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A comparison of three free and total PSA assays

HJ Roth[1], SC Stewart[2] & MK Brawer[3]

[1]*Department of Endocrinology and Oncology, Laboratory Group Heidelberg;* [2]*Clinical Studies Specialist, Chiron Diagnostics and* [3]*Northwest Prostate Institute, Seattle, Washington*

Prostate-specific antigen (PSA) has emerged as the most predictive test of whether or not a man has prostatic carcinoma. The free to total PSA ratio provides important enhancement in specificity, thus obviating unnecessary negative biopsies. In the absence of an international standard for total PSA, much less free PSA, variation between manufacturers may cause confusing results. We sought to compare three different manufacturer's free and total PSA assays in a population consisting of consecutive patients who had PSA testing in a reference laboratory in Germany. Between April 1994 and July 1996, serum specimens from 240 men were evaluated with three different free and total PSA assays. Indications for PSA determination were based on the referring physician, who also provided the clinical diagnosis. Total and free PSA were measured on the same freeze–thaw cycle with Chiron Diagnostics, Enzymun Boehringer Mannheim, and Hybritech Tandem-R assays. Seventy-nine men had carcinoma of the prostate, 120 had clinical evidence of benign prostatic hyperplasia and 27 were without evidence of prostatic disease. The Chiron ACS: 180 free to total ratio compared very well with the Tandem-R assay at the 95% sensitivity level, affording 17 and 22% specificity respectively. Using the range of total PSA of 4–10 ng/ml, the increase in specificity of the free to total PSA is quite significant, and the specificity of the Enzymun assays is greatly improved. (Specificity of 49%, 29% and 25% at 95% sensitivity for ACS, Enzymun and Tandem respectively.) This data, based on 'real world' clinical experience, shows significant variation between different manufacturers' assays. There was significant equivalence between the Chiron and Hybritech assays. The Enzymun assay performed well only when data from the total PSA range of 4–10 ng/ml was included. Clinicians must be aware of which manufacturers' assays for both the free and total PSA their laboratory staff is utilizing, and laboratory technicians must provide meaningful outcome data based on the patient population they serve with respect to the performance of these assays.

Keywords: prostate-specific antigen; isoforms; free; total

Introduction

Prostate-specific antigen (PSA) has revolutionized our approach to the diagnosis, staging and monitoring of patients with prostatic carcinoma. Its role in early detection and screening is well established and has resulted in the recommendations of organizations including the American Cancer Society, as well as the American Urological Association, for serum determinations of PSA in conjunction with carefully performed digital rectal examination in men seeking evaluation for the possibility of prostatic carcinoma.

Despite the impressive yield of a serum PSA greater than 4.0 ng/ml as an indication for further work-up, approximately 3 out of 4 men will have negative biopsies.[1–4] This lack of specificity has resulted in considerable efforts to improve the performance of this most important of all tumour markers.

Three methods— PSA density (adjusting the serum PSA by the size of the prostate or more recently the volume of the transition zone), PSA velocity (the change in PSA over time)

Correspondence: Dr MK Brawer, Director, Northwest Prostate Institute, Seattle, Washington, USA 98133.
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and age-specific PSA cutoff values have been extensively investigated. A review of these approaches is beyond the scope of this manuscript but may be found in works by Nixon *et al*[5] and Brawer and Kirby.[6]

Percent free-to-total PSA

Probably the most promising development in enhancing the performance of PSA testing involves measuring the amount of different molecular forms of the PSA protein in serum. In 1990, two groups independently observed that PSA exists in different forms in the systemic circulation, PSA occurs in the free form and forms complexes with α_1-antichymotrypsin (ACT) and α_2-macroglobulin (AMG) along with other protease inhibitors. Five distinct immunoreactive epitopes have been identified on the free PSA molecule available for immunoreactive detection of serum levels. Christensson *et al* reported that the proportion of PSA-ACT was greater in patients with prostate cancer than in patients with BPH.[18] These early studies demonstrated that measuring the free-to-total PSA ratio had the potential to enhance the performance of PSA testing. Recent research has focused on characterizing the clinical utility of this new method and its range of effectiveness.[1,7–16]

Before this assay is used routinely, further studies are needed to help delineate the most appropriate free-to-total ratio in a large scale population-based study. Furthermore, we have observed considerable differences in the measurement of the free PSA when different manufacturers' assays are compared.[17] In this present investigation we sought to evaluate the three different manufacturers' free and total assays in a mixed population evaluated by a single clinical reference laboratory.

Material and methods

Between April 1994 and July 1996, serum specimens from 241 men were sent to our laboratory for determination of serum PSA level. Blood specimens were spun and serum decanted, and all specimens stored at -20°C for up to one year.

Serum samples were collected from patients at sites in Germany and the US. Classification by diagnosis was based on the clinical situation of the patient as well as DRE, TRUS and biopsy results; all cancer patients were confirmed by biopsy. Twenty-eight of the cancer patients had been treated with prostatectomy, radiotherapy, hormonal therapy or orchiectomy and were being monitored for progression or recurrence of disease. BPH patients were DRE and biopsy or TRUS negative for cancer. Normal control patients had no evidence of any disease by a physical examination.

Blood samples were collected in Sarstedt monovetteÆ or Becton Dickinson vacutainerÆ serum collection tubes. Serum samples were separated within two hours of collection in all but a few cases and were then frozen at -20°C until analysed.

Free and total PSA reagents from three manufacturers were compared. ACS:180 PSA2 and Free PSA reagents were supplied by Chiron Diagnostics, East Walpole, MA and used with the Chiron Diagnostics ACS:180 instrument. EnzymunÆ PSA and Free PSA reagents were supplied by Boehringer Mannheim GmbH, Mannheim, Germany and used with the Boehringer Mannheim ES 700 Instrument. TandemÆ-R PSA and free SPA reagents were supplied by Hybritech, Inc., San Diego, CA. As TandemÆ-R is a manual assay, samples were pipetted into assay tubes manually or with a Tecan RSP robotic pipettor. During incubation, assay tubes and beads were placed on a GERHARDT rotator. Beads were manually washed and bound radioactivity was measured using an LKB 1277—Gamma Master gamma counter. PSA dose was calculated using RIACalc software by LKB. All tests were performed and the systems were used accordingly to the manufacturer's instructions.

Statistical analysis

Least squares linear regression analysis for scattered plots were generated and the Pearson's correlation coefficients were calculated using Kaleidagraph 3.0 software; statistical comparisons of the data were performed using the same software. Receiver operating characteristic (ROC) plots were generated to graphically display the difference in diagnostic accuracy between total PSA and the free to total PSA ratio. Sensitivity is dfined as the percentage of true positvies in the affected (biopsy positive) population using a given cutoff, and the corresponding specificity is the percentage of true negatives in the unaffected population at the same cutoff. Data from samples missing one or more results was not used in any calculation or graphic representation. For those figures showing data from a specific range, the mean value obtained using all assays was used to select data included.

Results

Table 1 demonstrates the total PSA level by each of the three manufacturers stratified by patient diagnosis. There were 79 men that had carcinoma of the prostate, 120 with benign prostatic hyperplasia, and 27 had no evidence of prostatic disease. Only one prostatitis patient had free and total PSA results from all assays, and for that reason, this group of patients is not included in the table. As expected, the total PSA was greater in those men with malignancy than in those without. Table 2 demonstrates the free to total PSA ratio in each of the three disease categories, again, as expected, the men with prostatic carcinoma had lower free to total ratio with the ACS:180 and Tandem-R assays, intriguingly however, the free to total ratio did not stratify those men with cancer compared to those with benign prostatic hyperplasia when the Enzymun assay was employed. Also of interest was the signifcantly lower free to total ratio observed in those men with prostatitis as compared to other histologies. The small numbers, however, preclude making a conclusion in this regard.

Figure 1 shows a regression over the 0–12 ng/mL range of the total PSA assays. As is demonstrated, the ACS:180 resulted in about a 1% higher value than the Tandem-R assay and the Enzymun resulted in a 1% lower value. As

Table 1 Results of total PSA by assay and histology

Histology	Assay	Total (*n*)	Mean (ng/mL)	Median (ng/mL)	Range (ng/mL)	s.d.
CaP	ACS:180 PSA2	79	10.3	6.6	0.0–72.2	11.2
	Enzymun PSA	79	8.2	6.4	0.0–43.9	6.8
	Tandem-R PSA	79	9.5	7.0	0.4–68.0	9.2
BPH	ACS:180 PSA2	120	6.1	4.9	0.3–27.4	4.4
	Enzymun PSA	120	4.8	4.3	0.1–20.8	3.0
	Tandem-R PSA	120	5.6	4.7	0.9–20.7	3.3
NED	ACS:180 PSA2	27	1.4	0.9	0.4–6.8	1.5
	Enzymun PSA	27	1.3	0.7	0.2–6.0	1.3
	Tandem-R PSA	27	2.1	1.7	0.6–7.3	1.4

Table 2 Results of free to total PSA by assay and histology

Histology	Assay	Total (*n*)	Mean (ng/mL)	Median (ng/mL)	Range (ng/mL)	s.d.
CaP	ACS:180 F&T	79	11.0	7.7	0.0–90	13.2
	Enzymun F&T	79	22.3	9.2	3.3–725	81.4
	Tandem-R F&T	79	10.7	8.6	0–52	8.4
BPH	ACS:180 F&T	120	18.0	15.9	0–57	10.2
	Enzymun F&T	120	21.3	16.9	0–191	19.1
	Tandem-R F&T	120	19.0	15.9	0–61	10.9
NED	ACS:180 F&T	27	43.5	35.7	9.5–100	26.4
	Enzymun F&T	27	34.3	31.8	9.6–80	18.5
	Tandem-R F&T	27	15.7	15.2	3.7–29	6.2

expected, the correlation was quite good with all assays suggesting that indeed these assays are measuring the same analyte.

Figure 2 shows a similar comparison of the different manufacturers' free to total PSA ratio. Data from patients with total PSA between 4–10 ng/mLwas used for the comparison as that was the clinically relevant group. The comparison shows that the ACS: free to total PSA ratio is approximately 40% lower and the Enzymun free to total PSA ratio is 15% lower than the Tandem-R free to total PSA ratio.

Figures 3–5 demonstrate scatter plots of the free to toal PSA *vs* the total PSA for each manufacturer. Included in these figures are those men with carcinoma compared to those with benign histology. Owing to the small number, men with prostatitis were excluded from this figure. In general, with each of the manufacturers, there is an expected trend toward a lower free to total PSA in those

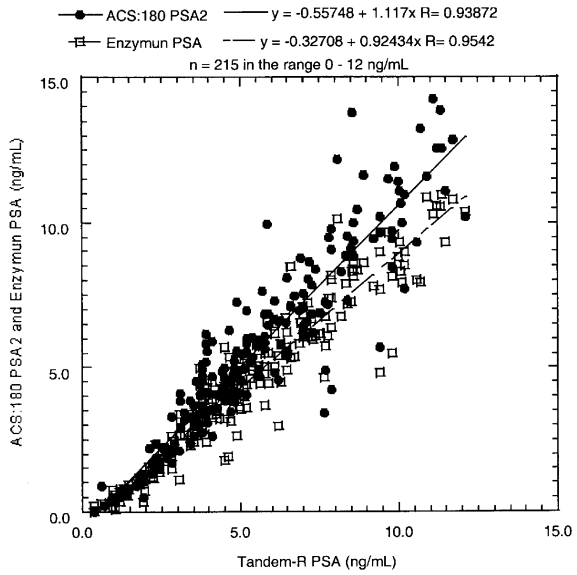

Figure 1 *Comparison of ACS:180 PSA2, Enzymun PSA and Tandem-R PSA.*

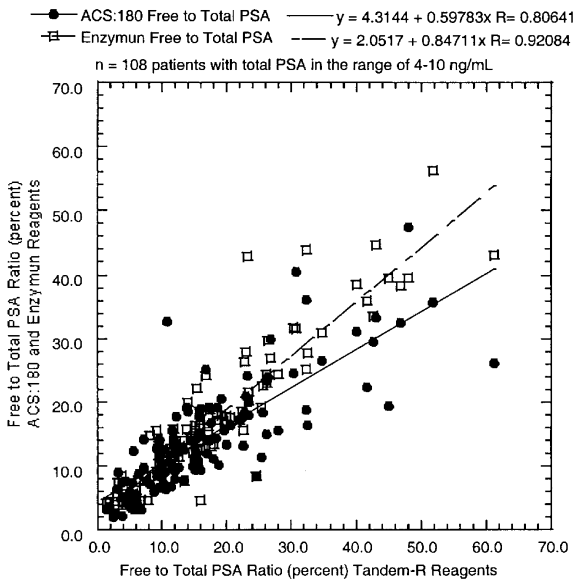

Figure 2 *Comparison of free to total ratio using ACS:180 PSA2 and free PSA Enzymun PSA and free PSA, and Tandem-R PSA and free PSA.*

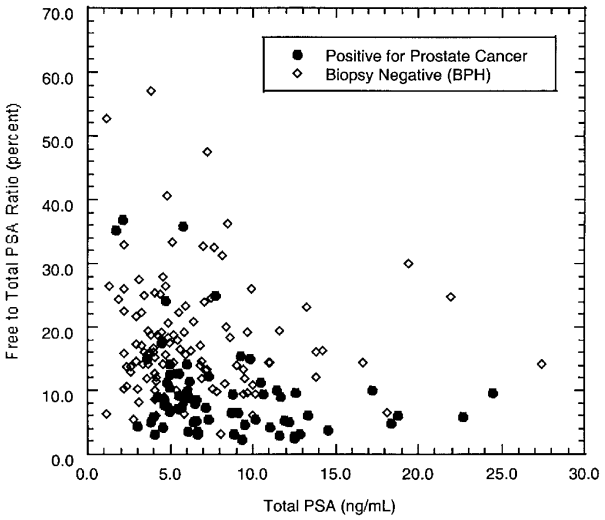

Figure 3 *Comparison of total PSA and free to total PSA ratio in prostate cancer and BPH patients ACS:180 PSA2 and free PSA.*

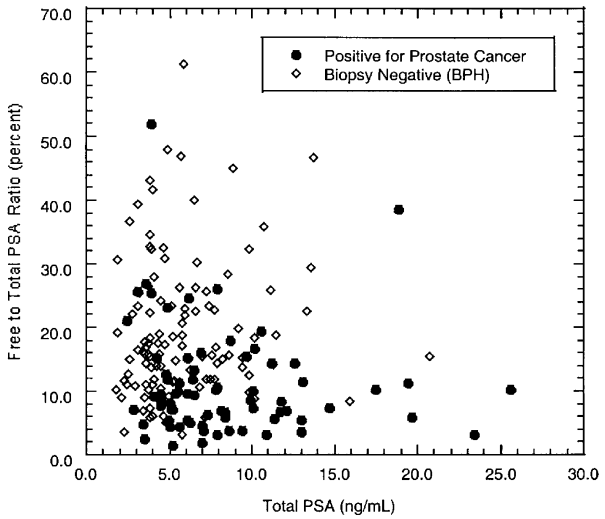

Figure 4 *Comparison of total PSA and free to total PSA ratio in prostate cancer and BPH patients Tandem-R PSA and free PSA.*

men harboring malignancy. The cutoff for the x-axis was selected to show all results from biopsy negative individuals, although several individuals with prostate cancer had higher levels of total PSA.

Figures 6–8 demostrate the received operating characteristic curves generated from each of the manufacturers' assays over the PSA range of 4–10 ng/ml. This range was selected owing to the fact that this appears to be emerging as the most useful range for measuring the free to total PSA. These figures show data from patients with prostate cancer and BPH, but not from normal individuals or patients with prostatitis. As can be seen, the free to total PSA provided a significant enhancement in stratification of those men with or without malignancy.

Table 3 examines, at various sensitivities, the specificity afforded by the total PSA as well as the free to total PSA and demonstrates the cutoff necessary to generate these values. As is apparent, the ACS:180 free to total compared very well with the Tandem-R manufacturer at the 95% sensitivity level affording 17 and 22% specificity

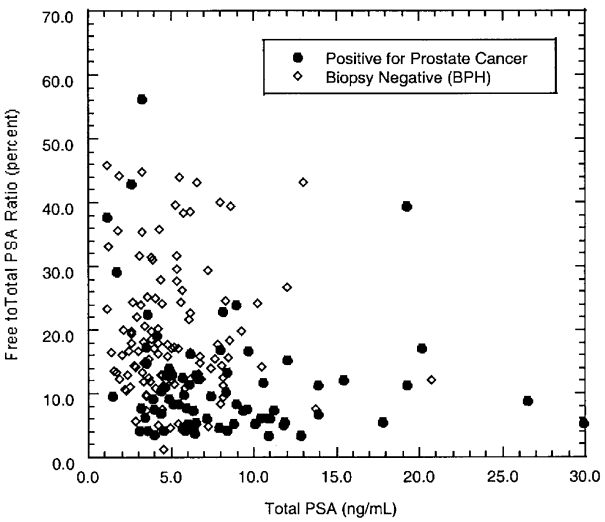

Figure 5 *Comparison of total PSA and free to total PSA ratio in prostate cancer and BPH patients Enzymun PSA and free PSA*

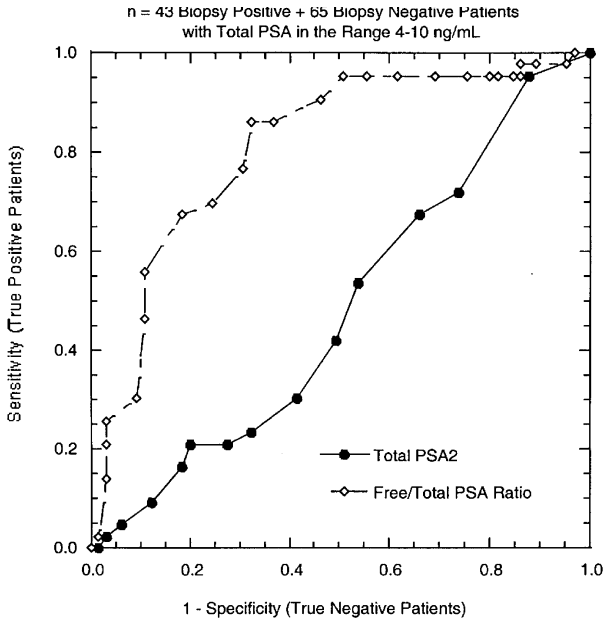

Figure 6 *ROC curve comparing sensitivity and specificity of ACS:180 PSA2 to free to total PSA ratio.*

respectively. The specificity of the Enzymun assay fell significantly at this sensitivity level.

Table 4 demonstrates the similar specificity analysis for total and free to total PSA utilizing the truncated range of total PSA of 4–10. Using values in this range, the increase in specificity of the free to total PSA is quite significant. The specificity of the Enzymun assays is greatly improved using data from this group of patients.

Discussion

It is quite clear that serum PSA determination has revolutionized virtually all aspects of the management of men with prostatic disease. Numerous efforts have been made

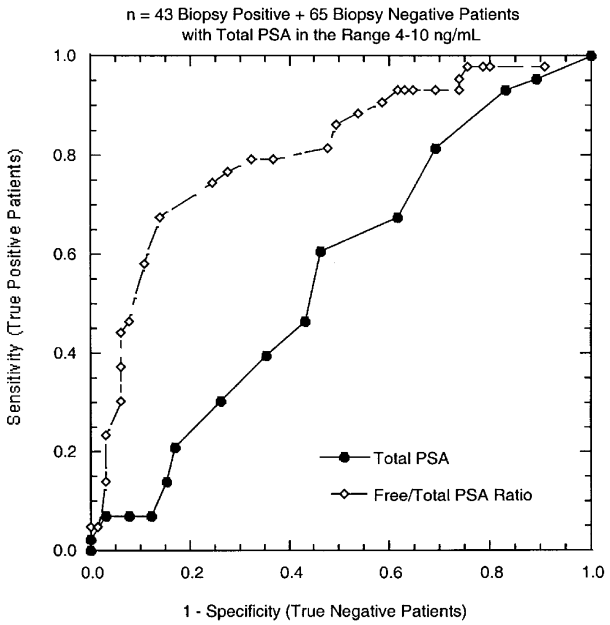

Figure 7 *ROC curve comparing sensitivity and specificity of Tandem-R PSA to free to total PSA ratio.*

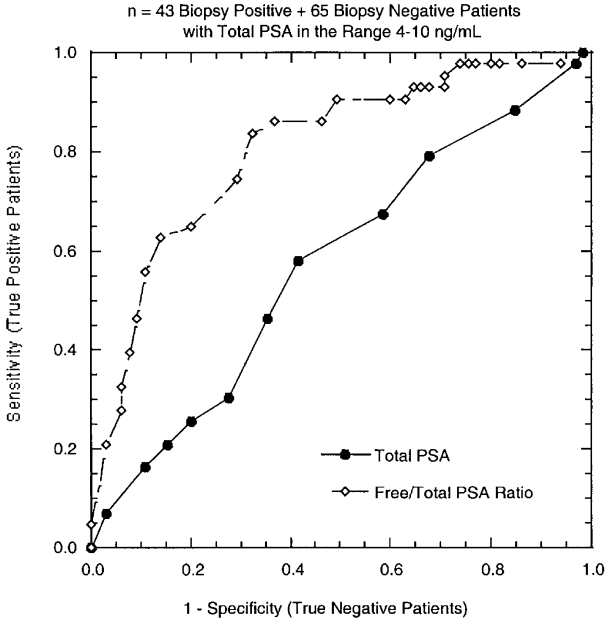

Figure 8 *ROC curve comparing sensitivity and specificity of Enzymun PSA to free to total PSA ratio.*

Table 3 Comparison of the sensitivity and specificity of total PSA and the free to total PSA Ratio. Data from all patients in the BPH and prostate cancer groups is included

Assay	Sensitivity (%)	Specificity (%) total PSA	Specificity (%) F/T PSA	Cut-point total PSA (ng/mL)	Cut-point F/T PSA (%)
ACS:180 PSA2 and free PSA	100	0	0	0.02	90
	95	10	17	1.7	25
	90	25	54	3.3	15
Enzymun PSA and free PSA	100	0	0	0.02	100
	95	5	7	1.1	43
	90	24	32	3.0	23
Tandem-R PSA and free PSA	100	1	1	0.4	52
	95	7	22	2.1	25
	90	17	31	3.4	21

to enhance the performance of this assay primarily related to improvement in specificity. This has stemmed from the realization that many men will have more than one PSA determination during the course of their lifetime and thus, given the slow growing nature of most prostatic carcinoma, a false-negative test is likely to revert to positive during the time when cure is still possible.

In contrast, lack of specificity with the high false-positive rate, is expensive both in an economic sense owing to the fact that it necessitates further evaluation

Table 4 Comparison of the sensitivity and specificity of total PSA and the free to total PSA Ratio. Data from all patients in the BPH and prostate cancer groups is included

Assay	Sensitivity (%)	Specificity (%) total PSA	Specificity (%) F/T PSA	Cut-point total PSA (ng/mL)	Cut-point F/T PSA (%)
ACS:180 PSA2 and free PSA	100	0	5	4.0	36
	95	12	49	4.5	16
	90	17	54	4.7	14
Enzymun PSA and free PSA	100	1.5	0	3.2	57
	95	3	29	3.6	24
	90	15	51	4.0	17
Tandem-R PSA and free PSA	100	3	2	3.8	52
	95	12	25	4.1	26
	90	17	38	4.5	20

including ultrasound and biopsy, and also because of the stress it creates for the patient and his family. Of the modalities to enhance PSA specificity, determination of the free to total PSA has emerged as the most promising.[1,7–16]

However, significant problems exist in this regard, including the optimum setting in which to apply this assay as well as the cutoff to utilize. Moreover, significant assay variation has emerged as a potential confounder when different manufacturers' assays are utilized. Given the fact the free to total PSA necessitates two assay determinations, potential manufacturers' biases possibly significantly enhance differences in results. For example, the free PSA assay of one manufacturer reads lower than that in the literature and the total PSA (perhaps by another manufacturer) has a positive bias. The quotient will vary considerably from reference or literature values.

Conclusion

In this present investigation we compared total and free to total PSA with three manufacturers' assays in a very 'real world' setting. We chosed the patients being evaluated in an active clinical laboratory setting rather than derive specimens from our archival serum bank to obviate potential storage artifacts as well as potential sampling bias. We observed substantial concordance between the ACS : 180 free to toal PSA and that of the Tandem free to total. The Enzymun assay performed well when only data from the total PSA range of 4–10 ng/mL was included.

These findings underscore the absolute requirement for each manufacturer, and indeed each laboratory, to provide the clinician actual outcome data in their setting. This will guide urologists in the proper selection of appropriate cut-points and allow them to counsel the patient with meaningful risk assessment of the likelihood of prostatic carcinoma.

Acknowledgements

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References

1 Catalona WJ *et al*. Measurement of PSA in serum as a screening test for prostate cancer. *NEJM* 1991; **324**: 1156–1161.
2 Brawer MK *et al*. Screening for prostatic carcinoma with PSA. *J Urol* 1992; **147**: 841–845.
3 Cooner WH *et al*. Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate-specific antigen. *J Urol* 1990; **143**: 1146–1152.
4 Babaian RJ, Camps JL The role of PSA as part of the diagnostic triad and as a guide when to perform a biopsy. *Cancer* 1991; **68**: 2060–2063.
5 Nixon RG, Brawer MK Refinements in serum prostate-specific antigen testing for the diagnosis of prostate cancer, In: O'Leary MP, Kirby R (eds.) *Recent advances in Urology.* Churchill Livingstone: London, 1998.
6 Brawer MK, Kirby RS (eds.). Fast Facts: PSA Health Press: London, 1998.
7 Luderer AA *et al*. Measurement of the proportion of free to total PSA improves diagnostic performance of PSA in the diagnostic gray zone of total PSA. *Urology* 1995; **46**: 187–194.
8 Catalona WJ *et al*. Evaluation of percentage of free serum PSA to improve specificity of prostate cancer screening. *JAMA* 1995; **274**: 1214–1220.
9 Woodrum DL *et al*. Interpretation of free prostate-specific antigen clinical research studies for the detection of prostate cancer. *J Urol* 1998; **159**: 5–12.
10 Bangma CH, Kranse R, Blijenberg BG, Schroder FH. The value of screening tests in the detection of prostate cancer. Part I: Results of a retrospective evaluation of 1726 men. *Urology* 1995; **46**: 773–778.
11 Bangma CH, Kranse R, Blijenberg BG, Schroder FH. The value of screening tests in the detection of prostate cancer: Retrospective analysis of free/total prostate-specific antigen ratio, age-specific reference ranges, and PSA density. *Urology* 1995; **46**: 779.
12 Chen Y *et al*. Using proportions of free to total prostate-specific antigen, age, and total prostate-specific antigen to predict the probability of prostate cancer. *Urology* 1996; **47**: 518–524.
13 Prestigiacomo AF *et al*. A compariosn of the free fraction of serum prostate-specific antigen in men with benign and cancerous prostates: The best case scenario. *J Urol* 1996; **156**: 350–354.
14 Elgamal A *et al*. Free-to-total prostate-specific antigen ratio as a single test for detection of significant stage T1c prostate cancer. *J Urol* 1996; **156**: 1042–1049.
15 Van Cangh PJ *et al*. Free to total prostate-specific antigen (PSA) ratio is superior to total-PSA in differentiating benign prostate hypertrophy from prostate cancer. *Prostate Suppl* 1996; **7**: 30.
16 Partin AW *et al*. Analysis of percent free PSA for prostate cancer detection: Influence of total PSA, prostate volume and age. *Urology* 1996; **48**: 55–61.
17 Nixon RG, Gold MH, Blase AB, Meyer GE, Brawer MK. Comparison of three investigative assays for the free form of prostate-specific antigen. *J Urol* 1998; **160**: 420–425.
18 Christensson AG, Abrahamsson PA, Bjork T, Cockett AT, Lilja H. An increased proportion of the serum PSA complexed to alpha-1 antichymotrypsin discriminates prostate cancer from benign prostatic hypertrophy. *J Urol* 1992; **147**: 706.