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Original Paper

Percentage of Free Serum Prostate-specific Antigen: a New Tool in the Early Diagnosis of Prostatic Cancer

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Prostate-specific antigen (PSA) is a protease able to bind to serum antiproteases as alpha 1 antichymotrypsin (ACT). Free PSA (FPSA) corresponds to the fraction of total PSA (TPSA) which is unbound to ACT. Specific detection of the FPSA seems to be a valuable tool in the distinction between prostatic cancer (PCa) and benign prostatic hyperplasia (BPH). Our aim was to evaluate retrospectively the FPSA/TPSA ratio in comparison to TPSA or FPSA determination, using two new immunoradiometric assays (PSA-RIACT and FPSA-RIACT, CIS bio international, Gif Sur Yvette, France) in the early diagnosis of PCa. 256 men, with TPSA levels between 0.7 and 44.7 ng/ml (median age = 69 years), including 164 sera obtained from patients with BPH and 92 sera from patients with untreated PCa were assayed. All diagnoses were histologically confirmed and patients tested before any adjuvant treatment. The evaluation of the median FPSA/TPSA ratio in the two groups showed significantly different values (BPH group: 24.2%, PCa group: 12.1%, $P < 0.0001$). By R.O.C. (Receiver-Operating-Characteristics) analysis, we show that the FPSA/TPSA ratio is the method of choice for discriminating BPH and PCa, since the area under curve is the greatest for the FPSA/TPSA ratio curve, as compared to the TPSA or FPSA curves ($P < 0.0001$). The best accuracy (number of true positive + true negative/total = 82.4%) was obtained with a FPSA/TPSA ratio $\leqslant 15\%$ with high odds ratio (20.5; confidence interval (CI): 11.2; 37.7). Of interest, similar results were also confirmed even in the subpopulation with serum TPSA levels between 2.5 and 10 ng/ml (161 patients including 99 BPH and 62 PCa). We thus confirm that combined serum measurement of FPSA and TPSA is of particular interest in the early diagnosis of PCa for patients with non-suspicious digital rectal examination and a TPSA value between 2.5 and 10 ng/ml. In those patients, biopsy should be reserved to the cases with FPSA/TPSA below 15%, which allows significant odds ratio (12.8; CI: 5.2; 31.4). Otherwise, to avoid the risk of missing any PCa, usual follow-up with combined TPSA and FPSA determination would be required with the same criteria of biopsy (i.e. FPSA/TPSA ratio $\leqslant 15\%$ when TPSA value is between 2.5 and 10 ng/ml; or TPSA > 10 ng/ml).

Key words: free prostate-specific antigen, immunoassay, prostatic neoplasms, prostatic hypertrophy

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INTRODUCTION

PSA (PROSTATE-SPECIFIC ANTIGEN) is an organ-specific marker, but is not specific of cancer since it is also elevated in benign diseases such as benign prostatic hyperplasia

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(BPH) [1–3]. Its usefulness in the management of prostatic cancer (PCa) is now clearly established, and when used alone, PSA is recognised to be the most sensitive tool in detecting early malignant disease [4, 5]. Nevertheless, the screening of prostatic cancer remains highly controversial due to an uncertainty regarding any real benefit in survival, and difficulties in choosing the most appropriate treatment and cost [6].

From its identification in 1979, it has been shown that this serine protease-related protein may be present in different forms in serum [7, 8]. Three have been identified, one a 25–40 kD protein recognised as free PSA (FPSA), one 80–90 kD (alpha 1 antichymotrypsin (ACT)-bound PSA) and one inaccessible to immunoassays but detected by electrophoresis and referred to as alpha 2 macroglobulin-bound PSA [9]. The description of the PSA epitope mapping allows the choice of antibodies for the development of kits measuring FPSA. Until now, only the measurement of PSA referred to as total PSA (TPSA) was possible.

The first clinical studies using the results from the measurement of FPSA and TPSA in serum showed that the ratio FPSA/TPSA was a better parameter to discriminate between PCa and BPH than PSA alone or PSA–ACT alone [10–15]. This application could be of real interest when the clinical diagnosis is difficult due to what is called an 'intermediate' level of TPSA, between 2.5 and 10 ng/ml for a patient older than 40 years [2, 16]. In this range of TPSA values, a clear-cut clinical behaviour is not evident .

We designed this study to investigate the clinical value of FPSA and the ratio FPSA/TPSA, compared with TPSA, in the differential diagnosis of prostatic cancer from BPH.

PATIENTS AND METHODS

There were 256 consecutive patients including 164 benign prostate hypertrophy (BPH) and 92 untreated prostatic cancer (PCa) (34 T1, 49 T2 and 9 T3, according to the UICC classification) from the Urology Department of St Louis Hospital, Paris and the Prostatic Disease Observation Centre of Agen (Lot et Garonne). The median age of the BPH and PCa patients was 69 years, with no significant difference between the groups ($P = 0.93$). All BPH patients had non-suspicious digital rectal examination (DRE) and 6 negative transrectal ultrasound guided needle biopsies (3 in each lobe) or negative histological findings after transurethral resection of prostate (TURP). Diagnosis of PCa was made on positive biopsy or after TURP, and confirmed after radical prostatectomy in operated patients. Patients with prostatitis or other cancers were excluded. All sera were included retrospectively; they were routinely sampled prior to the biopsy and before any adjuvant treatment, and stored at −20°C from February 1992 to November 1994.

Total PSA (TPSA) and free-PSA (FPSA) were assayed using two new commercially available radioimmunological kits, respectively, PSA-RIACT and FPSA-RIACT (CIS bio international, BP 32, 91192-Gif sur Yvette, France). These assays are immunoradiometric sandwich type assays, each made with two different mouse monoclonal antibodies; the solid phase is a coated tube and the tracer, iodine 125. The results are expressed in ng/ml for TPSA and FPSA. The *R* ratio (FPSA/TPSA) was also calculated in each case and expressed as a percentage. The interassay coefficients of variation were $\leqslant 6\%$ for the PSA-RIACT kit and $\leqslant 5\%$ for the FPSA-RIACT kit. Three samples with TPSA concentrations between 36.8 and 76 ng/ml were serially diluted up to four times, the recovery range being between 82 and 100%; similarly, three samples with FPSA concentrations between 4.23 and 14.30 ng/ml were serially diluted up to four times, the recovery range being between 98 and 101%. PSA-RIACT and FPSA-RIACT are, respectively, well correlated with Tandem-R PSA ($r^2 = 0.97$, regression line is $y = 0.95x - 0.13$, in which y is the Tandem-R PSA value and x is the corresponding value measured with PSA-RIACT reagent), and Tandem-R free PSA ($r^2 = 0.87$, regression line is $y = 0.98x + 0.08$, in which y is the Tandem-R free PSA value and x is the corresponding value measured with FPSA-RIACT reagent). The stability of TPSA as measured in fresh and frozen stored serum samples was evaluated on 22 samples with TPSA concentrations ranging from 1.12 to 41 ng/ml: mean coefficient of variation (± S.D.) TPSA concentration in fresh and stored pairs was very weak: 1.98% (±5.56%). In order to establish a normal range of values, blood samples from 143 healthy male donors were assessed; none had previous prostatic history and none showed any urinary symptoms at the time of sampling. This population was divided into four groups according to age: 20–30 years ($n = 28$), 31–40 years ($n = 59$), 41–50 years ($n = 36$) and > 50 years ($n = 20$). The median age was 39 years. A constant increase of the 95th percentile from the first age group to the last for TPSA and FPSA was observed, whereas the 95th percentile of the *R* ratio remained between 78.4% and 90.0%, without constant increase or decrease (Table 1).

Comparisons were conducted using the Mann–Whitney *U*-test. Statistical significance was considered as $P < 0.05$. Risk of cancer was estimated with odds ratio with 95% confidence interval. The area under R.O.C. (Receiver-Operating-Characteristics) curves was calculated with GraphROC for Windows Software [17] according to the Hanley method [18].

Table 1. Normal levels of TPSA, FPSA and R *(FPSA/TPSA) ratio measured in blood from healthy male donors with no previous prostatic history and no urinary symptoms*

	md* (5th–95th)†	md* (5th–95th)†	md* (5th–95th)†	md* (5th–95th)†
Age (years)	20–30 ($n = 28$)	31–40 ($n = 59$)	41–50 ($n = 36$)	>51 ($n = 20$)
TPSA‡	0.67(0.22–1.1)	0.75(0.32–1.4)	0.98(0.45–1.9)	0.89(0.10–3.6)
FPSA‡	0.38(0.20–0.62)	0.35(0.16–0.70)	0.42(0.24–0.83)	0.35(0.04–1.1)
R§	60.2(35.0–90.0)	48.7(30.0–78.4)	48.1(43.0–80.0)	36.5(11.8–87.9)

* md, median; † 5th–95th percentile; ‡ (ng/ml); § (%).

Table 2. TPSA, FPSA and R *(FPSA/TPSA) ratio in BPH and PCa patients*

	BPH (n = 164)		PCa (n = 92)		
	md* (5th–95th)†	Range	md* (5th–95th)†	Range	P
Age (years)	69(58–85.3)	48–98	69(55.2–84)	46–92	0.93
TPSA‡	4.9(1.74–25.2)	1.33–44.7	6.68(1.45–21.3)	0.7–27.9	0.049
FPSA‡	1.1(0.42–6.3)	0.35–12.4	0.77(0.17–3.3)	0.07–6.08	<0.0001
R§	24.2(12.4–43.9)	8–62	12.1(6.6–32)	2–55	<0.0001

* md, median; † 5th–95th percentile; ‡ (ng/ml); § (%).

RESULTS

TPSA, FPSA and R *ratio results (Table 2)*

Median TPSA was lower in the BPH group (4.9 ng/ml) than in PCa group (6.68 ng/ml), but the difference was weak ($P = 0.049$). Conversely, median FPSA was significantly higher in the BPH group ($P < 0.0001$). The median R ratio was significantly higher in the BPH (24.2%) than the PCa group (12.1%, $P < 0.0001$).

Differences of the R *ratio between BPH and PCa*

The determination of R according to TPSA values between 0.7 and 44.7 ng/ml is shown in Figure 1 for BPH and PCa samples; most of the BPH are in the area where TPSA is below 10 ng/ml and the R ratio is above 15%, whereas PCa are, in the majority, in the area where the R ratio is below 15%.

In order to assess the performances of the different biological parameters, sensitivity and specificity were calculated and reported on R.O.C. curves (Figure 2). The best accuracy (number of true positive + true negative/total = 82.4%) was obtained with a cut-off R ratio at 15%, with specificity of 90.8% and sensitivity of 67.4%; the risk of cancer is then very high (odds ratio (OR) = 20.5; confidence interval (CI): 11.2; 37.7) (Table 3). Furthermore, the area under curve (AUC) of R ratio was more important than AUC of TPSA ($z = -5.64$, $P < 0.0001$) or FPSA ($z = -5.18$, $P < 0.0001$). Moreover, the R.O.C. analysis showed that a specificity of 90.8% was obtained either for $R \leqslant 15\%$ or TPSA $\geqslant 18$ ng/ml; but for this TPSA value, the sensitivity was very low (7.6%).

As we are interested in the clinical usefulness of the different parameters tested here for a range of TPSA between 2.5 and 10 ng/ml, we evaluated performances of the R ratio in this range for the 161 concerned patients (62 PCa (26 T1, 32 T2 and 4 T3) and 99 BPH). Results, comparable to the previous ones in the whole population, were confirmed in this subpopulation (Table 4). Of interest, we also found that the AUC remained greater for the FPSA/TPSA curve compared to the TPSA ($z = -4.04$, $P < 0.0001$).

When patients with non-suspicious DRE and TPSA values between 2.5 and 10 ng/ml were examined (125 patients: 99 BPH and 26 PCa), an R ratio $\leqslant 15\%$ gave a significant odds ratio of 12.8 (95% CI 5.2–31.4), and 88.9% (88/99) prostatic biopsies should be avoided. However, the sensitivity was only 61.5% (16/26 T1 PCa).

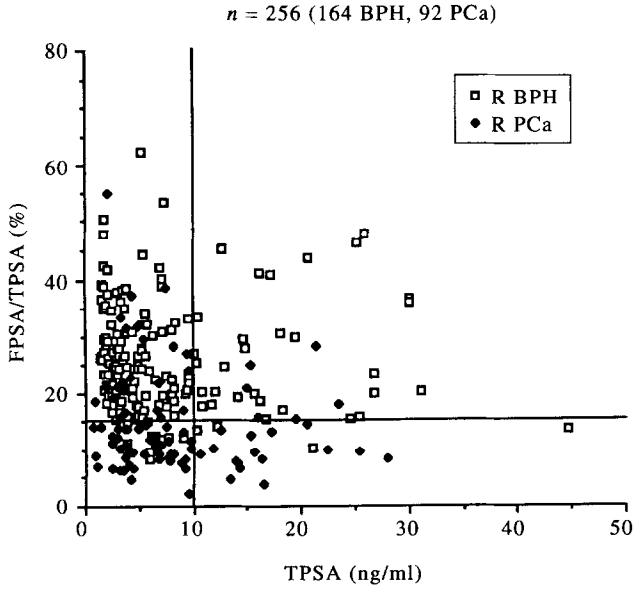

Figure 1. Distribution of R ratio as a function of TPSA (0.7–44.7 ng/ml) in the 256 patients (164 BPH, 92 PCa). The 10 ng/ml TPSA and 15% R ratio thresholds are indicated.

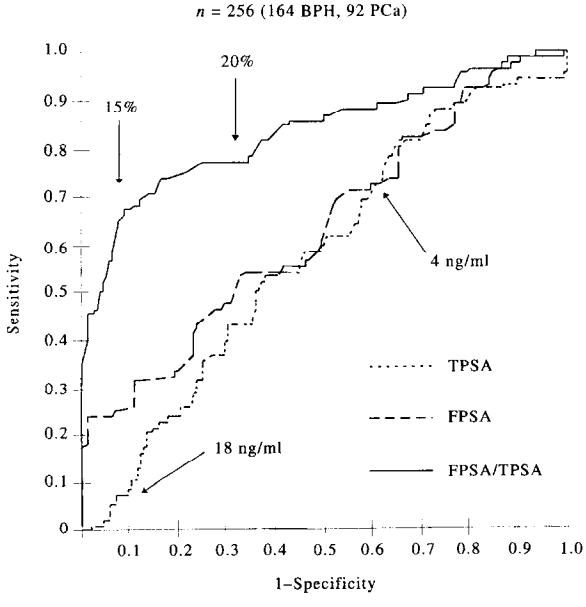

Figure 2. Representation of the R.O.C. (Receiver Operating Characteristics) analysis of the 256 patients (164 BPH, 92 PCa), with TPSA between 0.7 and 44.7 ng/ml. Different cut-off points are indicated on the TPSA and R ratio curves. AUC under FPSA/TPSA is greater than AUC under FPSA and TPSA ($P < 0.0001$ in each case). The same specificity (90.8%) was observed either with a R ratio of 15% or a TPSA value of 18 ng/ml, but the sensitivity was much lower with TPSA (7.6%).

Table 3. Performance of R *(FPSA/TPSA) ratio (92 PCa versus 164 BPH)*

	Sensitivity	Specificity	PPV	NPV	Accuracy	Odds ratio (CI 95%)
$R \leqslant 10\%$	41.3	98.2	74.9(77.1)*	92.7(83.1)*	77.7	37.8(16.0; 89.4)
$R \leqslant 15\%$	67.4	90.8	80.5(70.7)*	83.2(88.9)*	82.4	20.5(11.2; 37.7)
$R \leqslant 20\%$	77.2	65.8	55.9(44.8)*	83.7(90.0)*	69.9	6.5(3.7; 11.4)
$R \leqslant 25\%$	88.0	45.7	47.6(35.1)*	87.2(92.0)*	60.9	6.2(3.2; 11.9)

PPV, positive predictive value; NPV, negative predictive value. * Corrected by the prevalence of PCa in the general population, which is 0.25.

DISCUSSION

To improve early diagnosis of PCa, different parameters have been studied. Veneziano and associates [19] and Benson and associates [20, 21] have proposed the ratio of TPSA to prostatic volume (PSA density), but subsequent studies have shown that PSA density is no better than TPSA alone [22–26]. Similarly, the interpretation of TPSA level as a function of age is controversial. Oesterling and colleagues [27] showed that TPSA thresholds increase with age, thus modifying indications of prostatic biopsy. However, Catalona and coworkers disagreed using a higher cut-off point to avoid a decrease in the sensitivity of the test [28]. As the median age in the BPH and PCa groups was the same in our study, we avoided any bias from this parameter.

TPSA measurement alone and for a cut-off of 4 ng/ml provides a sensitivity of 69.6%, but with a low specificity (42.1%) (data not shown). This result is in agreement with previously established results [29]. Taking into account the median age of BPH and PCa patients of our study (69 years), a higher cut-off point (5.2 ng/ml) is recommended by Jacobsen and Oesterling [30]. This cut-off, corresponding to the 95th percentile of TPSA at 69 years, gives intermediate TPSA specificity (51.8%) and sensitivity of 58.7%.

Only a very weak difference was observed between TPSA value of BPH and PCa populations ($P = 0.049$), but median FPSA and FPSA/TPSA ratios were both highly different ($P < 0.0001$) (Table 1). When comparing the three methods (TPSA alone, FPSA alone and FPSA/TPSA ratio), the best accuracy (82.4%) was obtained for a FPSA/TPSA cut-off of 15% which provides high specificity (90.8%) (Table 3). We evaluated the benefit provided by the FPSA/TPSA ratio compared to TPSA measurement alone; with TPSA measurement alone, an identical specificity of 90.8% (corresponding to the specificity obtained with a R ratio $\leqslant 15\%$) was obtained for a high TPSA cut-off at 18 ng/ml, but with a very low sensitivity (7.6%) (Figure 2). In our study with age matched patients and a very weak difference between TPSA in both populations, TPSA is of no interest since its curve is close to the 45° diagonal. The greatest AUC is observed for FPSA/TPSA ($P < 0.0001$ versus FPSA or versus TPSA), thus making it the method of choice. Our results are in agreement with the literature, in spite of the great variability of the TPSA and FPSA assays [13, 15, 31–35].

In current urological practice, diagnosic problems appear particularly when TPSA measurements gives a value between 2.5 ng/ml and 10 ng/ml. In our study, the ratio (FPSA/TPSA) with a cut-off of 15% was the most accurate (79.5%), with high specificity (88.9%) and a high odds ratio (14.6; CI: 6.9; 30.5) (Table 4). Furthermore, the area under the FPSA/TPSA curve remained the highest ($P < 0.0001$ versus FPSA or versus TPSA), even in this subpopulation.

Thus, it seems possible to refine indications of prostatic biopsies for patients with an intermediate TPSA value ($\leqslant 10$ ng/ml); using this FPSA/TPSA ratio, Catalona and colleagues proposed a higher cut-off (23.4%) which would eliminate more than one third of the useless prostatic biopsies when 90% of the cancers were diagnosed [13, 32]. They thus 'improve' the sensitivity of the test. In our study, when TPSA was below 10 ng/ml, we found that 90% of the cancers were diagnosed with a FPSA/TPSA ratio below 28%, which could also allow us to avoid 35% of prostatic biopsies, using the criteria used by Catalona and colleagues (i.e. TPSA $\leqslant 10$ ng/ml and in our study FPSA/TPSA $\leqslant 28\%$) [13]. However, with a sensitivity of 90%, no benefit was observed using FPSA/TPSA determination compared to TPSA alone, since both R.O.C. curves were very close to each other, so we propose to maintain the 15% cut-off R ratio which allows high specificity and furthermore the best accuracy even when TPSA is between 2.5 and 10 ng/ml (Table 4). Our results are in agreement with Filella and coworkers who recently found similar results (sensitivity of 44%, specificity of 95%) in the subgroup between 4 and 20 ng/ml, using different TPSA and FPSA immunoassays [15].

Table 4. Performance of R *(FPSA/TPSA) with TPSA between 2.5 and 10 ng/ml (62 PCa versus 99 BPH)*

	Sensitivity	Specificity	PPV	NPV	Accuracy	Odds ratio (CI 95%)
$R \leqslant 10\%$	38.7	98.0	92.3(85.1)*	71.8(81.8)*	75.2	30.6(10.3; 91.1)
$R \leqslant 15\%$	64.5	88.9	78.4(64.9)*	80.0(87.4)*	79.5	14.6(6.9; 30.5)
$R \leqslant 20\%$	74.2	63.6	56.1(38.8)*	79.8(86.1)*	67.7	5.0(2.6; 9.9)
$R \leqslant 25\%$	85.5	38.4	46.5(31.4)*	80.8(88.2)*	56.5	3.7(1.7; 8.1)

PPV, positive predictive value; NPV, negative predictive value. * Corrected by the prevalence of PCa in the general population, which is 0.25.

There is still a need to detect PCa while it remains confined to the gland because radical prostatectomy is the most effective therapy. Furthermore, it would be of great interest to be able to discriminate BPH from PCa patients using a blood sample. In our opinion, determination of the FPSA/TPSA ratio can help clinicians as a biopsy indicator, with a significant odds ratio of 12.8 obtained with an *R* ratio $\leqslant 15\%$. However, because the sensitivity is low (61.5%), annual clinical and biological follow-up would be required with the same criteria of biopsy (i.e. FPSA/TPSA ratio $\leqslant 15\%$ when TPSA value is between 2.5 and 10 ng/ml; or TPSA > 10 ng/ml) in order to avoid missing any PCa.

Nevertheless, a methodological bias in our results cannot be completely excluded, as in the studies by Catalona and associates and Filella and associates [13, 15], since PCa cannot be strictly eliminated in a case of BPH whose diagnosis is based on both non-suspicious rectal examination and negative biopsy or TURP. PCa can be missed on biopsy in around one third of patients, as has been shown with rebiopsy [36]. Work is in progress to evaluate the percentage of FPSA with a new methodological approach.

In conclusion, combined measurements of FPSA and TPSA appear to be useful in early diagnosis of prostatic cancer, especially in the management of patients with non-suspicious rectal examination and a TPSA value between 2.5 and 10 ng/ml where determination of FPSA/TPSA ratio can help clinicians as an indicator of prostatic biopsies. Therefore, it is a new and early diagnostic tool for prostatic cancer.

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