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Original Paper

Clinical Evaluation of Free PSA/Total PSA (Prostate-specific Antigen) Ratio in the Diagnosis of Prostate Cancer

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The objective of this study was to evaluate the utility of the free/total prostate-specific antigen (PSA) ratio (per cent free PSA) in the diagnosis of prostate cancer. Serum total PSA and free PSA concentrations were measured in 156 patients with benign prostate hyperplasia (BPH) and 74 patients with prostate cancer using Hybritech Tandem immunoradiometric assays. Patients with prostate cancer had a significantly lower free/total PSA ratio than patients with BPH, although the distributions across study groups overlapped. In patients with a total PSA level between 4 μg/l and 25 μg/l, free/total PSA demonstrated better diagnostic utility than total PSA alone. © **1997 Published by Elsevier Science Ltd.**

Key words: PSA, free PSA, prostate cancer

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INTRODUCTION

PROSTATE-SPECIFIC ANTIGEN (PSA) is a 33 kDa glycoprotein produced by the prostatic epithelium [1] and has been widely used in the follow-up of patients with prostate cancer. However, PSA utility in prostate cancer diagnosis still remains controversial due to lack of specificity [2].

Recently, different PSA forms have been described in serum [3–5]. Circulating PSA is found to occur in complexed and free forms. Complexed PSA is predominantly bound to the protease inhibitors alpha-1-anti-chymotrypsin (PSA-ACT) and alpha-2-macroglobulin (PSA-AMG). PSA-ACT is detected in all current commercial immunoassays while PSA-AMG is not. Non-complexed PSA (free PSA) is found in lower concentrations than complexed PSA and is measured in various degrees by all commercial PSA assays. Total PSA determination in those assays corresponds, therefore, to combined measurements of PSA-ACT and free PSA. Free PSA can also be measured specifically by immunoassays using free PSA specific antibodies.

Preliminary studies have shown that the percentage of free PSA is different in prostate cancer and benign prostate hyperplasia (BPH), suggesting that determination of the free PSA form has a diagnostic utility [4]. The objective of this study was to evaluate the utility of free PSA measurement in prostate cancer diagnosis.

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PATIENTS AND METHODS

A retrospective study was carried out based on the evaluation of 230 serum samples of patients with prostate disease from our serum bank. The study included 156 patients with BPH and 74 untreated prostate cancer patients. The diagnosis of prostate cancer was confirmed by histological examination. Prostate volume was determined by transrectal ultrasonography in 57 patients with cancer. To avoid the inclusion of occult cancer, all the patients with BPH included in the study demonstrated negative rectal digital examination and negative transrectal ultrasonography. Transurethral resection was performed in all patients, with the possible existence of an unknown tumour being excluded by anatomopathological examination. Likewise, after a minimum follow-up of 1 year, none of the patients had developed prostate cancer.

Venous blood was obtained by venipuncture and serum was stored at −20°C until assayed. In no case was the prostate gland manipulated during the 48 h prior to blood withdrawal. Total PSA and free PSA were measured with the Tandem-R PSA and Tandem-R free PSA assays, respectively (Hybritech Europe, Liège, Belgium).

Tandem-R free PSA is a newly developed double antibody immunoradiometric assay. One monoclonal antibody coated on to polystyrene beads recognises free and bound PSA equally. A second monoclonal antibody highly specific to free PSA is labelled with 125-iodine and used as a tracer. After formation of the solid phase capture antibody-free

Table 1. Distribution of results of PSA and free/total PSA ratio by patient group

	BPH Number of patients (%)	Cancer Number of patients (%)
Free/total PSA		
Mean	0.195 ± 0.09	0.100 ± 0.06
<0.110	16 (10%)	45 (61%)
0.110–0.150	31 (20%)	18 (24%)
0.151–0.200	51 (33%)	6 (8%)
0.210–0.250	24 (15%)	1 (1%)
0.251–0.3	21 (13%)	4 (5%)
>0.3	13 (8%)	0 (0%)
PSA (μg/l)		
Mean	4.77 ± 4.42	75.14 ± 289.28
0–4	83 (53%)	4 (5%)
4.1–10	58 (37%)	17 (23%)
>10	15 (10%)	53 (72%)
Total patients	156	74

PSA–tracer sandwich, beads are washed to eliminate unbound excess tracer and bound radioactivity, as measured in a gamma counter, is proportional to the free PSA concentration in the sample. In all cases the free/total PSA ratio was determined. To analyse the results, we chose 0.13 (corresponding to the cut-off with the higher efficacy in the group of patients with PSA between 4 and 25 mg/l) and 0.15 and 0.2 as proposed by other groups [6].

RESULTS

Table 1 summarises the distribution characteristics of total PSA and free/total PSA across study groups (BPH and prostate cancer). Total PSA concentration and per cent free PSA differed across groups. In general, the free/total PSA ratio was found to be lower in the group of patients with prostate cancer compared to BPH, while total PSA was higher.

As shown in Table 2, when all subjects were included, a higher percentage of men with prostate cancer than with BPH had a free/total PSA ratio below 0.113 irrespective of the total PSA range considered.

Receiver-operating characteristic (ROC) curve analysis was used to evaluate the reciprocal relationship between sensitivity and specificity and to compare total PSA with free/total PSA in terms of distinguishing between prostate cancer and BPH. When all subjects were included (Figure 1), ROC curve analysis revealed that there was almost similar performance between total PSA and the free/total PSA ratio. In contrast, when patients with total PSA concentrations between 4 and 25 μg/l were selected, an improved performance for the free/total PSA ratio was found compared to total PSA (Figure 2). The free/total PSA ratio had a higher sensitivity than total PSA for all specificity levels. Similarly, Table 3 compares total PSA and the free/total PSA ratio at various

Table 2. Percentage of patients with free/total PSA ratio lower than 0.113 classified according to PSA levels

	Total PSA 0–4 μg/l	4.1–10 μg/l	10.1–25 μg/l	> 25 μg/l
BPH	12/83 (14%)	6/58 (10%)	1/15 (7%)	
Cancer	2/4 (50%)	8/17 (47%)	17/21 (81%)	26/32 (81%)

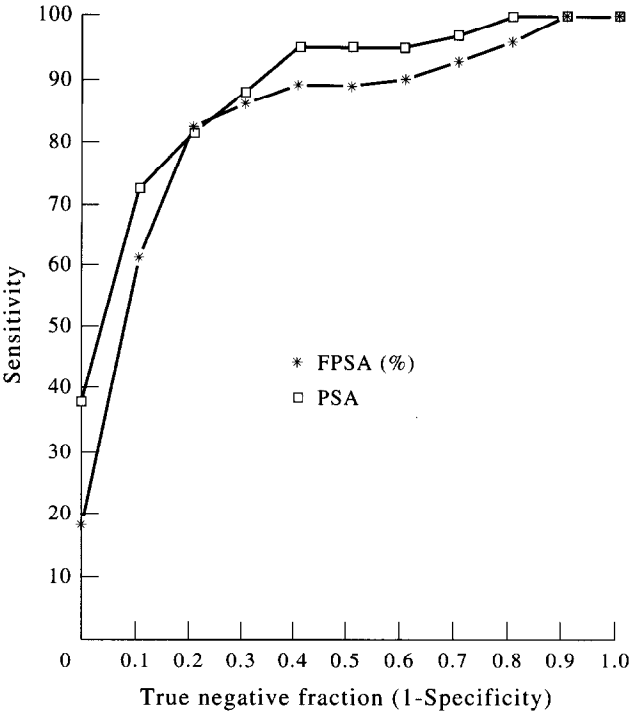

Figure 1. ROC curve for PSA and free/total PSA ratio.

specificity levels, ranking from 100% to 50%, in terms of sensitivity and efficiency in subjects with total PSA between 4 and 25 μg/l. An optimal 82% efficiency was obtained with a free/total PSA ratio cut-off of 0.113. At all specificity values, sensitivity and efficiency were higher for the free/total PSA ratio than total PSA.

To investigate further the clinical utility of the free/total PSA ratio, cut-off points were compared for predicting cancer in patients with total PSA between 4 and 10 μg/l. As illustrated in Table 4, a free/total PSA ratio cut-off of 0.113

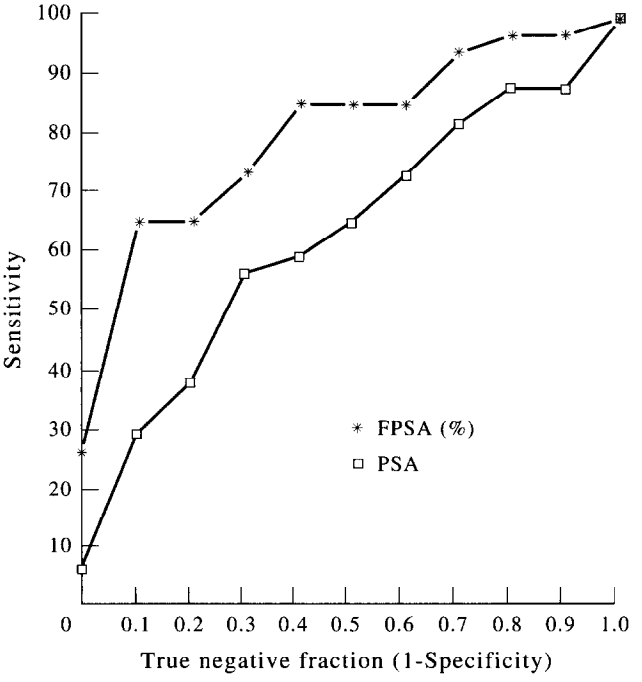

Figure 2. ROC curve for PSA and free/total PSA ratio in patients with total PSA levels between 4 and 25 μg/l.

Table 3. Diagnostic value of free/total PSA ratio and total PSA in patients with a total PSA level between 4 and 25 μg/l

	Free/total PSA			PSA		
Specificity	Cut-off	Sensitivity	Efficiency	Cut-off (μg/l)	Sensitivity	Efficiency
100	0.077	26	76	18	6	69
98	0.081	35	78	17	9	69
95	0.086	42	77	14	29	73
90	0.113	65	82	13	29	70
80	0.126	65	75	10	38	67
70	0.142	73	71	8.3	56	66
60	0.159	85	72	7.7	59	60
50	0.167	85	64	6.7	65	54

provided a positive predictive value (PPV) of 57%. The PPV decreased when the free/total PSA ratio cut-off was increased. The same results are shown in Table 5 for patients with total PSA ratios between 10 and 25 μg/l.

Finally, Table 6 shows that the sensitivity of per cent free PSA was related to the estimated prostate volume. The free/total PSA ratio had a higher sensitivity in the cancer group characterised by a small (<50 cm^3) compared to large (≥50 cm^3) prostate volume.

DISCUSSION

BPH is a frequent cause of increased PSA levels. Between 25 and 50% of patients with BPH show PSA concentrations above the upper limit of the normal range (4 μg/l) [2, 7–9]. As a consequence, the specificity of an elevated PSA is often considered as being too low for prostate cancer diagnosis. For instance, in a recent study of early diagnosis of prostate cancer, we found that only 24% of 504 patients with PSA levels above 4 μg/l and 46% of 208 patients with PSA levels above 10 μg/l had histologically confirmed prostate cancer [10]. Conversely, among patients with BPH, 47% and 10% were shown to have PSA levels above 4 μg/l and 10 μg/l, respectively.

Different strategies have been proposed to improve PSA specificity, including the use of PSA density (PSAD) and PSA velocity [11, 12]. However, shortcomings exist related to either a lack of precision in prostate volume measurement or falsely elevated PSA concentrations due to BPH complications or biological and analytical variability in serial PSA determinations.

More recently, different groups reported that the free/total PSA ratio was lower in patients with prostate cancer compared to BPH [4, 13, 14]. It has been suggested that the use of per cent free PSA instead of PSA alone is likely to improve the specificity for detection of prostate cancer [6].

Our results are in agreement with this view. The mean free/total PSA ratio was found to be lower in the group of patients with prostate cancer compared to BPH. However, the distributions of individual values show an overlap across groups (Table 1). Consequently, when all subjects included in our study were considered, the free/total PSA ratio did not provide any significant advantage over PSA in terms of diagnostic efficacy. This was confirmed by ROC curve analysis (Figure 1) comparing the free/total PSA ratio and PSA in the whole study population and revealing very similar performance characteristics. The distribution overlap of the free/total PSA ratio between prostate cancer and BPH groups may be due, at least in part, to coexisting hyperplasia and carcinoma areas within the same prostatic gland. More than 80% of patients with prostate cancer would also have histological BPH [15]. It may be hypothesised that carcinomas associated with important BPH might not lead to a significant decrease in the free/total PSA ratio compared to BPH alone. Our finding that the sensitivity of the per cent free PSA was less in cancer patients with larger prostate volumes (Table 6) is in agreement with this hypothesis. Furthermore, this finding suggests that the sensitivity of the free/total PSA ratio may be greater when applied to early diagnosis of the disease than when applied to the differential diagnosis between BPH and cancer.

In general, patients with BPH present PSA levels of less than 10 μg/l. However, in those cases of BPH with urinary infection and/or acute urine retention, levels greater than 10 μg/l may be observed, although they are usually lower than 25 μg/l. In our experience, slightly more than 25% of the patients with complicated BPH present with PSA levels greater than 10 μg/l [16, 17]. Accordingly, we believe that use of the free/total PSA ratio will be most effective in men with PSA values not exceeding 25 μg/l. In this study, we found that the performance characteristics of the free/total PSA ratio improved significantly compared to PSA alone when patient groups were restricted to subjects with PSA values between 4.0 and 25.0 μg/l. In this subset of patients, ROC curve analysis clearly showed that the free/total PSA ratio is a better predictor of carcinoma than PSA. An optimal efficency was obtained with a free/total PSA ratio cut-off of 0.113 with 90% specificity and 65% sensitivity.

Table 4. Usefulness of free/total PSA ratio in patients with a total PSA level between 4 and 10 μg/l

	Cut-off <0.113	Free/total <0.15	PSA ratio <0.2
Prostate cancer	8/17 (47%)	12/17 (71%)	16/17 (94%)
BPH	6/58 (10%)	20/58 (34%)	38/58 (66%)
PPV	57%	37.5%	30%

PPV, positive predictive value.

Table 5. Usefulness of free/total PSA ratio in patients with PSA between 10 and 25 μg/l

	Cut-off <0.113	Free/total <0.15	PSA ratio <0.2
CaP	17/21 (81%)	19/21 (90%)	20/21 (95%)
BPH	1/15 (7%)	5/15 (33%)	10/15 (67%)
PPV	94%	79%	67%

PPV, positive predictive value.

Table 6. Percentage of patients with prostate cancer and free/total PSA ratio lower than 0.113 classified according to volume of prostate

	Number of patients	(%)
Volume <50 cm^3	26/32	81%
Volume ≥50 cm^3	15/25	60%

Most patients with curable prostate cancer have PSA levels below 10 μg/l [18]. For this reason, we also re-evaluated free/total PSA ratio diagnosis utility for patients with PSA values between 4 and 10 μg/l. This PSA range corresponds to the 'grey zone' where the differential diagnosis of prostate cancer is more problematic. In this subgroup, we calculated that biopsy would reveal histologically confirmed prostate cancer in only 23% of patients. This result emphasises the high false-positive rate produced by PSA when it is used alone to predict potentially curable prostate cancer and highlights the need to improve the specificity of the test in order to decrease the number of unnecessary biopsies. Our results clearly show that use of the free/total PSA ratio leads to such an improvement.

In Table 4, we calculated the PPV that would have been obtained with different free/total PSA ratio cut-off points in those patients with PSA between 4 and 10 μg/l. We determined that a per cent free PSA cut-off of 0.113 would have led to a PPV of 57% with 47% sensitivity and 90% specificity. Compared to a PSA cut-off of 4 μg/l, a free/total PSA cut-off of 0.113 would thus have decreased the false-positive rate from 47% to 10%. Increasing the free/total PSA ratio cut-off would result in higher sensitivity but at the expense of a loss of specificity. Using a cut-off point of 0.2 would have resulted in 94% sensitivity and 34% specificity. Consequently, based on this data set, less than 10% of the cancer group would have been missed, while 34% of unnecessary biopsies would have been spared in the BPH group. The use of a cut-off of greater than 0.113 increases the sensitivity, but decreases the specificity of the test, thus leading to some doubts (economic cost, unnecessary performance of biopsies) as to the suitability of the test as an indicator for biopsy when the results range from 0.113 to 0.2. The use of a higher cut-off may only be indicated in patients under the age of 65 years, who may particularly benefit from radical treatment. However, the greater sensitivity of the free/total PSA ratio observed in patients with smaller prostate volumes (Table 6) indicates that the free/total PSA ratio may be of greater diagnostic efficacy in the younger group of patients (thus, with smaller prostates). Complicated BPH (urinary infection and/or acute urine retention) is the most common cause of false positives of PSA levels greater than 10 μg/l. We believe that a cut-off of the free/total PSA ratio of approximately 20% should be used as an indicator of biopsy. In our series, the use of a 20% cut-off in patients with PSA between 10 and 25 μg/l allows a 33% reduction in necessary biopsies with hardly any loss of sensitivity (Table 5).

In summary, our results show that use of the free/total PSA ratio improves PSA-based differential diagnosis of prostate cancer in patients with PSA values between 4 and 25 μg/l. Per cent free PSA can also lead to more specific identification of potentially curable prostate cancer in patients with PSA levels below 10 μg/l, reducing unnecessary prostate biopsies. However, further prospective studies are needed to investigate the combined use of free PSA, total PSA and rectal examination for prostate cancer diagnosis, to confirm these results and define the most appropriate cut-off for the free/total PSA ratio. This should allow for earlier diagnosis of prostate cancer and potentially improve the prognosis of the disease.

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