

The Use of Complexed PSA for the Early Detection of Prostate Cancer

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Abstract. *The advent of complexed PSA (cPSA) raised great expectations concerning the role of this parameter for improving the early detection of prostate cancer. Materials and Methods: Total PSA (tPSA), free PSA (fPSA) and cPSA were evaluated from the serum of 178 of our clinic's patients (74 patients with prostate carcinoma, 104 patients with benign prostate illness) prior to prostate histology. ROC curves were calculated for all of these parameters as well as for the ratios f/t-PSA, c/t-PSA and f/c-PSA. Results: The ROC analysis for the whole examined PSA area and PSA levels of 4 to 10 ng/ml showed a statistically significant difference between the AUCs of the ratios on the one hand and the cPSA and tPSA parameters on the other hand. However, there was no difference between these parameters in PSA levels of up to 6 ng/ml. In the comparison of specificities at PSA levels of 4 to 10 ng/ml, the best results were achieved for the c/tPSA ratio. Neither in the PSA level area between 4 and 10 ng/ml, nor in the whole examined PSA area, could a difference between the cPSA and tPSA parameters be detected. Conclusion: Firm conclusions regarding low PSA concentrations cannot be drawn because of the small number of cases included in our study. However, 5 out of 13 patients with prostate carcinoma, whose tPSA values were still in the employed method's reference area, would have been identified as carcinoma-suspicious and brought to further diagnosis by determining the cPSA value with a recommended cut-off of 2.5 ng/ml.*

As compared with the pre-PSA era, the introduction of routine PSA analysis led to an increased detection of prostate carcinomata, which are still limited to the organ at the time of diagnosis and can be subject to curative treatment (1). It is, however, generally acknowledged that PSA is not an ideal tumor marker. Many false-positive

results follow from PSA's lacking tumor specificity (benign prostate illnesses also cause a PSA increase). At PSA levels of 4 to 10 ng/ml, these amount to approx. 70%. On the other hand, approx. 20% of patients with prostate carcinoma show a PSA value which is lower than the usual "normal value" (2, 3). Therefore, different methods for improving the diagnostic value of PSA are under scrutiny. Among these are the analysis of the speed of PSA increase and PSA density, the use of age-specific PSA reference areas and also the measurement of different molecular forms of PSA. This last approach was introduced after it had been found that the distribution of complexed and free PSA molecules in the serum of patients with prostate cancer was different when compared to patients with benign prostate hyperplasia (4).

The main fraction of PSA within the serum is bound to other molecules, forming a complex. Measurement of complexed PSA (cPSA), therefore, means the measurement of a sum of different, immunologically recordable PSA complexes. The smaller PSA fraction is unbound (free) within the serum and not homogeneous either. The measurement of the free PSA (fPSA) fraction in serum is difficult, especially because of its low stability, which makes it necessary to observe precise pre-analytical conditions (5, 6). Some studies showed that cPSA is more stable than fPSA and also less susceptible to trouble caused by manipulations of the prostate. Because of these features and the advantage of the concentration in the serum, it should be possible to obtain a more precise analysis through a PSA value which is independent of the fluctuation of an incorrectly measured fraction of fPSA (7, 8).

On the basis of our own patients' data, we examined the controversial question of whether the diagnostic validity of PSA for the early diagnosis of prostate cancer can be improved by measuring complexed PSA forms.

Materials and Methods

Patients. The study included a total of 178 inpatients and outpatients of our clinic. Of these, 74 had an untreated prostate carcinoma and 104 a benign prostate illness. The diagnosis was based in every case on histological findings, by means of either a

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Key Words: PSA, complexed PSA (c PSA), free PSA (f PSA), prostate cancer.

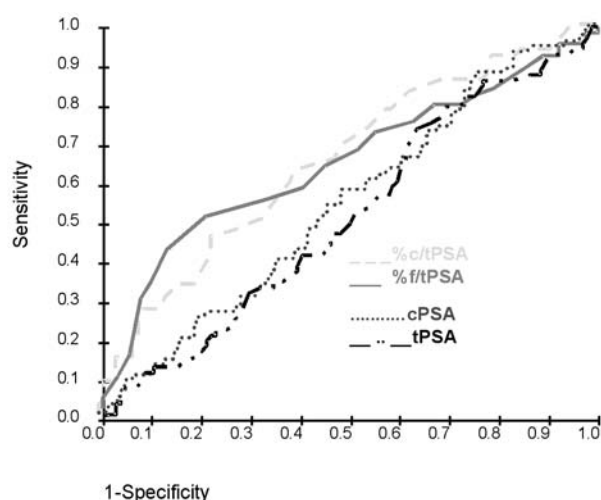


Figure 1. ROC curves for tPSA (Immulate), cPSA, f/tPSA and c/tPSA: PSA levels of 1 to 27 ng/ml.

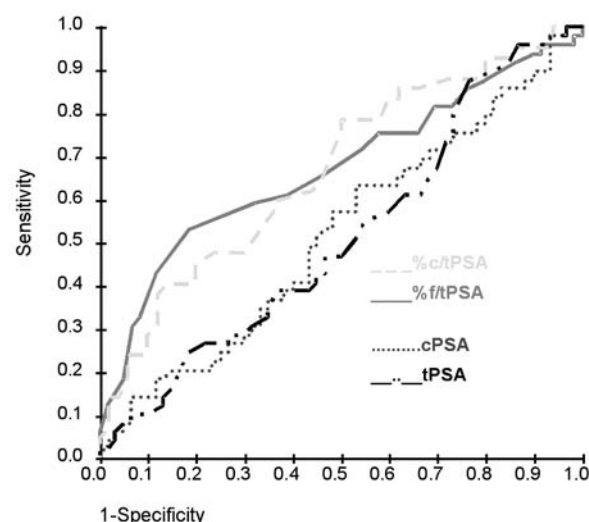


Figure 2. ROC curves for tPSA (Immulate), cPSA, f/tPSA and c/tPSA: PSA levels of 1 to 10 ng/ml.

prostate puncture cylinder, a tissue sample obtained through transurethral resection or adenoma ectomy of the prostate or the prostate ectomy preparation.

Preparation of samples. Blood sampling was carried out prior to any manipulation of the prostate. After a coagulation time of at least 30 minutes, the blood samples were centrifuged and the serum separated immediately. In cases where the measurement of the different parameters could not be carried out directly after serum preparation, the samples were stored until the analysis at -80°C .

PSA measurement. The measurement of fPSA and total PSA (tPSA) through Immulite (DPC Biermann), as well as of the cPSA and tPSA through ACS:180 (Bayer Diagnostics), were carried out

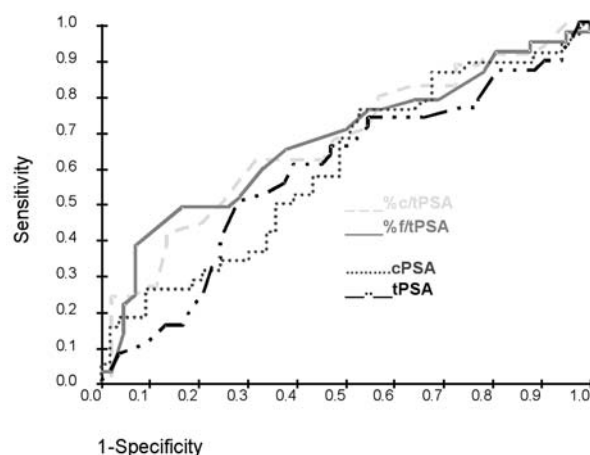


Figure 3. ROC curves for tPSA (Immulate), cPSA, f/tPSA and c/tPSA: PSA levels of 1 to 6 ng/ml.

according to the instructions of the immunoassay manufacturers. The ratios f/tPSA (Immulate), c/tPSA (ACS:180) and f/cPSA (Immulate/ACS:180) were then calculated on the basis of the measured parameters. Measurements were performed between April and September 2003 in our clinic's laboratory.

Statistics. The software programmes SPSS and GraphROC were used for carrying out the statistical analysis. A significance level of $p < 0.05$ was regarded as a statistically significant difference between two results.

Results

The measured values of tPSA (Immulate) of the 74 patients with prostate cancer were between 1.6 and 25 ng/ml (average: 6.94, median: 5.95 ng/ml) and those of the 104 patients with benign prostate illness between 1.1 and 26.6 ng/ml (average: 6.80, median: 5.95 ng/ml).

ROC curves and the respective AUC values for different tPSA areas were calculated in order to compare the diagnostic validity of the parameters tPSA (Immulate and ACS:180), cPSA and the ratios f/tPSA, c/tPSA and f/cPSA: 1.1 to 26.6 ng/ml tPSA (Immulate) (74 patients with prostate cancer, 104 patients with benign prostate illness); 4 to 10 ng/ml tPSA (Immulate) (49 patients with prostate cancer, 59 patients with benign prostate illness); 1 to 6 ng/ml tPSA (Immulate) (38 patients with prostate cancer, 53 patients with benign prostate illness).

Figure 1 shows the ROC curves for the total of the tPSA area, Figure 2 for the tPSA area between 4 and 10 ng/ml and Figure 3 for the tPSA area between 1 and 6 ng/ml. The accordingly calculated AUC values are represented in Table I.

In the whole tPSA area we examined there was a statistically significant difference between the AUCs of the ratios (0.66 for f/cPSA, 0.65 for f/tPSA and f/cPSA) on the

Table I. AUC values of the different PSA parameters.

PSA level (ng/ml)	Carcinoma (n)	Benign prostate illness (n)	AUC					
			tPSA (Imm)	f/tPSA	cPSA	tPSA (ACS)	c/tPSA	f/cPSA
1-6	38	53	0.58	0.66	0.60	0.54	0.66	0.67
4-10	49	59	0.51	0.68±	0.51	0.48	0.67	0.66
1-27	74	104	0.52	0.65*	0.54	0.49	0.65*	0.66**

*significantly higher than tPSA (Immulite) and tPSA (ACS:180)

**significantly higher than tPSA (Immulite), tPSA (ACS:180) and cPSA

± significantly higher than tPSA (Immulite) and cPSA

Table II. Comparison of specificities of tPSA, cPSA, f/tPSA, c/tPSA and f/cPSA at given sensitivities at PSA levels of 1 to 27 ng/ml.

Sensitivity (%)	t-PSA (Imm)		f/tPSA		c-PSA		t-PSA (ACS)		c/tPSA		f/cPSA	
	Cut-off	Specificity	Cut-off	Specificity	Cut-off	Specificity	Cut-off	Specificity	Cut-off	Specificity	Cut-off	Specificity
	(ng/ml)	(%) (CI 95%)	(ng/ml)	(%) (CI 95%)	(ng/ml)	(%) (CI 95%)	(ng/ml)	(%) (CI 95%)	(ng/ml)	(%) (CI 95%)	(ng/ml)	(%) (CI 95%)
80	≥4.15	27.9 (20-38)	≤0.18	27.5* (19-38)	≥3.10	26 (18-36)	≥3.76	22.7 (6-24)	≥0.71	40.9* (31-52)	≤0.24	29.7* (21-40)
85	≥3.60	23.1 (15-32)	≤0.20	20.9 (13-31)	≥2.76	25 (17-35)	≥3.48	20.5 (3-19)	≥0.69	31.8* (21-41)	≤0.28	24.2* (16-34)
90	≥2.94	11.5 (6-19)	≤0.21	14.3 (8-23)	≥2.42	17.3 (11-26)	≥3.29	17 (2-15)	≥0.65	21.6* (14-32)	≤0.37	12.1 (6-21)
95	≥2.35	4.8 (2-11)	≤0.26	7.7 (3-15)	≥2.11	12.5 (7-20)	≥2.68	9.1 (0-11)	≥0.56	8 (3-16)	≤0.40	9.9 (5-18)

CI 95% = 95% confidence interval

*statistically higher than tPSA (ACS:180)

one hand and the parameters tPSA (ACS:180) (0.49) and tPSA (Immulite) (0.52) on the other hand. The difference between the ratio and cPSA (0.54) was clear but not significant. Similar results were achieved in the PSA area between 4 and 10 ng/ml: AUC values of the ratio were higher than those of the individual parameters tPSA (Immulite and ACS:180) and cPSA. However, there was no provable statistical difference between PSA parameters in the area between 1 and 6 ng/ml.

Comparing the diagnostic validity with the help of ROC diagrams and the respective AUC values is one possible way of assessing laboratory parameters. However, clinically more relevant findings are obtained by the direct comparison of sensitivities and specificities. By looking at the ROC curves (Figures 1-3) from this point of view, there were only slight differences between the curves in the area of desired sensitivities starting from approx. 80%.

Tables II to IV represent the specificities of the PSA parameters at sensitivities of 80, 85, 90 and 95%, again separated according to the PSA areas "total", "4-10 ng/ml" and "1-6 ng/ml". Statistically significant differences could only be found between the ratios f/t-, f/c-, c/tPSA and tPSA (ACS:180) in the total PSA area, as well as between c/tPSA and cPSA, tPSA (ACS:180) in the PSA area 4-10 ng/ml, and between the ratios f/t-, f/c-, c/tPSA and tPSA (Immulite) in the PSA area 1-6 ng/ml. However, these differences were not provable for every given sensitivity value from 80 to 95%.

The specificities of cPSA at the chosen sensitivity levels, calculated on the basis of the ROC curves, showed no differences compared to the other PSA parameters, except in the PSA area 4 to 10 ng/ml. Here, the specificity of the c/tPSA ratio at a sensitivity of 80 and 85% was significantly higher than that of cPSA.

Table III. Comparison of specificities of tPSA, cPSA, f/tPSA, c/tPSA and f/cPSA at given sensitivities at PSA levels of 4 to 10 ng/ml.

Sensitivity (%)	tPSA (Imm)		f/tPSA		c-PSA		t-PSA (ACS)		c/tPSA		f/cPSA	
	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)
80	≥4.76	26.7 (16-40)	≤0.17	30.5 (19-44)	≥3.5	20 (11-32)	≥4.18	8.2	≥0.75	42* (28-57)	≤0.24	25.4 (15-39)
85	≥4.75	22 (13-36)	≤0.19	23.7 (14-37)	≥3.41	16.7 (8-29)	≥3.91	4.1	≥0.72	38** (25-53)	≤0.30	13.6 (6-25)
90	≥4.64	18.3 (10-31)	≤0.20	13.6 (6-25)	≥3.2	10 (4-21)	≥3.76	4.1	≥0.65	20 (10-34)	≤0.37	10.1 (4-21)
95	≥4.35	13.3 (6-25)	≤0.25	8.5 (3-19)	≥2.85	6.7 (2-16)	≥3.60	4.1	≥0.62	14 (6-27)	≤0.44	6.8 (2-17)

CI 95% = 95% confidence interval

*statistically higher than tPSA (ACS:180) and cPSA

**statistically higher than cPSA

Table IV. Comparison of specificities of tPSA, cPSA, f/tPSA, c/tPSA and f/cPSA at given sensitivities at PSA levels of 1 to 6 ng/ml.

Sensitivity (%)	tPSA (Imm)		f/tPSA		c-PSA		t-PSA (ACS)		c/tPSA		f/cPSA	
	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)	Cut-off (ng/ml)	Specificity (%) (CI 95%)
80	≥2.86	22.6 (12-36)	≤0.19	35.7 (21-52)	≥2.32	32 (20-46)	≥3.34	38.6* (24-55)	≥0.7	43.2* (28-59)	≤0.26	38.1* (24-55)
85	≥2.85	18.9 (9-32)	≤0.21	21.4 (10-37)	≥2.28	32.1 (20-46)	≥3.25	29.6 (17-45)	≥0.66	27.3 (15-43)	≤0.27	33.3 (19-50)
90	≥2.35	9.4 (3-21)	≤0.22	19* (9-34)	≥2.11	24.5 (14-38)	≥2.8	18.2* (8-33)	≥0.63	20.5* (10-35)	≤0.32	21.4 (10-37)
95	≥1.94	3.8 (0-13)	≤0.26	11.9 (4-26)	≥1.40	5.7 (1-16)	≥2.4	11.4 (4-25)	≥0.56	9 (2-22)	≤0.40	16.7 (7-32)

CI 95% = 95% confidence interval

*statistically higher than tPSA (Immuline)

A small number of our patients showed PSA values <4 ng/ml (tPSA Immuline) (13 patients with prostate cancer, 28 patients with benign prostate illness). Five carcinoma were additionally identified within this group using the recommended cPSA limit value of 2.5 ng/ml. In this way, the overall sensitivity of cPSA was higher than that of tPSA (Immuline) at almost the same specificity.

Although a similar sensitivity could have been achieved by lowering the cut-off for tPSA to 3 ng/ml (this would have meant only 8 false-negative results), this would have caused a considerable loss of specificity in return (see Table V).

Discussion

The introduction of PSA analysis into diagnostics led to a distinct shift in the stage of detected carcinoma of the prostate towards early detection of tumors, still limited to the organ and curable. The search for parameters that allow for a lower rate of unnecessary biopsies (increase of diagnostic specificity) and earlier identification of patients with clinically relevant carcinoma (increase of diagnostic sensitivity) is on going. Additionally, since many prostate cancer patients with "normal" PSA already have an

Table V. Diagnostic validity of cPSA and tPSA at PSA levels of 1 to 27 ng/ml.

Sensitivity and Specificity versus cut-off			
	cut-off	Sensitivity (%)	Specificity (%)
tPSA (Imm)	4.0 ng/ml	82.4	24.0
tPSA (Imm)	3.0 ng/ml	89.2	11.5
cPSA	2.5 ng/ml	89.2	21.2

cPSA: 5/13 carcinoma positive (t-PSA <4 ng/ml)

aggressive tumor, low PSA concentrations have increasingly become subject to studies.

Several publications controversially discussed whether the evaluation of complexed PSA alone or of the ratios of free or complexed PSA to total PSA improves diagnostic validity, as compared to the "simple" evaluation of total PSA, at PSA levels of 2 to 10 ng/ml, which are important for the early detection of prostate cancer. While some authors stated an advantage of cPSA over tPSA as well as over the ratio f/t-, c/t- or f/cPSA, others concluded that cPSA and tPSA are equivalent, but PSA ratios obtained a higher specificity at the same sensitivity (9-13). There are multicenter studies with large patient numbers for both PSA levels of 4 to 10 ng/ml as well as for low PSA concentrations (2 to 4 or 2 to 6 ng/ml). However, conclusions drawn from these studies are discordant (14-16). Table VI presents the results from some published studies.

Lein *et al.* (17) found a higher AUC value for cPSA at PSA levels of 2.5 to 4 ng/ml, but no increase of specificity compared with tPSA and the AUCs of the ratios. On the other hand, Partin *et al.* (18) concluded that cPSA is superior to tPSA and equivalent to the ratios.

These diverging findings and interpretations may be caused by different patient groups. The use of different PSA measurement methods and, therefore, different examined PSA levels, can also add to the contradictions. The manner of interpretation of the obtained data is also important. One point of criticism of Lein *et al.* (17) was the lack of indication of confidence intervals for the calculated specificities in other studies.

Our own results, especially the higher sensitivity of cPSA compared to tPSA at an almost similar specificity (see Table V), seemed to indicate at first that cPSA could be used to differentiate between prostate cancer and benign prostate illness at low total PSA concentrations. However, taking into account the small number of patients with PSA levels below 4 ng/ml, this assumption can not be regarded as verified. This also holds true for

Table VI. Comparison of results from selected multicenter studies for specificities of tPSA, cPSA, f/tPSA and c/tPSA at given sensitivities.

	% Sensitivity		% Specificity	
	Lein 2003 (n=253)	Partin 2003 (n=214)	Partin 2003 (n=452)	Okihari 2002 (n=131)
tPSA (ng/ml)	2.5-4	2-4	2-6	2.5-6
90	6.8	15	25.9	15
85	11	21.2	32.4	26
cPSA				
90	4	20	32.1	14
85	16.9	35	40.3	25
f/tPSA				
90	12.7	20	19	31
85	19.5	26	25	34
c/tPSA				
90	4.2	20	19	28
85	9.3	25	26.6	35

the other PSA levels examined, which can only be compared with the results of multicenter studies to a limited extent.

Our study did not reveal any advantage of cPSA evaluation for the whole examined PSA area as well as for PSA levels of 4 to 10 ng/ml, but rather equivalence to total PSA. A part of the AUC values of PSA ratios in these groups was significantly higher than those of cPSA and tPSA. The comparison of specificity at a given sensitivity showed no differences between cPSA and other PSA parameters. Only at PSA levels of 4 to 10 ng/ml was there an advantage of the ratios of cPSA and tPSA to cPSA.

Conclusion

Our results did not reveal any advantage of evaluating cPSA over tPSA at PSA levels of 4 to 10 ng/ml, but rather equivalence. However, the diagnostic value of PSA ratios seem to be superior to that of the separate PSA parameters cPSA and fPSA.

The question of whether an improvement of diagnostic value can be expected from cPSA evaluation can not be fully answered here because of the small size of the study. Since two multicenter studies came to very different conclusions, we accept that this question has yet to be answered.

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Received August 2, 2004

Accepted February 8, 2005