were 46-50 years old, 292 patients were 51-75 years old and 58 patients were above 75 years old. The median ages of patients with PCa and normal prostate biopsy were 69.0 (62-76) and 63.0 (57-69) years, respectively and were statistically different ($p < 0.001$).

In PCa group ($n=162$) tPSA was measured as 11.16 ng/mL (6.13-23.93 ng/mL) and % fPSA ratio was 11 (8-18). In normal prostate biopsy group ($n=202$) median tPSA level was 6.51 ng/mL (4.75-9.81 ng/mL) and % fPSA ratio was 20 (15-24). Both variables were significantly different between the two groups (Mann-Whitney test, $p < 0.001$, $p < 0.001$, respectively).

The subjects were first grouped into three according to their tPSA levels (<4 ng/mL, 4-10 ng/mL or >10 ng/mL), and then each group was subdivided according to %fPSA ratios (≤ 15 or > 15) (Table 1).

PCa patients were stratified according to their Gleason scores. In the low risk group ($n=75$); the median age was 68 (63-74) years, tPSA concentration was 7.09 (4.81-13.73) ng/mL and %fPSA was 14 (10-19). In the intermediate risk group ($n=41$) the median age was 67 (60-77) years, tPSA concentration was 13.30 (8.43-28.40) ng/mL and %fPSA was 10 (8-13). Finally in the high risk group ($n=46$) the median age was 71 (65-78) years, tPSA concentration was 19.37 (8.38-151.80) ng/mL and %fPSA was 10 (8-13). The median ages were not different ($p=0.210$), however, tPSA levels and % fPSA ratios differed significantly between low and intermediate risk groups ($p < 0.001$, $p=0.008$), and between low and high risk groups ($p < 0.001$ and $p=0.014$, respectively). A mild positive correlation between tPSA level and Gleason score ($r=0.416$, $p < 0.001$) and a weak negative correlation between %fPSA and Gleason score ($r=-0.249$, $p=0.002$) were obtained. For tPSA values <4 ng/mL of 18 PCa patients, 61% ($n=11$) had Gleason score <7, 16.7% ($n=3$) had =7 and 22.3% ($n=4$) had >7.

To demonstrate the sensitivity and the specificity of tumor markers, ROC curves were performed. In the total of 364 patients ROC curve revealed a sensitivity of 86.8% and a specificity of 13.1% at tPSA 4 ng/mL. The AUC was calculated to be 0.656 with $p < 0.001$. For 15%fPSA ratio, ROC curve revealed a 66% sensitivity and a 77% specificity. The AUC was calculated to be 0.731 with $p < 0.001$.

In figure 1 ROC curves for different levels of tPSA (<4 ng/mL, 4-10 ng/mL and >10 ng/mL) are given. For tPSA levels <4 ng/mL; the $AUC_{\%fPSA}$ and AUC_{tPSA} were statistically nonsignificant. For tPSA levels 4-10 ng/mL; The ROC curves demonstrated the beneficial use of %fPSA ratio. ($AUC_{\%fPSA} > AUC_{tPSA}$). These data once again confirm the essential use of % fPSA ratio in the gray zone. For tPSA levels >10 ng/mL, the AUCs for tPSA and %fPSA were nearly the same, indicating if tPSA levels >10 ng/mL, then further testing of %fPSA ratio is not necessary.

To evaluate the diagnostic performance of % fPSA in the gray zone, diagnostic odds ratios for different cut-off values were calculated and summarized in table 2. It is clearly seen that for % fPSA cut-off value 15, the sensitivity was 0.763, and the specificity was 0.607 and DOR was found to be 4.067 (95% CI 2.076-7.96). % fPSA cut-off value of 16 had a slightly less sensitivity (0.703) with a higher specificity (0.656) and DOR (4.254) than 15 % fPSA. 10 % fPSA had the highest DOR (8.795) and sensitivity (0.941) but at this cut-off value the specificity was 0.262 which was considered as very low. It was concluded that the most efficient %fPSA cut-off value to be reported in our laboratory should be 16%.

DISCUSSION

In our study, in which we aimed to assess the diagnostic performance of tPSA and %fPSA in PCa, we concluded that for % fPSA a cut-off value of 16 instead of 15 will be more beneficial to collimate patients to TRUS guided biopsies in our region. Our data, considering 364 Turkish men, confirm the beneficial use of % fPSA ratio in the gray zone levels, 4-10 ng/mL of tPSA.

PCa is the 3th cause of cancer among men (5.3%) in Turkey and it is observed much lower than the Western countries (13, 14). This retrospective study, to our knowledge, is one of the largest series run in Turkey for evaluating the diagnostic performance of % fPSA in patients with different tPSA levels and histologically confirmed TRUS guided biopsy materials (15-17).

Owing to the problems of false negative results of tPSA measurements, fPSA is being used as an adjunct for PCa screening (10,18). The initial reports concerning the

Table 1
The distribution of tPSA levels and % fPSA ratios determined in patients confirmed as PCa or normal biopsy

	Patients with TRUS guided biopsy	
	PCa, n (%)	Normal biopsy, n (%)
tPSA <4 ng/mL	18 (11.1%)	37 (18.3%)
%fPSA ≤15 (n)	10 (56%)	10 (27%)
%fPSA >15 (n)	8 (44%)	27 (73%)
tPSA 4-10 ng/mL	61 (37.7%)	119 (58.9%)
%fPSA ≤15 (n)	39 (64%)	36 (30.3%)
%fPSA >15 (n)	22 (36%)	83 (69.7%)
tPSA >10 ng/mL	83 (51.2%)	46 (22.8%)
%fPSA ≤15 (n)	65 (78.3%)	12 (26.1%)
%fPSA >15 (n)	18 (21.7%)	34 (73.9%)
Total	162 (100%)	202 (100%)

n: number of patients; TRUS: transrectal ultrasound

Table 2
The sensitivity, specificity and DOR of %fPSA at different cut-off levels between tPSA levels 4-10 ng/mL

% fPSA cut-off	Sensitivity	tPSA 4-10 ng/mL		
		Specificity	DOR	95% CI
10	0.941	0.262	8.795	3.300- 23.43
14	0.805	0.475	4.667	2.349- 9.27
15	0.763	0.607	4.067	2.076- 7.96
16	0.703	0.656	4.254	2.157- 8.39
17	0.669	0.705	2.943	1.508- 5.74
18	0.585	0.705	2.529	1.281- 4.99
19	0.500	0.721	3.298	1.572- 6.92
20	0.458	0.803	2.500	1.186- 5.27
22	0.305	0.803	2.027	0.910- 4.51
25	0.220	0.852	1.585	0.620- 4.04

DOR: Diagnostic odds ratio; CI: Confidence interval

%fPSA ratio, for improvement in specificity of tPSA, were demonstrated first in selected group of patients, and also recently in general population (19-22). In this retrospective study, 180 patients with tPSA level 4-10 ng/mL were sent to TRUS guided prostate biopsies. Only 61 of them were confirmed as PCa and 64% of them had % fPSA <15. For

tPSA levels 4-10 ng/mL, the AUC of %fPSA was found to be greater than AUC of tPSA confirming the beneficial use of %fPSA testing in the gray zone. In order to decide for an appropriate cut-off level, we performed ROC analysis, and it resulted with a DOR of 4.254 against 4.067 in our population. For tPSA >10 ng/mL, the AUCs for tPSA and

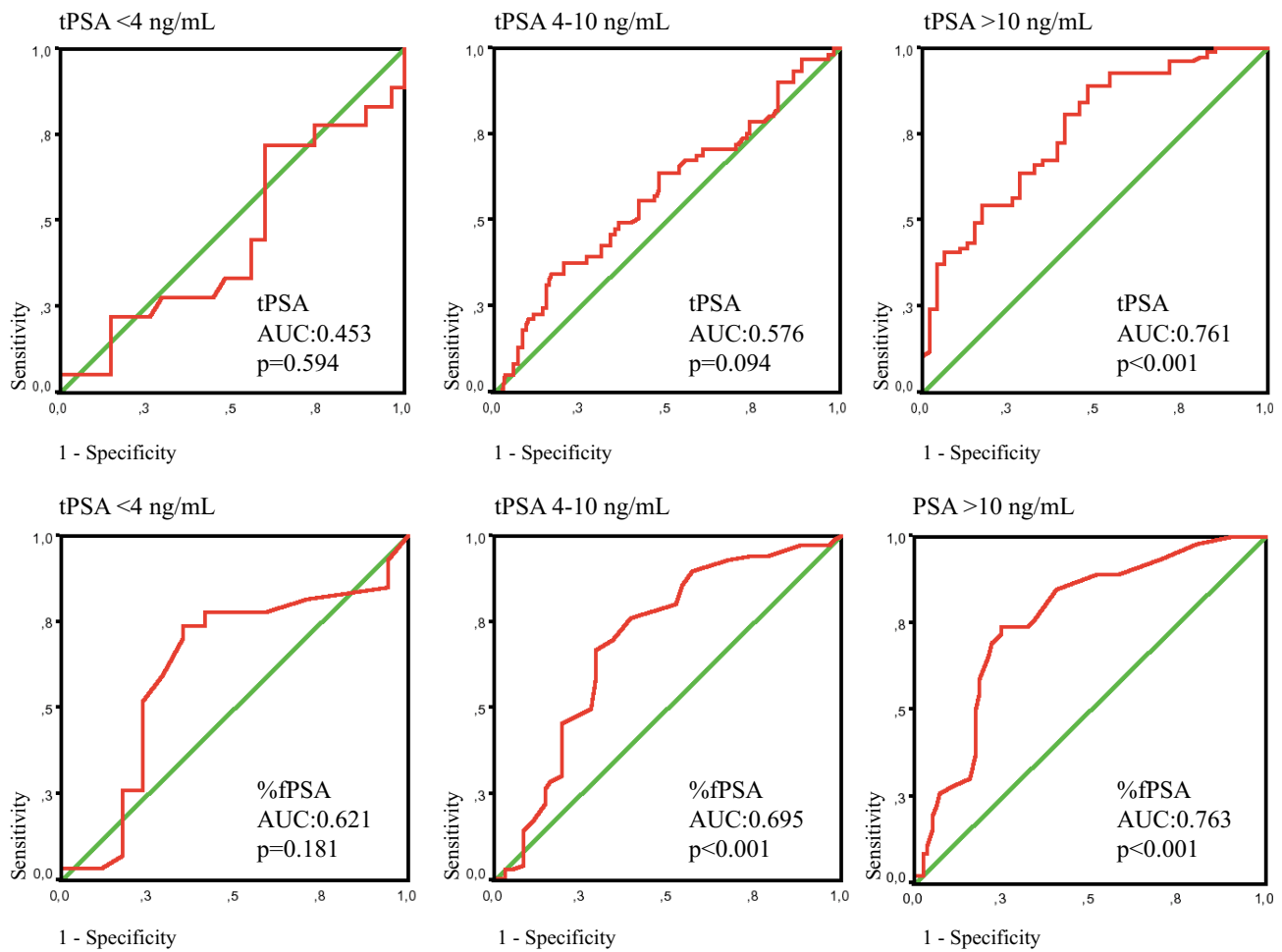


Fig 1. ROC curves of tPSA and %fPSA for different tPSA groups (<4, 4-10 and >10 ng/mL)
ROC curves: Receiver operating characteristics curves; AUC: area under curve

%fPSA were nearly the same, indicating that further testing of %fPSA ratio is not necessary. However, our results suggest that in contrast to step wise testing, the clinicians request tPSA and %fPSA measurements simultaneously. Since these types of requests are not cost-effective, they may increase the burden of the disease.

The worldwide use of a threshold tPSA level differs greatly between 2.5 and 4 ng/mL (10). Although, lowering the threshold improves the sensitivity, it also increases the unnecessary biopsy number such as demonstrated in Roehl et al.'s study (23). Also lowering the cut-off value ends up with a falsely elevated PCa incidence, because you may detect cancer in men who would never be diagnosed during their life time (24). If we apply lower threshold levels to our study, PCa was seen in 11.1% of patients with tPSA 4 ng/mL, and this percentage increases

to 17.35 when tPSA levels 2.5 ng/mL are considered. Although using tPSA 4 ng/mL as a cut-off value is more widely used in clinical practice, several studies indicated a rather low specificity with tPSA level of 4 ng/mL as a cut-off level. Rydén et al. (25) and Babaian et al. (26), both found similar percentages of PCa, in men with tPSA <4 ng/mL, which were 23% and 24.5%, respectively. In Adriole et al.'s study (27), they estimated that only 25-40% of men will develop PCa, meanwhile 60-75% of them will go under unnecessary biopsy when the serum tPSA measurement is 4-10 ng/mL. Furthermore, tPSA between 4-10 ng/mL is still not highly specific for PCa.

In contrast to above mentioned low specificity and unwanted biopsy number, some authors, such as Lujan et al. (28), believe lowering the cut-off value may increase the chance of recognizing PCa. They showed that an im-

portant number of PCa were detected in tPSA <4 ng/mL and detection rates in the range 1.0-2.99 ng/mL and 3.0-3.99 ng/mL were 9.4% and 21.4%, respectively. For the same cut-off tPSA level, our results showed a detection rate of 11.1% which is lower than their results. Similar to our study, in a multicenter Korean study, Yang et al. (29) detected 12.4% of PCa in men with tPSA <4 ng/mL in their cohort. There may be two possible explanations of this difference; it is either the majority of urologists in the daily clinical practice rely almost exclusively on tPSA levels or that they perform inadequate DRE during PCa screening. However, in our study design it was accepted that the screening guidelines were carefully followed by urologists in our hospital.

Despite all different arguments on choosing the best threshold, in the European Randomized Study of Screening for Prostate Cancer (ERSCP) (24) authors concluded there was no safe range of tPSA levels in detecting PCa, even at <0.5 ng/mL values cancer diagnosis is possible.

In the highlight of above mentioned data it is clear that, results from selected populations may differ and cannot be applied worldwide. The importance of collecting own data and reassessing the diagnostic performance of screening tests is once more emphasized.

In our study, of the 364 patients who fulfill the inclusion criteria and underwent TRUS guided biopsy, 44.5% were diagnosed as PCa and 55.5% had normal prostate biopsy result. When literature was reviewed for other Turkish population based studies, three studies for PCa detection were found. One of them, conducted by Eskiçorapçı et al. (15), based on 303 consecutive patients and an extended biopsy protocol, showed that the overall detection rate was 31%, lower than ours. Dinçel et al. (16), in their prospective study, found a detection rate of 21% in histologically proven PCa patients and, another study group, Yenişol et al. (17), showed that among 194 patients with tPSA between 4-20 ng/mL, 13.4% had PCa on biopsy. Owing to different patient selection protocols, detection rates could have been different and all these data will not reflect the real PCa incidence in Turkey. This is a worldwide problem and one of the pitfalls in screening PCa. For example in a study from Sweden, among 361 consecutive men the overall detection rate for PCa was 52% (25),

whereas in the meta-analysis of studies from China, Wang et al. (30) concluded a detection rate of 18.8%.

Gleason score correlates with tumor aggressiveness, prognosis and effects of interventional treatment and tPSA values (31). In general, serum tPSA levels correlate with larger tumor volume, advanced pathologic stages and higher grades. Although higher grade cancer produces less tPSA per cell as compared to lower grade tumors, overall, poorly differentiated tumors are associated with higher PSA levels as these tumors tend to be larger and of more advanced stage (32,33). Consistently, in our study, a mild positive correlation between tPSA level and Gleason score and a weak negative correlation between %fPSA and Gleason score were obtained. We found 11 PCa patients (61.1%) with tPSA levels <4 ng/mL and they had presented with Gleason score of <7. Similar to our study, many studies detected PCa at low tPSA levels emphasizing that aggressive PCa can be detected in low tPSA levels (18,25,28,34). PCa with low tPSA levels should be of concern, because it raises the question that there are some PCa patients that cannot be detected with tPSA based screening (35). When tPSA levels <4 ng/mL, even % fPSA ratio use is not efficient enough to decide on prostate biopsy and there is still a critically high number of patients with PCa. How PCa screening gap at low levels of tPSA should be resolved remains as a major problem.

PCa occurrence increases with aging (36). Likewise, in the current study the average age was 66 years (median age was 65) at biopsy, whereas in PCa patients the mean age was 69 years. It is interesting that, although our population is mainly Caucasian origin, our data are similar to Nationwide Multicenter Study of Korean, in which the mean age of PCa patients was 69.2 years and the total group mean age was 66.1 years, but it was different from Prostate Cancer Prevention Trial (PCPT) (30,37).

One of our limitations was that a restricted population of patients was analyzed; patients with tPSA levels of >4.0 ng/mL and/or with % fPSA of <15%, or a palpable prostate gland in DRE. On this issue, one of the best appreciated study is the ERSCP study. It is considered as a true screening study covering approximately 100,000 men aging 45 to 70 years or life expectancy greater than 10 years. However, even in this well-known large-scaled

screening program patients were referred to biopsy if they have fulfilled certain criteria; such as elevated tPSA (>4 ng/mL until 1998, or present protocol ≥ 3 ng/mL), abnormal DRE or abnormal TRUS findings in the first screening round and tPSA >3 ng/mL, regardless of DRE and/or TRUS (22). Therefore, to achieve realistic value of prostate cancer prevalence and detection rate of tPSA and % fPSA, biopsy should be performed regardless of selected criteria, but this is not practical. Another important limitation of our study is that, although 6-8 core biopsy is limited nowadays, it is still our urologists' choice and performing sextant biopsy to prostate less than 30 cc and 8 core biopsy in men with larger prostate may lead to missed detection of prostate cancer.

CONCLUSION

In summary, we retrospectively evaluated the tPSA and %fPSA results obtained at the time of TRUS guided needle biopsy performance. In a country, in which PCa incidence is lower than Western countries, like Turkey, our study once more proved the beneficial diagnostic performance of % fPSA in 4-10 ng/mL tPSA ranges. Within this range the use of %fPSA can offer reduction in the number of unnecessary biopsies while still maintaining a high cancer detection rate. We concluded that in our laboratory, pertaining tPSA levels between 4-10 ng/mL, 16 should be the most accurate value for %fPSA cut-off to decide on TRUS guided biopsy.

References

- Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43-66.
- Surveillance, Epidemiology, and End Results (SEER) Program SEER*Stat Database: Incidence—SEER 9 Regs Limited-Use, Nov 2006 Sub (1973–2004), National Cancer Institute (NCI), DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2007, based on the November 2006 submission. www.seer.cancer.gov.
- Smith RA, von Eschenbach AC, Wender R, et al. American Cancer Society guidelines for the early detection of cancer: update of early detection guidelines for prostate, colorectal, and endometrial cancers. Also: update 2001--testing for early lung cancer detection. *Cancer J Clin* 2001;51:38-75.
- Schroder FH, Denis LJ, Roobol M, et al. The story of the European Randomized Study of Screening for Prostate Cancer. *BJU Int* 2003;92(Suppl 2):1-13.
- Saito S. Prostate-specific antigen cut-off point of 2.5 ng/mL and increasing the number of prostate biopsies results in the detection of curable prostate cancer even in Japanese population. *Int J Urol* 2007;14:709-12.
- Ankerst DP, Thompson IM. Sensitivity and specificity of prostate-specific antigen for prostate cancer detection with high rates of biopsy verification. *Arch Ital Urol Androl* 2006;78:125-9.
- Partin AW, Kattan MW, Subong EN, et al. Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer. A multi-institutional update. *JAMA* 1997;277:1445-51.
- Noboru Hara, Yasuo Kitamura, Toshihiro Saito, et al. Total and free prostate-specific antigen indexes in prostate cancer screening: value and limitation for Japanese populations. *Asian J Androl* 2006;8:429-34.
- Vukotic V, Cerovic S, Kozomara M, et al. The predictive value of PSA in diagnosis of prostate cancer in non screened population. *Acta Chir Jugosl.* 2005;52:81-7.
- Lee R, Localio AR, Armstrong K, et al. A metaanalysis of the performance characteristics of the free prostate-specific antigen test. *Urology* 2006;67:762-8.
- Catalona WJ, Partin AW, Slawin KM, et al. Use of the Percentage of Free Prostate-Specific Antigen to Enhance Differentiation of Prostate Cancer From Benign Prostatic Disease A Prospective Multicenter Clinical Trial *JAMA* 1998;279:1542-7.
- Scherr D, Swindle PW, Scardino PT. National Comprehensive Cancer network Guidelines for the management of Prostate cancer. *Urology* 2003;61(suppl 2A):14-22.
- The study of National Disease Burden and Cost effectiveness, Appendix 1-6. The Ministry of Health, School of Sanitation, 2003.
- Ferlay J, Bray F, Pisani P, et al. *Globocan 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0.* IARC Press: Lyon, 2001. <http://www.iarc.fr/globocan/globocan.html>.
- Eskicorapci SY, Baydar DE, Akbala C, et al. An extended 10-core transrectal ultrasonography guided prostate biopsy protocol improves the detection of prostate cancer. *Eur Urol* 2004;45:444-9.
- Dincel C, Caskurlu T, Tasci AI, et al. Prospective evaluation of prostate specific antigen, (PSA), PSA density, free-to-total PSA ratio and a new formula

- (prostate malignancy index) for detecting prostate cancer and preventing negative biopsies in patients with normal rectal examinations and intermediate PSA levels. *Int Urol Nephrol* 1999;31:497-509.
17. Yenişol CO, Bozkaya G, Çavuşoğlu A, et al. The relation of prostate biopsy results and ratio of free to total PSA in patients with a total PSA between 4-20 ng/mL. *Int Urol Nephrol*. 2001;33:503-6.
 18. Schröder FH, Van der Crujisen-Koeter I, de Koning HJ, et al. Prostate cancer detection at low prostate specific antigen. *J Urol* 2000;163:806-12.
 19. Egawa S, Soh S, Ohori M, et al. The ratio of free to total serum prostate specific antigen and its use in differential diagnosis of prostate antigen and its use in differential diagnosis of prostate carcinoma in Japan. *Cancer* 1997;79:90-8.
 20. Magklara A, Scorilas A, Catalona WJ, et al. The combination of glandular kallikrein and free prostate-specific antigen (PSA) enhances discrimination between prostate cancer and benign prostatic hyperplasia in patients with moderately increased total PSA. *Clin Chem* 1999;45:1960-6.
 21. Reissigl A, Klocker H, Pointner J, et al. Usefulness of the ratio free/total prostate-specific antigen in addition to total PSA levels in prostate cancer screening. *Urology* 1996;48:62-6.
 22. Postma R, Schröder FH, van Leenders GJ, et al. Cancer detection and cancer characteristics in the European Randomized Study of Screening for Prostate Cancer (ERSPC)--Section Rotterdam. A comparison of two rounds of screening. *Eur Urol* 2007;52:89-97.
 23. Roehl KA, Antenor JA, Catalona WJ. Robustness of free prostate specific antigen measurements to reduce unnecessary biopsies in the 2.6 to 4.0 ng/ml range. *J Urol* 2002;168:922-5.
 24. Draisma G, Boer R, Otto SJ, et al. Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. *J Natl Cancer Inst* 2003;95:868-78.
 25. Rydén L, Egevad L, Ekman P, et al. Prevalence of prostate cancer at different levels of serum prostate-specific antigen (PSA) and different free: total PSA ratios in a consecutive series of men referred for prostate biopsies. *Scand J Urol Nephrol* 2007; 41: 302-7.
 26. Babaian RJ, Johnston DA, Naccarato W, et al. The incidence of prostate cancer in a screening population with a serum prostate specific antigen between 2.5 and 4.0 ng/mL. Relation to biopsy strategy. *J Urol* 2001;165:757-60.
 27. Andriole GL, Levin DL, Crawford ED, et al. Prostate cancer screening in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial: findings from the initial screening round of a randomized trial. *J Natl Cancer Inst* 2005;97:433-8.
 28. Lujan M, Paez A, Miravalles E, et al. Prostate cancer detection is also relevant in low prostate specific antigen ranges. *Eur Urol* 2004;45:155-9.
 29. Yang WJ, Lee DH, Chung BH, et al. Detection of prostate cancer on biopsy according to serum prostate-specific antigen in Korean men: A multicenter study. *Urology* 2006;67:333-6.
 30. Wang Y, Sun G, Pan JG, et al. Performance of tPSA and f/tPSA for prostate cancer in Chinese. A systematic review and meta-analysis. *Prostate Cancer and Prostatic Diseases* 2006;9:374-8.
 31. Stav K, Judith S, Merald H, et al. Does prostate biopsy Gleason score accurately express the biologic features of prostate cancer? *Urol Oncol* 2007;25:383-6.
 32. Partin AW, Carter HB, Chan DW, et al. Prostate specific antigen in the staging of localized prostate cancer: influence of tumor differentiation, tumor volume and benign hyperplasia. *J Urol* 1990;143:747-52.
 33. Sandblom G, Ladjevardi S, Garmo H, et al. The impact of prostate-specific antigen level at diagnosis on the relative survival of 28,531 men with localized carcinoma of the prostate. *Cancer* 2008;112:813-9.
 34. Recker F, Kwaitkowski M, Huber A, et al. Prospective detection of clinically relevant prostate cancer in the prostate specific antigen range 1 to 3 ng/mL combined with free-to-total ratio 20% or less. The Aurau experience. *J Urol* 2001;166:851-5.
 35. Sandblom G, Ladjevardi S, Garmo H, et al. The impact of prostate-specific antigen level at diagnosis on the relative survival of 28,531 men with localized carcinoma of the prostate. *Cancer* 2008;112:813-9.
 36. Choi YD, Kang DR, Nam CM, et al. Age-specific prostate-specific antigen reference ranges in Korean men. *Urology* 2007;70:1113-6.
 37. Seo HK, Chung MK, Ryu SB, et al. Detection rate of Prostate cancer according to Prostate-specific antigen and digital rectal examination in Korean men: A nationwide multicenter study. *Urol* 2007;70:1109-12.