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Ratio of Free or Complexed Prostate-specific Antigen (PSA) to Total PSA: Which Ratio Improves Differentiation between Benign Prostatic Hyperplasia and Prostate Cancer?

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Background: **The aim of this study was to compare the diagnostic utility of a new assay that measures all forms of prostate-specific antigen complexed (cPSA) to serum proteins except α_2-macroglobulin with the assay of free PSA (fPSA) and the corresponding ratios to total PSA (tPSA) to improve the differentiation between benign prostatic hyperplasia (BPH) and prostate cancer (PCa).**

Methods: **Serum samples were collected from 91 men without prostate disease and with normal digital rectal examination (controls), 144 untreated patients with PCa, and 89 patients with BPH. tPSA and cPSA were measured using the Bayer Immuno 1 system; fPSA and the additional tPSA were measured with the Roche Elecsys system.**

Results: **The median cPSA/tPSA, fPSA/tPSA, and fPSA/cPSA ratios were significantly different between patients with BPH and PCa (78.7% vs 90.7%, 25.5% vs 12.1%, and 36.8% vs 14.3%, respectively; $P < 0.001$). No correlations of cPSA and its ratios to tumor stage and grade were found. ROC analysis showed that cPSA was not different from tPSA (areas under the curve, 0.632 vs 0.568), whereas the cPSA/tPSA ratio was similar to the fPSA/tPSA ratio in increasing discrimination between BPH and PCa patients with tPSA concentrations in the tPSA gray zone between 2 and 10 μg/L (areas under the curve, 0.851 vs 0.838).**

Conclusions: **Compared with tPSA, the fPSA/tPSA and cPSA/tPSA ratios both improve the differentiation between BPH and PCa comparably and are similarly effective in reducing the rate of unnecessary biopsies, whereas cPSA alone does not have any effect.**

Prostate-specific antigen (PSA)[4] is the most useful marker for the early detection of prostate cancer (PCa). When the conventional PSA cutoff of 4 μg/L is used as the discrimination limit between cancer and nonmalignant 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 *(1)*. However, the differentiation between BPH and PCa can be improved by determination of the serum PSA isoforms *(2)*.

PSA occurs in serum in different molecular forms *(2–4)*. Approximately 70–90% is bound to α_1-antichymotrypsin (ACT), and a small amount is complexed with α_1-antitrypsin and protein C. An additional portion of PSA that is complexed with α_2-macroglobulin can be measured only if the complex is opened and the PSA epitopes become accessible. Of the total PSA (tPSA) in serum, 10–30% is not bound to serum proteins and is called free PSA (fPSA). Numerous studies have demonstrated a lower ratio of fPSA to tPSA in PCa patients, calculated as the percentage of fPSA [reviewed in Ref. *(5)*]. This ratio has been considered a promising tool for distinguishing between PCa and BPH. It has also been shown that ACT-PSA and the corresponding ACT-PSA/tPSA ratio improved the specificity and sensitivity for PCa *(2, 3, 6)*. However, several analytical difficulties im-

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[4] Nonstandard abbreviations: PSA, prostate-specific antigen; PCa, prostate cancer; BPH, benign prostatic hyperplasia; ACT, α_1-antichymotrypsin; tPSA, total PSA, both free and complexed forms; fPSA, free, noncomplexed form of PSA; and cPSA, complexed forms of PSA.

pair accurate ACT-PSA measurement *(7)*. For example, overrecovery of the ACT-PSA complex results when anti-PSA antibodies are used on the solid phase to trap ACT-PSA complex and when anti-ACT antibodies are applied to detect this complex. This overrecovery is caused by the presence of ACT-cathepsin G complex in serum, which also binds to the solid phase *(8)*. Various approaches have been suggested to eliminate these technical problems *(7–9)*. The use of monoclonal antibodies specific against ACT-PSA with low cross-reactivities to the cathepsin G-ACT complex, ACT, and fPSA *(9)*, the addition of heparin *(8)*, and the application of special blocking reagents to reduce the nonspecific binding of anti-ACT antibodies *(7)* have been recommended. The recently introduced novel PSA assay that measures all complexed PSA (cPSA) except PSA complexed with α_2-macroglobulin also offers a promising possibility to eliminate the technical obstacles for reliable measurement *(10)*.

We used this new test in patients with BPH and PCa to evaluate the assay with the following aims: (*a*) to evaluate the diagnostic performance of the assay in comparison with tPSA; (*b*) to compare cPSA and fPSA alone or as corresponding ratios to tPSA for differentiating between patients with PCa and BPH; and (*c*) to evaluate the relationship between cPSA and clinical characteristics of the patients.

Materials and Methods

STUDY GROUPS

The study was performed retrospectively with 324 sera. All of the men from whom the sera had been collected had been investigated in the Department of Urology at the University Hospital Charité and were divided into three groups. The study was performed in accordance with ethics standards of the Helsinki Declaration 1975, as revised in 1985.

Control group. This group consisted of 91 men (median age, 54 years; range, 21–76 years) with normal digital rectal examinations. The individuals were either patients hospitalized in our department or attending our outpatient department because of nonprostatic diseases (erectile dysfunction, hydrocele, stone disease without obstruction, and infection).

BPH group. This group included 89 untreated patients (median age, 65 years; range, 49–85 years). The diagnosis of BPH was established clinically by digital rectal examination and/or transrectal ultrasonography. In 48 of these 89 patients, the BPH was histologically confirmed either using tissue obtained by ultrasound-guided sextant prostate biopsy or by transurethral resection of the prostate. Because there were no differences in the fPSA/tPSA or cPSA/tPSA ratios between the two BPH subgroups diagnosed by clinical or histological examination, both groups were considered as one group.

PCa group. The PCa group included 144 patients (median age, 65 years; range, 48–88 years) diagnosed histologically with blood samples taken before different treatment regimens (radical prostatectomy, radiotherapy, or hormonal therapy). The cancer stage was assigned according to the TNM system, and the histological grade was classified as grades 1, 2, and 3, as described in detail previously *(11)*. The pathological stages and grades of 75 patients were as follows: pT2 pN0M0 (n = 51); pT3 pN0M0 (n = 24); G1 (n = 5); G2 (n = 45); and G3 (n = 25). The remaining 69 of the 144 patients were clinically staged with the following results: T1 (n = 5; 2 with pN0M0 and 3 with N0M0); T2 (n = 34; 13 with pN0M0, 19 with N0M0, 1 with pN1M0, and 1 with pN1M1); T3 (n = 30; 12 with pN0M0, 13 with N0M0, and 5 with pN1M0); G1 (n = 10); G2 (n = 40); G3 (n = 19).

SAMPLE COLLECTION

Blood samples were taken before diagnostic procedures, transurethral resection of the prostate, or 4 weeks (at the earliest) after digital rectal examination, prostatic biopsy, and transrectal ultrasound to avoid possible errors caused by the release of PSA from the prostate and the different elimination kinetics of their forms from blood. The samples were collected in evacuated tubes (Monovette 03.1528; Sarstedt) and centrifuged at 1600*g* for 15 min at 4 °C after the blood was allowed to clot for 1 h at room temperature. The sera were frozen at −80 °C within 2 h after collection and were tested within 12 weeks. Female sera as negative controls, in-house serum pools, and control sera from the producers of the test kits and from Bio-Rad were used as control materials.

PSA ASSAYS

tPSA was measured with both the Bayer Immuno 1 PSA Assay (product no. T01-3450-5; Bayer Diagnostics, Tarrytown, NY) and the Roche Elecsys PSA Immunoassay (product no. 1731262; Roche Diagnostics) according to the instructions of the manufacturers. fPSA was measured with the Roche Elecsys Free PSA Immunoassay (product no. 1820800; Roche Diagnostics) on the Elecsys analyzer 1010. For determining cPSA, a recently introduced immunoassay (product no. T01-3982-51) for the Bayer Immuno 1 system was used *(10)*. This assay is based on the unique binding properties of the capture monoclonal antibody MM1 used in the Bayer Immuno 1 assay for tPSA. That antibody fails to bind fPSA in the presence of antibodies specific against epitope E, which is exposed only in fPSA, so that all cPSA forms such as ACT-PSA and minor forms except PSA complexed to α_2-macroglobulin are detected.

STATISTICAL ANALYSIS

Data were analyzed using the statistical software packages SPSS 8.0 for Windows (SPSS) and GraphPad Prism 3.00 for Windows (GraphPad Software). The Kruskal–Wallis nonparametric ANOVA, the Mann–Whitney *U*-test, and the calculation of rank correlation coefficients

according to Spearman (r_S) were performed. The software GraphROC 2.1 for Windows was used to analyze the ROC. Patients for the ROC analysis were selected by the randomization procedure used in SPSS 8.0. Regression analysis for methodical evaluation was performed using the software EVAPAK 3.01 for Windows according to Passing and Bablok *(12)*. $P < 0.05$ was considered statistically significant.

Results

ANALYTICAL PERFORMANCE OF THE PSA ASSAYS

The intra- and interassay imprecision was assessed using both in-house serum pools and commercial control materials. Fig. 1 synoptically demonstrates the corresponding interassay imprecision profiles, which show comparable reproducibility for all four assays.

The Bayer Immuno 1 PSA assay for tPSA has shown to be an equimolar test *(13)*. According to the manufacturer's information, the Roche tPSA assay also measures on an equimolar basis. Data of a recent IFCC standardization study confirmed that the results obtained with the Roche test were comparable with the Tandem® test from Hybritech *(14)*. The method comparison *(12)* of the two tPSA assays showed similar slopes and intercepts between the groups so that the regression line was estimated for all 324 samples combined. The equation (95% confidence intervals in parentheses), $y_{Roche} = 1.097\ (1.072–1.136)x_{Bayer} + 0.057\ (-0.003\ to\ 0.124)$, showed that the tPSA concentrations measured with the Roche Elecsys assay were ~10% higher than those measured with the Bayer Immuno 1 assay. The values of fPSA plus cPSA in BPH and PCa patients related to the tPSA measured with the Bayer Immuno 1 assay and the Roche Elecsys assay were comparable between the patients but were different between the assay systems, being 111% vs 107% (Bayer) and 98.3% vs 101% (Roche), respectively. Therefore, the fPSA/cPSA ratio, although measured by two different assay systems, was used as an additional variable, whereas the fPSA/tPSA and cPSA/tPSA ratios were calculated using the data of the corresponding Bayer or Roche assays.

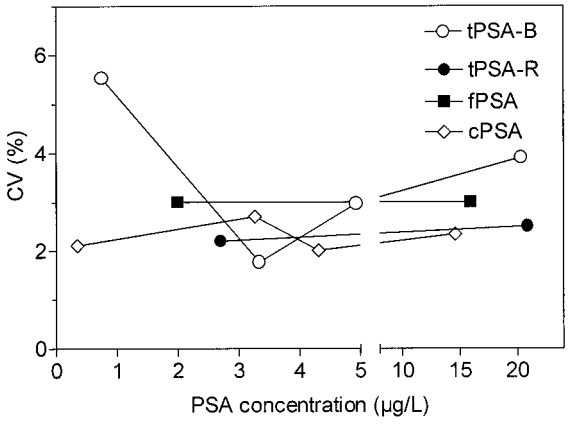

Fig. 1. Interassay imprecision of tPSA, fPSA, and cPSA.

Study materials were either control materials or pooled human serum (n = 7–25). *tPSA-B*, Bayer Immuno 1 assay; *tPSA-R*, Roche Elecsys assay.

tPSA, fPSA, cPSA, AND THEIR RATIOS IN THE STUDY GROUPS

Of the 324 subjects studied, 137 (84 controls, 36 BPH patients, and 7 PCa patients) had tPSA values between 0 and 2 µg/L, 124 (7 controls, 47 BPH patients, and 81 PCa patients) had values between 2.01 and 10 µg/L, and 43 (6 BPH and 37 PCa patients) had values between 10.1 and 20 µg/L; of the remaining 19 PCa patients, 18 had tPSA values between 20.1 and 100 µg/L, and 1 patient had a value >100 µg/L. Fig. 2 shows the scatter plots and medians for tPSA (only the Bayer test), fPSA, cPSA, and the fPSA/tPSA (Roche tests), cPSA/tPSA (Bayer tests), and fPSA/cPSA ratios. The mean ages of the BPH (65 years) and PCa (65 years) patients did not differ but were somewhat higher than in the controls (54 years). Significant differences between the groups were shown by Kruskal–Wallis nonparametric ANOVA (Fig. 2). PCa patients showed higher tPSA and cPSA concentrations than controls and BPH patients, whereas they were characterized by lower fPSA/tPSA and fPSA/cPSA ratios and a higher cPSA/tPSA ratio, respectively (Fig. 2, D-F).

RELATIONSHIP BETWEEN cPSA AND OTHER PSA FORMS, TUMOR STAGING, AND GRADING

The cPSA values were more closely related to the tPSA values (corresponding $r_S = 0.959$, 0.991, and 0.994 for controls, BPH patients, and PCa patients, respectively) than to the fPSA values ($r_S = 0.542$, 0.724, and 0.647, respectively). As described previously for fPSA and the fPSA/tPSA ratio *(11)*, no correlations of cPSA and the isoform ratios to the pathological tumor stage and the histological grading were found. For example, the median values of the cPSA/tPSA ratio in patients with stages of pT2 (n = 51) and pT3 (n = 24) or grades G1 (n = 5), G2 (n = 45), and G3 (n = 25) did not differ significantly (92.3% vs 93.3%, 91.3%, 91.7%, and 93%, respectively). Similarly, the cPSA/tPSA, fPSA/tPSA, and fPSA/cPSA ratios were not different between patients with lymph node stages of pN0 (n = 101) and pN1 (n = 7; 91.5% vs 96.9%, 12.1% vs 10.8%, and 14.2% vs 11.2%, respectively), whereas tPSA and cPSA concentrations were significantly increased in patients with metastatic lymph nodes (8.03 vs 26.9 µg/L and 7.1 vs 25.3 µg/L, respectively; $P < 0.001$).

ROC ANALYSIS AND DIAGNOSTIC VALIDITY

We performed ROC analyses in patients with BPH and PCa for the entire tPSA range (0.33–365 µg/L) and for the particularly characteristic range of overlapping tPSA concentrations in both groups (2–10 µg/L). When the entire tPSA range was considered, areas under the ROC curves for cPSA, fPSA, and the respective ratios did not show any statistical differences compared with the area under the tPSA curve [e.g., mean area under the curve ± SE, 0.870 ± 0.024 for tPSA (Bayer); 0.888 ± 0.022 for cPSA;

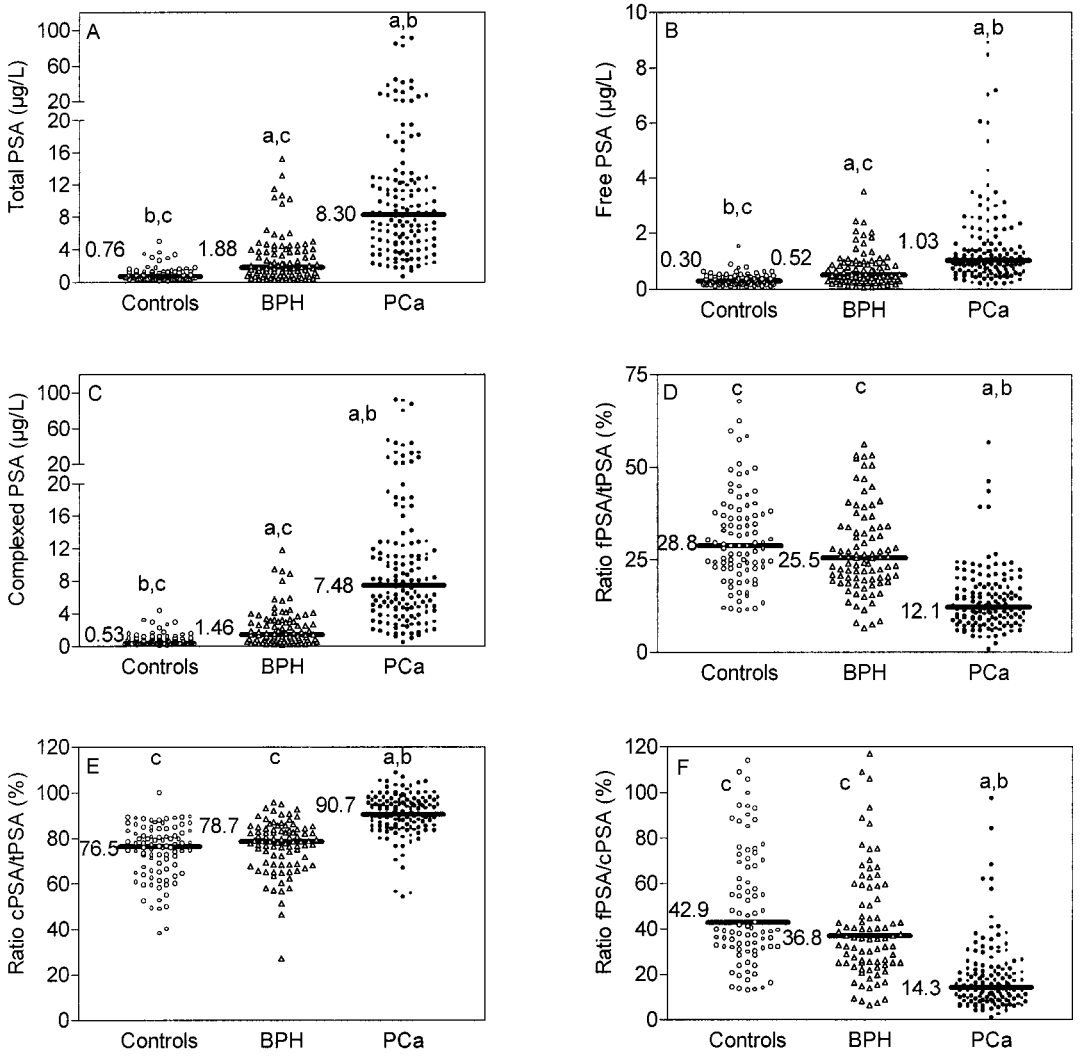

Fig. 2. Scatter plots of tPSA (*A*), fPSA (*B*), cPSA (*C*) and the percentage ratios fPSA/tPSA (*D*), cPSA/tPSA (*E*), and fPSA/cPSA (*F*) in controls, patients with BPH, and patients with PCa.

The study included 91 controls (○), 89 patients with BPH (△), and 144 patients with PCa (●). Median values of the respective groups are shown as *horizontal lines*. Only tPSA values measured with the Bayer Immuno 1 assay are presented. Significant differences between the groups (Kruskal–Wallis nonparametric ANOVA) are indicated: *a*, significantly different from controls; *b*, significantly different from patients with BPH; *c*, significantly different from patients with PCa.

0.859 ± 0.028 for fPSA/tPSA]. To simulate the characteristics of overlapping tPSA values in both groups of patients, equal numbers of corresponding patients were randomly selected from the larger group to match the number of patients in the smaller group at 1-µg/L tPSA intervals within the tPSA range mentioned above, using the randomization procedure of SPSS 8.0. Thus, 40 BPH and 40 PCa patients were selected. A similar matching procedure was applied recently to avoid the influence of tPSA as a confounding factor *(15)*. The areas under the ROC curves for tPSA (both assays), cPSA, and fPSA were not significantly different ($P > 0.005$; Fig. 3A), but the areas under the curves of all ratios (Fig. 3B) were significantly higher ($P \leq 0.05$) than those of the PSA concentrations. No significant differences ($P > 0.05$) between the areas under the curves of the fPSA/tPSA, cPSA/tPSA, and fPSA/cPSA ratios were observed.

The diagnostic validity criteria sensitivity, specificity, and efficiency of tPSA, cPSA, and their ratios at different decision limits of the ROC curves are shown in Table 1. cPSA alone did not improve the sensitivity or specificity compared with tPSA to differentiate between patients with BPH or PCa. However, the specificity and the sensitivity, respectively, increased by ~30–50% compared with tPSA or cPSA if one of the three ratios, fPSA/tPSA, cPSA/tPSA, or fPSA/cPSA, was used as discriminatory indicator at the decision limit with the highest efficiency or a sensitivity or specificity of 90%.

No statistical differences in the specificity and sensitivity, respectively, were found at the selected 90% sensitivity or specificity between the two ratios fPSA/tPSA and cPSA/tPSA ($P > 0.05$). On the basis of these data, the usefulness of the two ratios was demonstrated by calculating the true-negative and false-positive results in BPH

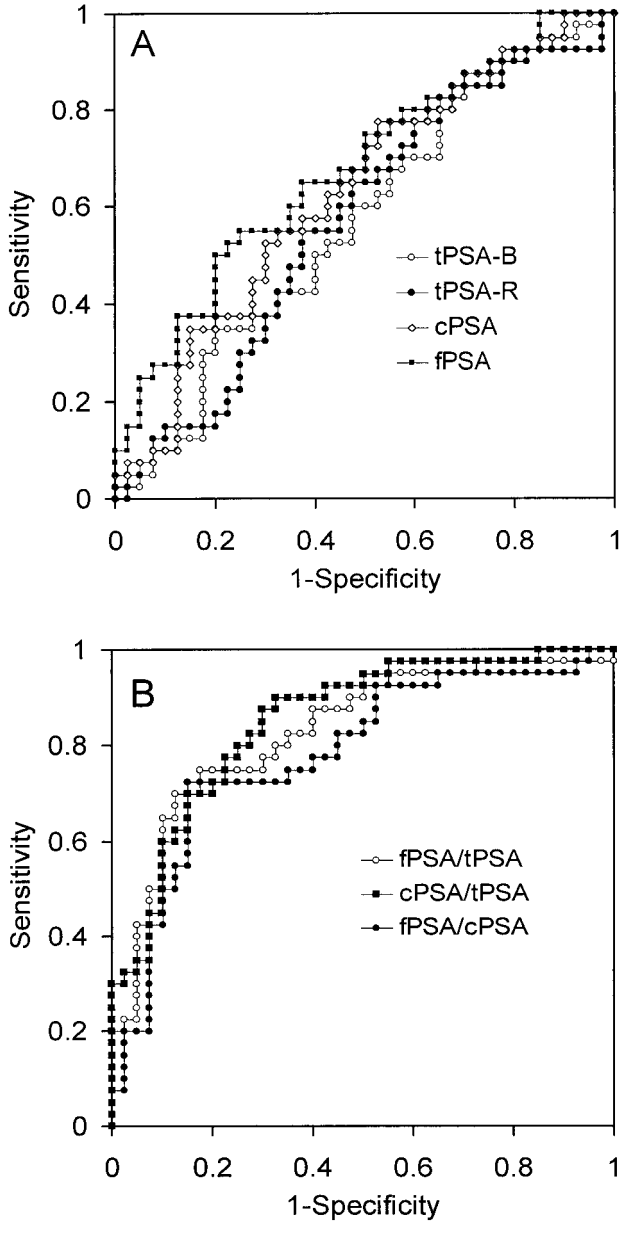

Fig. 3. ROC curves for patients with tPSA concentrations 2–10 μg/L.

To simulate the characteristics of overlapping tPSA values in both groups of patients, equal numbers of corresponding patients were randomly selected from the larger group to match the number of patients in the smaller group at 1-μg/L intervals within the 2–10 μg/L tPSA interval. The analysis included 40 patients with BPH and 40 with PCa. (*A*), mean areas under the curve ± SE: Bayer Immuno 1 assay (*tPSA-B*; ○), 0.568 ± 0.065; Roche Elecsys assay (*tPSA-R*; ●), 0.573 ± 0.065; fPSA (◇), 0.643 ± 0.059; cPSA (■), 0.632 ± 0.062; ($P > 0.05$). (*B*), mean areas under the curve ± SE: fPSA/tPSA (○), 0.838 ± 0.047; cPSA/tPSA (■), 0.851 ± 0.043; fPSA/cPSA (●), 0.784 ± 0.053. There were no statistically significant differences among the ratios ($P > 0.05$), but differences between all ratios and tPSA, fPSA, and cPSA were significant ($P < 0.05$).

patients and the true-positive and false-negative results in PCa patients, respectively (Table 2). It is obvious that unnecessary biopsies could have been avoided in ~65% of BPH patients, whereas ~8% of cancers would had been missed.

Table 1. Diagnostic validity of tPSA, cPSA, and the fPSA/tPSA, cPSA/tPSA, and fPSA/cPSA ratios to distinguish PCa and BPH patients.[a]

	Sensitivity, %	Specificity, %	Efficiency, %
tPSA, μg/L			
4.00[b]	45	60	53
2.71[c]	83	35	59
2.29[d]	90	18	54
6.02[e]	10	90	50
3.79[f]	53	53	53
cPSA, μg/L			
2.60[c]	78	48	63
1.88[d]	90	25	58
5.69[e]	10	90	50
3.03[f]	58	58	58
cPSA/tPSA, %			
82.9[c]	90	68	79
81.3[d]	90	57	74
89.9[e]	58	90	74
84.4[f]	78	78	78
fPSA/tPSA, %			
17.5[c]	75	83	79
22.5[d]	90	55	73
13.2[e]	55	90	73
19.2[f]	75	75	75
fPSA/cPSA, %			
21.1[c]	73	85	79
34.1[d]	90	48	69
14.3[e]	45	90	68
25.0[f]	73	73	73

[a] Data from ROC analysis performed with 40 matched pairs of BPH and PCa patients with tPSA values between 2 and 10 μg/L (see Fig. 3).

[b] Value of 4 μg/L was selected as the conventional upper reference limit.

[c] Threshold with the highest diagnostic efficiency.

[d] Threshold with diagnostic sensitivity of 90%.

[e] Threshold with diagnostic specificity of 90%.

[f] Threshold at the equivalent point, i.e., at the point with similar sensitivity and specificity.

Discussion

It has been suggested that there are clinical and analytical reasons to determine ACT-PSA rather than fPSA *(16)*. However, reported data on the usefulness of ACT-PSA are controversial because in some studies ACT-PSA performed better than fPSA and the fPSA/tPSA ratio *(2, 6, 17)*, whereas in others it was less reliable *(9, 18)*. Therefore, it was the special aim of this study to evaluate the clinical utility of the alternative approach of Bayer Immuno 1 assay for determining cPSA in comparison with the established fPSA/tPSA ratio.

The interassay imprecision for all assays was generally <5% (Fig. 1) and made it possible to compare the diagnostic validity of the various assays and derivatives. It is of interest that although the Bayer and Roche assays have been calibrated against the Stanford 90:10 reference preparation *(14)*, there were differences of ~10% between tPSA concentrations measured by both assays. Reasons other than the properties of the calibrator, e.g., the selec-

Table 2. fPSA/tPSA and cPSA/tPSA[a] ratios as potential tools to reduce the rate of biopsies.

	BPH[b] (n = 48)				PCa[b] (n = 144)			
	fPSA/tPSA	cPSA/tPSA	fPSA/tPSA	cPSA/tPSA	fPSA/tPSA	cPSA/tPSA	fPSA/tPSA	cPSA/tPSA
Cutoff	<13.2%	>89.9%	>22.5%	<81.3%	<13.2%	>89.9%	>22.5%	<81.3%
	False positive		True negative		True positive		False negative	
No. of patients	3 of 48 (6.3%)	5 of 48 (10.4%)	32 of 48 (66.6%)	31 of 48 (64.6%)	86 of 144 (59.2%)	88 of 144 (61.4%)	12 of 144 (8.3%)	11 of 144 (7.6%)

[a] Cutoffs for 90% sensitivity and specificity, respectively, were taken from Table 1.
[b] Patients with BPH confirmed histologically (n = 48) and all patients with PCa (n = 144) were included in this calculation.

tivity of antibodies, the equilibration time during the measurement, and the format of the assay, must be considered as influencing the final result.

Our findings clearly indicate that cPSA testing is not superior to tPSA testing alone (Fig. 3 and Table 1). These data are contradictory to the results of Brawer et al. *(19)*, who found that cPSA alone was a better discriminator between BPH and PCa than tPSA or the fPSA/tPSA ratio in the range between 4 and 10 μg/L. According to the suggestions of those authors, the determination of cPSA could replace the measurements of the two analytes tPSA and fPSA. However, both practical experience and the theoretical background do not give reason for such hope. Because cPSA strongly correlates with tPSA, a large overlapping range of cPSA concentrations consequently exists between PCa and BPH patients within in the gray zone of tPSA concentrations up to 10 μg/L. Thus, although the cutoff may be narrowed when cPSA is determined, the general problem of the overlapping PSA concentrations characteristic of these two groups of patients cannot be solved by such an approach. A cutoff for cPSA of 3.75 μg/L was chosen to obtain a sensitivity for PCa detection equal to that achieved with the tPSA cutoff limit of 4 μg/L *(10)*; that choice may emphasize that the high expectation concerning the sole determination of cPSA is not very realistic. For example, a median cPSA/tPSA ratio of ~80% in BPH patients means that patients with tPSA concentrations >4.7 μg/L generally exceed the limit of 3.75 μg/L for cPSA and thus belong to the patient group with tPSA concentrations in the gray zone. Two studies reported in abstracts were also unable to confirm that cPSA was better than the fPSA/tPSA ratio in discriminating biopsy-negative from biopsy-positive men *(20, 21)*. In addition, because the area under the ROC curve for the percentage of fPSA did not show the typical value higher than that for tPSA, it was assumed that Brawer et al. *(19)* studied inappropriate cohorts *(22)*.

Our results (Figs. 2 and 3) confirm our own findings and the data of numerous studies that the fPSA/tPSA ratio is statistically different between patients with PCa cancer and BPH [reviewed in Ref. *(5)*]. Because the diagnostic validity of that ratio is not superior to tPSA over an expanded range *(23)*, we included in the ROC analysis only patients with tPSA concentrations between 2 and 10 μg/L. Selecting in our ROC curves the points at 90% sensitivity, at the highest diagnostic efficiency, or at the equivalent point of sensitivity and specificity, threshold values were 22.5%, 17.5%, and 19.2%, respectively (Table 1). These cutoff points for the fPSA/tPSA ratio roughly correspond to values given by other authors in the gray zone of PSA between 2 and 20 μg/L *(24–28)*, although assay-specific compatibility of these values should be considered *(29)*. Thus, our results obtained with cPSA and the corresponding ratios were based on an appropriate patient selection. The matching approach as applied in our study avoided a possible confounding effect related to unequal tPSA values in the cohorts and should generally be used in such studies.

The cutoff point of 81.3% for cPSA/tPSA produced a diagnostic sensitivity of 90% to detect PCa patients within the tPSA range between 2 and 10 μg/L (Table 1). Our results correspond to data shown in a report on preliminary measurements of cPSA *(10)*. Using the same cPSA assay as the prototype test, those authors revealed that all men with PCa had cPSA >77%. Brawer et al. *(19)* also performed cPSA measurements using such a test but did not calculate the cPSA/tPSA ratio. The ROC analysis (Fig. 3B) and the specificity of the cPSA/tPSA ratio achieved at a selected sensitivity of 90% (Tables 1 and 2) compared with tPSA, cPSA, and other ratios underlines our view that the cPSA/tPSA ratio can be accepted as a real alternative to the fPSA/tPSA ratio for improving the differentiation between PCa and BPH patients. The cutoffs shown in Table 1 are provisional because of the limited number of patients investigated. However, the results show that the two ratios, fPSA/tPSA and cPSA/tPSA, are similarly effective in reducing the rate of unnecessary biopsies (Table 2), as demonstrated recently by other authors *(15, 23)*. It can be assumed that the use of the cPSA/tPSA ratio instead of the fPSA/tPSA ratio is also helpful to prepare cancer probability curves *(30)*. While this report was being prepared, an abstract was published that confirmed our results that the fPSA/tPSA and cPSA/tPSA ratios are equal in their diagnostic validity *(31)*. The obstacles observed with ACT-PSA assays as found in our previous study *(18)* and also by others *(9)* have apparently been overcome the new cPSA assay.

We also evaluated the fPSA/cPSA ratio, although both

components were measured by the two assay systems of Bayer and Roche. From the theoretical point of view, a better differentiation might be possible because the proportion of fPSA and cPSA is opposite in both groups of patients. Although the percentage of difference of the fPSA/cPSA ratio between patients with BPH and PCa was significantly higher ($P < 0.01$) than that of the fPSA/tPSA ratio (Fig. 2, D and F), a better discrimination was not achieved (Fig. 3 and Table 1). A general difference of ~10% between both tPSA concentrations with both assays perhaps partly explains the difference of the ratios without the expected improvement of diagnostic power. However, Demura et al. *(32)*, using different assays but comparing variables similar to those we studied, reported that the fPSA/cPSA ratio was the most powerful tool for diagnosis of PCa.

The fPSA/tPSA ratio has been examined as a method to predict the final pathological stage of PCa. The relationships between this ratio and the stage, grade, and aggressiveness of PCa are controversial at this point in time [reviewed in Ref. *(5)*]. Our data could not confirm that the ratios of fPSA/tPSA, cPSA/tPSA, and fPSA/cPSA depend on tumor stage and grade. Therefore, none of these ratios can be recommended as a predictive indicator for final pathological staging of PCa.

In summary, compared with the determination of tPSA, the differentiation of patients with BPH and PCa with tPSA concentrations in the overlapping range between 2 and 10 μg/L could be equally improved with the ratios of free or complexed PSA to tPSA, whereas cPSA alone does not have any additional discriminatory power. Thus, the cPSA/tPSA ratio can be considered as an alternative to the fPSA/tPSA ratio.

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