Serum tPSA, cPSA, Related Density Parameters and Chromogranin A as Predictors of Positive Margins after Radical Prostatectomy

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Abstract. *Serum levels of total prostate specific antigen (t-PSA) and PSA complexed to antichymotrypsin (PSA-ACT), as well as their corresponding density parameters were measured in prostate cancer (PC) candidates for radical prostatectomy. In these patients blood Chromogranin A (CgA) values were also recorded. The PSA-ACT recordings in presurgically characterized organ-confined disease were assumed to predict post-surgical staging better than t-PSA. If this proved correct the novel approach might contribute to the positive predictive value of Partin nomograms. In this prospective study 50 patients with clinically localized PC underwent staging pelvic lymphadenectomy and radical prostatectomy. The numerical values of the tPSA and PSA-ACT parameters were presurgically measured. The PSA and PSA-ACT densities (PSAD and ACTD) of the whole prostate were calculated by using transurethral ultrasound (TRUS) data. These preoperative results together with the CgA values were correlated with post-surgical pathological staging data. The relationships between serum tPSA, PSA-ACT, PSAD, ACTD, CgA and the final stage of prostatectomy specimens derived from the pathological data were analyzed. This preliminary study was performed on a relatively small number of patients who were characterized by a serum PSA <20 and a Gleason score (GS) ≤7. Nevertheless, the application of the logistic regression model showed both t-PSA and PSA-ACT to be superior to their density derivatives in predicting post-surgical pathological stage in PC patients who initially seemed to have localized prostate cancer. An elevation in serum CgA level, although rather infrequent at the early stages of PC is principally found in patients with higher Gleason score PC and was mostly associated with extracapsular tumor spread. Our results do not justify the substitution of PSA-ACT for t-PSA data in the Partin nomogram approach.*

Prostate-specific antigen (PSA) is the best tumor marker presently available. It detects, although with some limitations, a significant proportion of organ-confined prostate cancer (PC) (1). Improvements in detecting PC linked to PSA screening and various PSA derivatives, together with other new clinical technologies and approaches, led to the detection of many tumors in the early stages (2). The PSA parameter, however, is of limited help in differentiating between clinically relevant and dormant carcinomas and prostate confined *versus* extended disease and in predicting the true post-surgical PC stage (3). Thus these dilemmas require a constant search for new detection mode, but remain a difficult task to determine.

As early as 15 years ago Lilja *et al.* found that circulating PSA is inactivated in part by a covalent bonding to either alpha-2-macroglobuline (A2M) or alpha-1-antichymotrypsin (ACT) (5). Hence, PSA circulates in several molecular forms including free PSA and PSA complexed to α1-antichymotrypsin (PSA-ACT) and α2-macroglobulin (5-8). These reports related to several different molecular forms of the PSA marker have suggested the improved diagnostic significance of t-PSA leading also to an increased interest in this marker (5-11). The ACT complex is immunoreactive and is detectable in serum by using specific antibodies and related assays (12). The sera from patients with PC contain a higher proportion of PSA-ACT than subjects with benign prostatic hypertrophy (BPH) (9-10) and thus complexed PSA might be more closely associated with PC progression than the total PSA (t-PSA).

In addition, PSA-ACT is more stable than the free PSA (11). Thus, the higher stability of complexed PSA compared to the respective free form seemed to favor the application of PSA-ACT in PC diagnosis and also as the

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method of discrimination between organ confined and extraprostatic disease (11, 21).

The PSA density has been used as a standard tool in clinical practice for over fifteen years (13). Although t-PSA density (PSAD) failed to fulfill many hopes for a more accurate diagnosis of PC in the early stages (13) less data regarding the role of PSA-ACT density have been available.

Following radical prostatectomy (RP) a positive surgical margin will be found in 5-43% of the specimens (4). Numerous factors have an impact on the incidence of positive margins the most important of which are tumor volume and location, surgical and pathological techniques. Whether or not a positive margin should influence subsequent treatment and whether it independently or in a combination with as yet unknown genetic characteristics influences the prognosis is not known. Some of patients even with negative post-surgical margins develop PC dissemination. This observation emphasizes the importance of determining subclinical PC markers which are related to tumor progression.

The objective of this study was to determine the relative merits of t-PSA *versus* PSA-ACT and their respective density parameters (PSAD and ACTD) for presurgical determination of the PC margin status in clinically confirmed candidates for radical treatment. The role of PSA-ACT and the related density parameter when used together with the neuroendocrinological oncomarker Chromogranin A (CgA) (14) was also investigated. In parallel, the aim of the study was to evaluate the possible addition of these parameters to the Partin predictive values (PPV) in the same manner as immunoscintigraphy with capromab pendetide (15).

Patients and Methods

Between January 2004 and December 2005, 50 PC patients with clinically localized PC underwent pelvic lymphadenectomy and RP. The mean age of the PC patients was 65 years (range 55-74 y, SD 4.2 y) and mean prostate volume was 35.7 ml (SD 13.0 ml). The presence of the tumor was confirmed by sextant needle biopsy. The TNM criteria, digital rectal examination (DRE), abdominal computerized tomography (CT) (Shimadzu Model Intellect, Shimadzu Corporation, Tokio, Japan), scintigraphic bone scan, serum PSA measurement and transrectal ultrasonography (TRUS, Siemens Sonoline Prima, Multiplanar Probe Siemens P II, transducer 7.5 mHz, Siemens AG Munich, Germany) were used to analyze the clinical stage. The presurgical clinical stage distribution data were T1c 14 patients, T2a 19 patients and T2b 17 patients.

The final selection criteria were PSA<20 ng/mL, Gleason score (GS) 4-7, the absence of DRE, scintigraphic, TRUS or CT evidences of an extracapsular PC invasion. Venous blood samples for laboratory measurements were taken from these 50 PC patients, as well as from 15 controls with clinically proven benign BPH.

None of the PC patients had been given neoadjuvant hormone therapy while the BPH subjects had not received previous pharmacological treatment. All the PC patients had undergone pelvic lymph node dissection prior to the radical retropubic prostatectomy.

Patients over 75 years, those already referred to some other surgical treatment and patients with severe kidney insufficiency that might cause an increase in the blood CgA level were not included in this study.

Tumor marker assays. The serum t-PSA and PSA-ACT values were measured in the venous blood samples according to previously described procedures (10) by using chemiluminescence assays purchased from Bayer Corporation, Tarrytown, NY, USA, and were recorded by means of the Automated Chemiluminescence System (ACS) obtained from the same company. The free PSA present in the sample was prevented from reacting with the total PSA antibodies by incubating the sample with a free-PSA-specific monoclonal mouse antibody which blocks free PSA preventing it from reacting in the ACS: 180 assay. The complexed PSA in the sample was measured afterwards by a two-site sandwich ACS: 180 immunoassay using direct chemiluminometric technology and a constant amount of two antibodies (MM1 and MP2). The serum CgA was measured by using a RIA kit from CIS Biointernational, France (14).

The density values PSAD and PSA-ACTD were calculated by dividing the serum values by the prostate volume, determined by one of the authors (Z.C) from the TRUS findings. The prostate volume was calculated by using the formula: height x length x width x n/16.

Post-surgical pathology. The prostatectomy specimens were parafin fixed and whole-mount step section were cut transversely at 5 mm intervals from the apex of the prostate to the tips of the seminal vesicles. The sections examined for tumor location, capsular penetration, surgical margin involvement and seminal vesicle invasion. The clinical and pathological stages were determined according to the TNM classification.

Statistical analysis. The Mann-Whitney mean non-parametric *U*-test and the area under the receiver-operating characteristic (ROC) curves were completed by a computer program for medical statistics.

The predictive values of PSA-ACT and ACTD were analyzed with a logistic regression (Backward-Stepwise Wald Method) which included age, volume of the prostate, GS, t-PSA, PSA-ACT, C:T PSA ratio, PSAD and ACTD. Probability values of <0.05 were considered statistically significant.

Results

The subsequent pathological findings revealed an organ-confined disease in 34 patients (pT2, 68%) and extraprostatic tumor in 16 patients (>pT2, 32%). There were no significant differences between the tPSA and PSA-ACT values or the PSAD and ACTD recordings between these two groups.

In the ROC curves plotted to predict extracapsular tumor extension, tPSA parameter resulted in the highest (0.62) area under the curve (AUC), followed by PSA-ACT (0.61), ACTD (0.52), and PSAD (0.50), respectively (Figures 1 and 2).

Logistic regression of these data was statistically significant ($p<0.001$) between PSA serum data and between their density derivatives. The most reliable predictors of extracapsular extension of PC were PSA-ACT and tPSA data while both density parameters were of lower reliability (Table I).
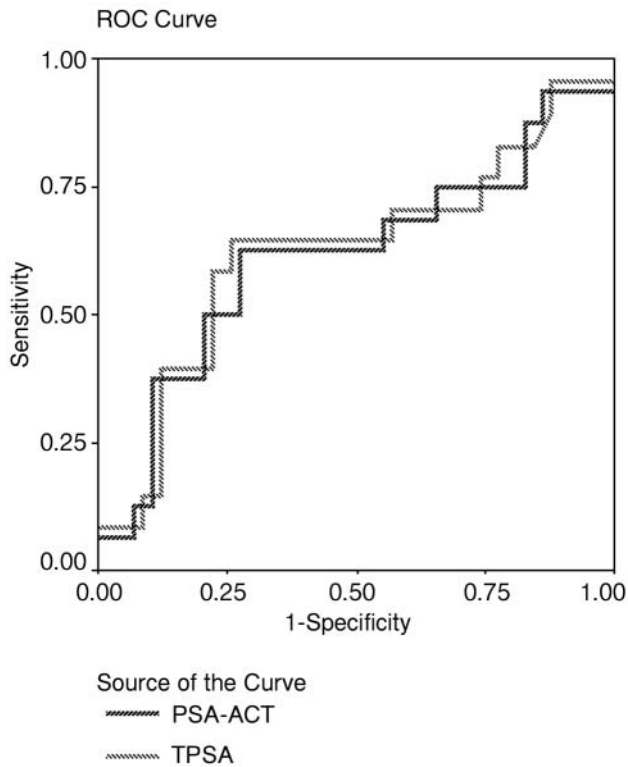
Figure 1. *ROC curves based on t-PSA and PSA-ACT data measured prior to RP on fifty PC patients. The related areas under the curve are 0.62 and 0.61, respectively.*
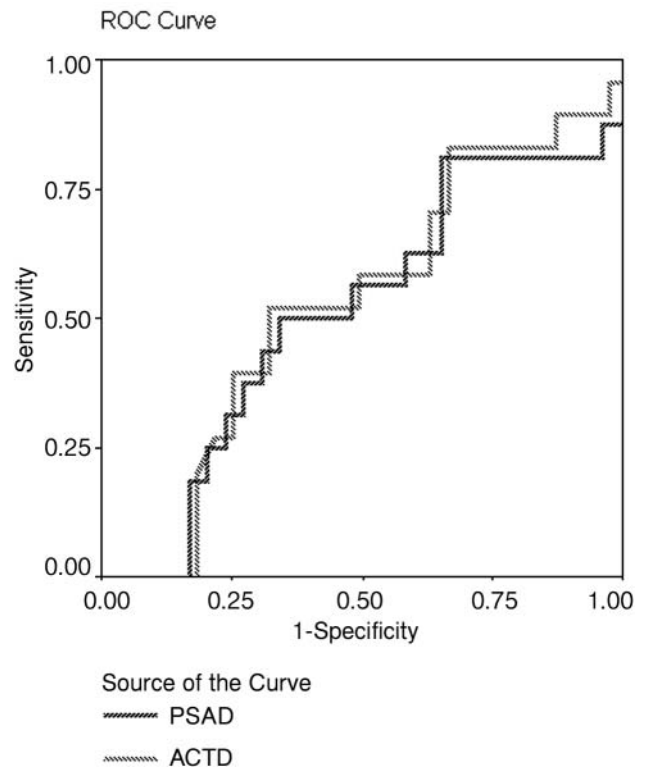

Figure 2. *ROC curves constructed from PSAD and ACTD recordings on fifty PC patients prior to RP. The related areas under the curve are 0.50 and 0.52, respectively.*

There were no significant differences in the tPSA ($p=0.188$) and PSA-ACT ($p=0.209$) values between the two PC patient groups. The value of PSAD ($p=0.943$) and ACTD ($p=0.822$) showed even less significance in differentiating between the two groups of PC patients.

In order to determine the predictive utility of all these parameters for extracapsular extension they were compared with an established combination of total PSA, PSAD, Gleason score, prostate volume, age, DRE, TRUS and C:t PSA ratio in a logistic regression analysis (Table II). The model of logistic regression has been shown to be statistically significant (-2 Log likelihood=34.2, χ^2=22.2, df=6, $p<0.001$). The variables such as DRE, prostate volume and GS were excluded from further analysis. The total reliability of the logistic regression model was 79%. According to the logistic regression model equally valid predictors for extracapsular extension of prostate cancer were PSA-ACT and t-PSA.

In the extracapsular disease group elevated CgA values were recorded in 1/6 GS 6 patients and in 2/7 GS 7 patients while in the localized PC group CgA elevation was found only in 1/9 GS 7 patients. The rate of elevated CgA for localized tumors was 1/32 (3%) and in extracapsular disease

3/15 or 20%. The overall CgA elevation in all studied PC patients (4/50) was 8% which was comparable to the value in the BPH controls (1/15, 6.7%). However, the CgA elevation in the patients with positive surgical margin was higher (20%).

Discussion

RP is the gold standard of potentially curative treatment for organ confined PC (16). However, several studies have reported that clinical staging before surgery fails to detect up to 40% of patients with extracapsular disease extension (17). A recurrence-free period of 10 years has been recorded in >90% of the patients with pathologically confirmed organ-confined disease in patients with a steady low post-surgical PSA concentration, whereas extra-prostatic extension markedly decreased survival and the recurrence-free period (17,18). Partin nomograms clearly show a very much higher chance of extracapsular extension with a rise in PSA level and a high GS (15). On the other hand elevated CgA concentration is a well-established risk factor for PC dissemination and poor prognosis (14,19). In addition, clinical exams are

Table I. *Serum PSA, PSA-ACT and CgA[a] concentrations and PSAD and ACTD values in PC patients prior to RP. Local or extracapsular disease was determined by post-surgical pathohistological analysis. Control group consisted of BPH patients. Serum concentrations expressed as ng/ml (SD).*

Conditions	PSA	PSA-ACT	CgAa	PSAD	ACTD
Local disease (34 patients)	8.22(3.81)	7.58(3.61)	31.1(12.8)	0.286(0191)	0.296(0.185)
Extracapsular disease (16 patients)	9.92(4.46)	9.30(4.41)	62.6(20.9)	0.273(0.144)	0.255(0.183)
Benign prost. hypertrophy (15 patients)	3.11(0.94)	2.33(0.34)	29.8(13.1)	0.077(0.010)	0.051(0.020)

[a]CgA concentrations were measured in 32 pts with a local PC and in 15 pts with the extended disease. Elevated CgA values in 3pts with extracapsular disease were 102.0, 119.3 and 131.2 ng/ml, in 1 pt with the local PC 98.8 and in 1 pt with BPH 104.4 ng/ml, respectively.

insufficient for the detection of micrometastases in patients with clinically localized PC and thus subclinical methods, such as CgA measurement, may emerge as important prognosticators (20).

The results indicated that neither PSAD nor PSA-ACTD being predictors of positive margins in PC candidates for RP at the time of preoperative PC diagnosis. In all studied patients the AUC was about 0.5, thus, demonstrating indeed the poor prediction. The study level of PSA-ACT also showed no improved prognostic significance over t-PSA in presurgical distinguishing extraprostatic disease (Tables I and II). T-PSA even showed a slightly larger AUC compared to that of PSA-ACT.

Our study, though limited to a small number of patients with PSA value <20 ng/ml and GS ≤7 revealed by logistic regression that the PSA-ACT value was a slightly more reliable predictor for PC extracapsular extension. However, the statistical differences between t-PSA and PSA-ACT data were not significant.

Although neuroendocrine differentiation (NED) expressed as a rise in serum CgA value is a rather late event in the natural history of PC its early appearance is a definite predictor of a poor prognosis (14, 22). A higher value of elevated CgA concentration was expectedly found in patients with GS 7 compared to lower PC GS. The addition of CgA assessment to the PSA studies may give the approach a new dimension since these two markers record different events during the development of PC. Among the patients with histologically organ-confined disease a rise in t-PSA level was observed, six months after surgery, only in one GS 7 patient who had an initially elevated CgA concentration. This observation confirms serum CgA elevation as an important predictor in PC patients (19) even when recorded in early stages of the disease (14). Similar conclusions have been recently reported in the literature in studies parallel to ours (23).

According to the data presented here a more accurate subclinical detection in the early stages PC cannot be derived from the use of PSA-ACT instead of T-PSA but

Table II. *Logistic regression analysis serum PSA and PSA-ACT concentrations (ng/ml) together with PSAD and ACTD values and other clinical parameters in 50 PC patients prior to RP. [a]In step 2 regression analysis age, Vp (ml) DRE, C/T PSA ratio and Gleason score were excluded.*

	-2 Log likelihood		Cox & Snell R Square		Nagelkerke R Square
	33.433		0.408		0.564
	B	S. E.	Wald	df	Sig.	Exp(B)
Step 1						
AGE	0.3774	0.1832	4.2445	1	0.0394*	1.4585
DRE	−0.5083	0.9632	0.2784	1	0.5977	0.6015
TRUS	−1.5791	1.1168	2.4809	1	0.1152	0.1722
PSA-ACT	5.0145	3.1911	2.4693	1	0.1161	50.5820
t-PSA	−3.9521	2.7436	2.0749	1	0.1497	0.0192
C/T	−4.3968	24.2378	0.0329	1	0.8561	0.0123
Vp (ml)	0.0113	0.0778	0.0213	1	0.8841	1.0114
PSAD	58.4106	44.3635	1.7335	1	0.1880	330E+25
ACTD	−78.667	49.3483	2.5412	1	0.1109	0.0000
GLEASON	0.4029	0.6733	0.3581	1	0.5496	1.4961
CONSTANT	−25.3651	23.6683	1.1485	1	0.2839	
Step 2						
AGE	0.3756	0.1753	4.5916	1	0.0321*	1.4558
TRUS	−2.0288	1.0176	3.9571	1	0.0462*	0.1315
PSA-ACT	4.6414	2.0654	5.0498	1	0.0246*	03.6921
t-PSA	−3.5702	1.7330	4.2442	1	0.0394*	0.0281
PSAD	58.1484	41.5413	1.9594	1	0.1616	793E+25
ACTD	−80.3827	47.8249	2.8250	1	0.0928	0.000

*Statistically significant data. [a]Serum CgA concentrations have not been statistically analyzed since data were lacking for some pts. Abbreviations: DRE, digital rectal examination; TRUS, transrectal ultrasound; PSA-ACT, PSA-alpha 1-antichymotripsin complex; t-PSA, total prostate specific antigen; C/T, C/T PSA ratio; Vp (ml), prostate volume; PSAD, PSA density; ACTD, PSA-ACT density.

may be achieved from the early detection of NED (following the t-PSA measurement) expressed as the elevation of CgA level. Thus, the use of PSA-ACT instead of t-PSA in Partin nomograms as an addition to Partin predictive values cannot be advocated.

Acknowledgements

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