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Complexed Prostate-specific Antigen for the Detection of Prostate Cancer

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Abstract. *Background: The description of a new method for the measurement of complexed prostate-specific antigen (cPSA) offers a new approach to the diagnosis of prostate cancer. Patients and Methods: We measured PSA (Hybritech and Bayer), free PSA (Hybritech) and complexed PSA (Bayer) in 72 patients with prostate cancer and 128 with benign prostate hyperplasia. Results and Conclusion: We found an increase of sensitivity using 2.5 and 7 ng/mL as cut-offs for cPSA in relation to total PSA using as cut-offs 4 and 10 ng/mL (96 and 36% vs. 92 and 35.5%). Similar differences were found for specificity (78% and 31% for cPSA vs. 73% and 29% for total PSA). Therefore, we defined a gray zone for patients with cPSA between 2.5 and 7 ng/mL for which the measurement of the free/complexed PSA ratio saves an important number of negative biopsies maintaining a higher sensitivity.*

Five years ago, Allard *et al.* (1) described a new method for the measurement of complexed prostate-specific antigen (cPSA) that improved on former attempts. Background problems observed in earlier assays were eliminated using a monoclonal antibody directed against free PSA (fPSA) that excluded this analyte from the subsequent reaction with anti-total PSA antibodies. The description of the different molecular forms of serum PSA in the early nineties (2,3), modified the traditional diagnostic scheme of prostate cancer. Several studies have demonstrated that the measurement of fPSA in relation to total PSA avoids the number of negative biopsies in the gray zone of PSA, with results between 4 and 10 ng/mL (4-7). Although fPSA has stability problems (8,9), its measurement has had important consequences both in terms of the increased costs and the morbidity associated with the biopsy.

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Following the description of the Allard group's method, various authors have pointed out advantages of measuring cPSA. Initially, Brawer *et al.* (10) reported that cPSA offers better results that total PSA in the early diagnosis of prostate cancer. Similar results have been presented by other authors recently (11,12). Nevertheless, the application of cPSA is not so clear in the differential diagnosis between benign prostatic hyperplasia (BPH) and prostate cancer. Our group, in a previous report, showed that cPSA improves the usefulness of total PSA only for patients with PSA around 4 ng/mL. So, in the differential diagnosis between prostate cancer and BPH, we have found that the use of PSA ratios improves the diagnostic accuracy of cPSA (13). We also reported, in a previous retrospective study, that the free/complexed PSA ratio offers the best results of all PSA ratios (14). Now, the aim of the present work was to study the clinical usefulness of cPSA in the differential diagnosis between prostate cancer and BPH, using a prospective evaluation of data. Also, with reference to previous studies, we evaluated the adaptation of cPSA measurement on the Advia Centaur analyzer, which includes certain changes in the master curve standard values.

Patients and Methods

We measured the serum levels of PSA, fPSA and cPSA in 72 patients with prostate cancer (mean age, 66.06; S.D., 7.44) and 128 patients with BPH (mean age, 67.60; S.D., 7.94; 94 of whom had prostatic symptoms), between September 2001 and June 2002.

All patients underwent an echography-guided biopsy by random octant technique. No patient with BPH developed prostate cancer in the 12 months following the measurement of tumor markers. In 43 of our patients, total PSA was lower than 4 ng/mL, while in another 43 patients cPSA was lower than 2.5 ng/mL. Criteria for entering in the study were PSA above 4 ng/mL, symptomatic BPH undergoing surgery or a pathologic digital rectal examination.

Determinations of total and fPSA were performed using the automated Access analyzer. These tests use two-site immuno-enzymatic assays employing monoclonal antibodies directed against different sites on the total or fPSA molecule, respectively. For both methods, a chemiluminiscent substrate was added to the reaction and the light generated was measured with a

Table I. *Results in patients with BPH and prostate cancer.*

	BPH Mean±S.D. Median (25% percentile; 75% percentile)	Prostate cancer Mean±S.D. Median (25% percentile; 75% percentile)	*p* value (BPH *vs.* prostate cancer)	AUC Area (95% C.I.)
PSA	8.24±8.43 5.94 (3.15; 10.8)	11.60±10.12 7.52 (5.63;13.19)	0.002	0.636 (0.557- 0.709)
cPSA	4.97±4.60 3.85 (1.82; 6.43)	8.02±7.24 5.44 (3.84; 9.19)	<0.001	0.672 (0.599-0.746)
free/ total PSA ratio	0.20±9.89 0.19 (0.13; 0.24)	0.12±6.07 0.12 (0.07; 0.16)	<0.001	0.754 (0.686-0.822)
complexed/ total PSA ratio	0.70±0.12 0.72 (0.64; 0.78)	0.79±8.50 0.80 (0.74; 0.86)	<0.001	0.731 (0.659-0.803)
free/complexed PSA ratio	0.37±0.29 0.31 (0.2; 0.45)	0.19±0.12 0.17 (0.10; 0.27)	<0.001	0.757 (0.688-0.825)

luminometer. The light production was directly proportional to the concentration of the analyte in the sample (Hybritech, Beckman, San Diego, CA, USA).

Total PSA was also measured using the fully automated Advia Centaur (Bayer Health Care, Tarrytown, NY, USA). This test is a two-site sandwich immunoassay using chemiluminometric technology, which employs a polyclonal goat anti-PSA antibody labelled with acridinium ester and a monoclonal mouse anti-PSA antibody, MM1, coupled to paramagnetic particles as solid phase. A similar design is used in the measurement of cPSA. fPSA present in the sample is prevented from reacting with total PSA antibodies by incubating the sample with a fPSA-specific monoclonal mouse antibody, which blocks fPSA so that it is non reactive in the cPSA assay. In both assays, the amount of relative light units detected by the system is proportional to the concentration of PSA or cPSA. The master curve standard values of cPSA on Advia Centaur were adjusted by the manufacturer to an average –9% in reference to the previous assay in Immuno-1 analyzer.

Separation of the serum from the clot was performed within three hours of sampling. Afterwards, we measured total and fPSA in the Access system. For determinations of total PSA and cPSA in the Advia Centaur system, serum samples were frozen at -80˚C until used. In all patients, the free/total PSA ratio (using the Hybritech assays), the complexed/total PSA ratio (using the Advia Centaur assays) and the free/complexed PSA ratio were calculated. The total PSA concentrations mentioned in the Results section refer to the Hybritech method.

The statistical analysis comprised the Mann-Whitney *U*-test to investigate whether there were significant differences between groups. We evaluated the reciprocal relation between sensitivity and specificity by plotting true-positive *versus* false-positive results in Receiver Operating Characteristic (ROC) curves. Areas under the curve (AUC) were measured and their 95 percent confidence intervals and significance calculated. Statistical analysis of the data was performed using STATA 7 software (STATA Corp. 2001).

Table II. *Percentage of patients with elevated PSA and cPSA serum levels.*

	Prostate cancer	BPH
PSA > 4	66/72 (92%)	91/128 (71%)
PSA > 10	27/72 (35.5%)	35/128 (27%)
cPSA > 2.5	69/72 (96%)	88/128 (69%)
cPSA > 7	26/72 (36%)	28/128 (22%)

Results

Table I shows the mean, median, 25 and 75 percentile and standard deviation of PSA, cPSA, the free/total PSA ratio, the complexed/total PSA ratio and the free/complexed PSA ratio. Significant differences between BPH and prostate cancer were found for all tests. In this Table, the AUC obtained by the ROC analysis is also shown. The best results found were for the free/complexed PSA and free/total PSA ratios (0.757 and 0.754, respectively). Also, we observed better results with cPSA (0.672) than total PSA (0.636), with significant differences between them ($p=0.0016$).

The percentage of patients with serum levels of PSA above 4 and 10 ng/mL is shown in Table II. Using the standard cut-off of 4 ng/mL, a sensitivity of 92% and a specificity of 29%, respectively, was observed. When we used a cut-off of 10 ng/mL, these results were 35.5% and 73%, respectively. This Table also shows the sensitivity and the percentage of false-positives found for cPSA. For this marker two cut-offs were selected with similar sensitivity and specificity to those obtained for PSA, using the cut-offs of 4 and 10 ng/mL. The values obtained were 2.5 and 7 ng/mL.

In Table III the results obtained with the current routine scheme for diagnosis of prostate cancer are shown. In short, biopsy is performed in patients with positive digital rectal examination (DRE), PSA greater than 10 ng/mL, or in patients with negative DRE and PSA between 4 and 10 ng/mL when the free/total PSA ratio is lower than 0.2. Twenty-three patients were excluded from this analysis with an indeterminate DRE. With this scheme, a sensitivity of 91% (63/69) and a specificity of 41% (44/108) were found.

Table III. *Results obtained using the current routine scheme.*

Positive DRE 42 patients	Negative DRE 135 patients		
	PSA < 4 34 patients	PSA 4-10 63 patients	PSA >10 38 patients
	Patients with cancer / Total patients		
Positive DRE	PSA <4	free/total PSA ratio >= 0.2	free/total PSA ratio < 0.2	PSA >10
29/42 (69%)	5/34 (15%)	1/16 (6%)	19/47 (40%)	15/38 (39%)

Table IV. *Results obtained introducing cPSA in the stratification of the subgroup of patients with negative DRE.*

Positive DRE 42 patients	Negative DRE 135 patients		
	cPSA < 2.5 35 patients	cPSA 2.5-7 66 patients	cPSA > 7 34 patients
	Patients with cancer / Total patients		
Positive DRE	cPSA <2.5	free/total PSA ratio >= 0.2	free/total PSA ratio < 0.2	cPSA >7
29/42 (69%)	3/35 (9%)	2/18 (11%)	21/48 (44%)	14/34 (41%)

Tables IV and V present the results obtained with two alternative schemes, in which we introduced cPSA as the first tool for the classification of patients with negative DRE. In these Tables, we defined a gray zone for cPSA for values between 2.5 and 7 ng/mL. For this subgroup of patients, the results obtained with the use of the free/total PSA ratio (Table IV) and the free/complexed PSA ratio (Table V) are shown. The use of the free/complexed PSA ratio with 0.32 as cut-off (Table V) offered better results than the free/total PSA ratio (Table IV), the sensitivity being 91% and the specificity 42%. In the same subgroup of patients, the sensitivity and specificity of the free/total PSA ratio was 91% and 37%, respectively.

Table V. *Results obtained introducing cPSA in the stratification of the subgroup of patients with negative DRE and the use of the free/complexed ratio in the subgroup of patients with cPSA between 2.5 and 7 ng/mL.*

Positive DRE 42 patients	Negative DRE 135 patients		
	cPSA < 2.5 35 patients	cPSA 2.5-7 66 patients	cPSA > 7 34 patients
	Patients with cancer / Total patients		
Positive DRE	cPSA <2.5	free/complexed PSA ratio >= 0.32	free/complexed PSA ratio < 0.32	cPSA >7
29/42 (69%)	3/35 (9%)	2/20 (10%)	21/46 (46%)	14/34 (41%)

Finally, we calculated the global results for our series using these alternative schemes. Using the free/total PSA ratio for patients with cPSA between 2.5 and 7 ng/mL and negative DRE resulted in a sensitivity of 93% (64/69) and a specificity of 44% (48/108). Using the free/complexed PSA ratio, a sensitivity of 95% (64/69) and a specificity of 46% (50/108) were obtained. Table VI presents a résumé of results for different tests, expressed in terms of number of cancer detected and number of patients biopsied to detect one patient with cancer.

Discussion

The high number of false-positives is the most important disadvantage in using PSA. PSA is greater than 4 ng/mL in 25-50% of patients with BPH (15). In the present series, we found that PSA was above 4 ng/mL in 71% of patients with BPH. We also detected that PSA was above 10 ng/mL in 27% of these patients. Various studies advocate the use of the free/total PSA ratio as a tool that avoids an important number of negative biopsies in the group of patients with PSA between 4 and 10 ng/mL. In this study, the results obtained with the introduction of the current scheme were based on biopsies performed on patients with positive DRE or PSA above 10 ng/mL, and for patients with the free/total PSA ratio lower than 0.2 when DRE is negative and PSA is between 4 and 10 ng/mL. On evaluating the patients with this scheme, we obtained a sensitivity of 91% and a specificity of 41%. Similar results have been reported in other studies, including a previous analysis of our group (4-7).

Several authors have reported that cPSA shows better results than total PSA or the free/total PSA ratio in the early diagnosis of prostate cancer (10-12), although Lein *et al.* (16) have presented opposing results. In a previous study, we found that the usefulness of the free/total PSA ratio improves the diagnostic accuracy obtained with total PSA or with cPSA in the differential diagnosis between prostate cancer and BPH (13). In our opinion, cPSA improves the diagnostic usefulness of total PSA only for patients with PSA around 4 ng/mL. Thus, in the differential diagnosis between BPH and prostate cancer, we found similar specificity problems with cPSA and with total PSA.

Table VI. *Number of patients biopsied to detect one patient with cancer for the different tests.*

	No. of patients	No. of cancer detected	No. of patients biopsied to detect 1 with cancer
PSA > 4	154	66 (42%)	2.37
PSA > 10	62	27 (43,5%)	2.29
cPSA > 2,5	157	69 (44%)	2.27
cPSA > 7	54	26 (48%)	2.07
PSA 4-10 & f/tPSA < 0.2	127	63 (49,6%)	2.01
cPSA 2.5-7 & f/tPSA < 0.2	124	64 (51,6%)	1.93
cPSA 2.5-7 & f/cPSA < 0.32	122	64 (52,4%)	1.90

In the present series, we evaluated our results using cPSA as the first-line test for the classification of patients with negative DRE (Tables IV and V). First, a gray zone of cPSA for patients with serum levels of this marker between 2.5 and 7 ng/mL was determined. For these cut-off limits, similar results were found to those obtained with total PSA using the standard concentrations of 4 and 10 ng/mL (Table II). Next, the results obtained in the current routine scheme were compared with those obtained using cPSA as a stratifying test for patients with negative DRE. This alternative procedure improved the sensitivity from 91% to 93%. Two alternative schemes were studied, evaluating the usefulness of the free/total PSA ratio and the free/complexed PSA ratio in order to decide whether to perform biopsy on patients with negative DRE and cPSA between 2.5 and 7 ng/mL. We found, respectively, a specificity of 44% and 46%. These are an improvement on the specificity of 41% obtained with the current routine scheme.

We must emphasize that the combination of cPSA and the free/complexed PSA ratio offered the best results in our series. The introduction of the free/complexed PSA ratio instead of the free/total PSA ratio for patients with negative DRE and PSA between 4 and 10 ng/mL improved the specificity from 37 to 42%, with the same sensitivity. There are few studies evaluating the usefulness of the free/complexed PSA ratio. Jung *et al.* (17) showed that this test has a lower AUC than the free/total PSA ratio, while Lein *et al.* (18,19) found no substantial differences between the ratios. In contrast, Wu and Liu (20) reported the advantage of the free/complexed PSA ratio and our group, in a previous retrospective study, found that it performed better (14). Differences in the results obtained by the different groups can be caused by the different methods used in the measurement of cPSA. It should be underlined that our data are consistent with the performance of different forms of PSA in the presence or absence of cancer. The concentration of fPSA is lower in patients with prostate cancer, while cPSA is higher in these patients. When both tests are used simultaneously, maximization of the differences between patients with prostate cancer and BPH can be obtained, as shown in the study.

The advantages of replacing the total PSA assay with the new assay for cPSA have been demonstrated. The choice of cPSA as the first-line test for the detection of prostate cancer defines a new strategy that improves the diagnostic accuracy obtained with the current routine scheme. In this sense, we have defined a new gray zone for patients with cPSA between 2.5 and 7 ng/mL, for whom measurement of the free/complexed PSA ratio saves an important number of negative biopsies maintaining a higher sensitivity. Obviously, further studies are required to decide whether the differences observed in this evaluation are sufficient to substitute the total PSA by the cPSA assay as first-line test in the diagnosis of prostate cancer.

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