Usefulness of Proprostate-specific Antigen in the Diagnosis of Prostate Cancer

XAVIER FILELLA[1], JOAN ALCOVER[2], RAFAEL MOLINA[1], PILAR LUQUE[2], JUAN M. CORRAL[2], JOSEP M. AUGÉ[1] and FRANCISCA COCA[1]

Departments of [1]Biochemistry and Molecular Genetics (CDB) and [2]Urology (ICNU), IDIBAPS, Hospital Clínic, Barcelona, Catalonia, Spain

Abstract. *Background: Free prostate-specific antigen (fPSA), the minor form of total PSA, contains different molecular subforms, including BPSA and proPSA. Whereas BPSA is associated with benign prostate hyperplasia, proPSA is associated with prostate tumor. Patients and Methods: The serum levels of PSA, fPSA and proPSA were measured using automated electrochemiluminescent immunoassays (Elecsys 2010, Roche Diagnostics) in 87 patients with prostate cancer and 138 patients with benign prostate hyperplasia. Also, we calculated the derived tests of these assays through the subtraction or the ratio between the measured tests. Results: Receiver operating characteristics curves were used for comparison of the diagnostic utility of tests assessed. The biggest areas were obtained for the free/total PSA ratio (0.705), the calculated Bfree PSA/total PSA ratio (0.719) and the calculated Bfree PSA/bound PSA ratio (0.726). Conclusion: Applying a multivariate logistic regression analysis, it was determined that the combination of the proPSA concentration, the proPSA/total PSA ratio and the calculated Bfree/total PSA ratio improves the area under the curve obtained for individual tests (0.753). ProPSA may be useful in the diagnosis of prostate cancer.*

Serum prostate-specific antigen (PSA) determination is the clinical laboratory test of choice for the diagnosis of prostate cancer. However, the use of PSA to correctly distinguish between cancer and benign prostate hyperplasia (BPH) remains imperfect (1-2). With the aim of improving specificity, different strategies have been described, including age-referenced PSA (3), PSA velocity (4) and the evaluation of different forms of circulating PSA (5-10).

Correspondence to: Dr. Xavier Filella, Department of Biochemistry and Molecular Genetics (CDB), Hospital Clínic, C/ Villarroel, 170, 08036 Barcelona, Catalonia, Spain. Fax : +34 3 227 93 76, e-mail: xfilella@clinic.ub.es

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Approximately 70-90% of the total PSA in serum is bound to protease inhibitors, such as alfa-1-antichymotrypsin, whereas 10-30% of total PSA is free PSA (fPSA) (5-6). The measurement of fPSA, expressed as the percentage of total PSA, represents an important improvement in the diagnosis of prostate cancer, allowing a significant reduction of biopsies for these patients with PSA values between 4-10 ng/ml (11-12).

Several studies have shown that fPSA contains different molecular subforms, including BPSA and proprostate specific antigen (proPSA) (13-14). Whereas BPSA is associated with BPH, proPSA is associated with prostate tumor. Thus, serum proPSA, expressed as a percentage of fPSA, may be more specific than other fractions of PSA. The determination of serum levels of proPSA can improve the specificity of prostate cancer detection (13-19).

This study tested the specificity of proPSA in the detection of prostate cancer and compared its performance with total and different fractions of PSA.

Patients and Methods

The serum levels of PSA, fPSA and proPSA in 87 patients with prostate cancer and 138 patients with BPH were measured. All patients underwent an echography-guided biopsy to detect patients with BPH who advanced to prostate cancer in the 12 months following the measurement of tumor markers.

Measurement of total PSA and fPSA were performed using the automated Elecsys™ 2010 analyzer (Roche Diagnostics, Mannheim, Germany). These are two-site immunoenzymatic assays using monoclonal antibodies directed against different sites on the total or free PSA molecule.

The proPSA test is a non-commercial assay in the electro-chemiluminescence immunoassay format. The combination of two monoclonal antibodies, directed against fPSA and the -5 and -7 propeptide parts of proPSA, allows the recognition of the fPSA subfractions [-5] proPSA and [-7] proPSA. Upon the addition of a chemiluminiscent substrate, light is produced in direct proportion to the amount of analyte in the sample. The analyse concentration is read from a stored calibration curve.

Measurements of total and free PSA in the Elecsys analyzer were performed on the same day with the extraction. However, for

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

	BPH Mean±S.D.	Prostate cancer Mean±S.D.	*p*-value
Total PSA	7.52±7.12	10.53±11.47	0.018
Free PSA	1.46±2.55	1.38±2.03	n.s.
Calculated bound PSA	6.06±5.38	9.14±9.79	0.003
Free / total PSA ratio	0.19±0.08	0.16±0.09	<0.001
ProPSA	0.35±0.23	0.49±0.91	n.s.
ProPSA / total PSA ratio	0.06±0.06	0.05±0.03	0.043
ProPSA / free PSA ratio	0.34±0.18	0.38±0.17	0.038
Free – proPSA (Bfree PSA)	1.11±2.49	0.88±1.36	n.s.
BfreePSA / total PSA ratio	0.12±0.06	0.08±0.05	<0.001
BfreePSA / bound PSA ratio	0.16±0.11	0.10±0.07	<0.001
ProPSA / Bfree PSA ratio	0.71±0.83	0.82±0.73	0.038

n.s. = not significant ($p>0.05$).

Table II. *Area under the curve obtained by receiver operating characteristic curves and p-value for univariate logistic regression (LR)*

Test	AUC (95% CI)	*p*-value (LR)
Total PSA	0.594 (0.520-0.668)	0.0258
Free PSA	0.535 (0.459-0.611)	0.8023
Bound PSA	0.617 (0.544-0.690)	0.0065
Free / total PSA	0.705 (0.636-0.775)	<0.0001
ProPSA	0.518 (0.440-0.595)	0.1414
ProPSA / total PSA	0.580 (0.504-0.657)	0.0318
ProPSA / free PSA	0.582 (0.505-0.659)	0.0575
Free – proPSA (Bfree PSA)	0.559 (0.482-0.634)	0.4624
BfreePSA / total PSA	0.719 (0.649-0.789)	<0.0001
BfreePSA / bound PSA	0.726 (0.657-0.795)	<0.0001
ProPSA / Bfree PSA	0.582 (0.505-0.659)	0.3136

the measurements of proPSA serum samples were frozen at –80˚C until used.

Bound PSA (fPSA subtracted from total PSA), and Bfree PSA (proPSA subtracted from fPSA) were calculated. Also, the ratios of free/total PSA, proPSA/total PSA, proPSA/free/PSA, Bfree PSA /total PSA and Bfree PSA/bound PSA were calculated.

The Mann-Whitney *U*-test was used for the statistical comparison of the results. The area under the curve (AUC) was calculated using receiver operating characteristics (ROC) analysis. Logistic regression analysis was also performed to obtain a model of variables to predict the disease outcome of prostate cancer versus BPH. All statistical calculations were carried out with the software package 9.1 (Cary, NC, USA).

Results

Table I shows the results obtained for the tests evaluated in this study, including the ratios obtained between different fractions of PSA. The maximum differences ($p<0.001$) between the patients with prostate cancer and those with BPH were observed for the free/total PSA ratio, the calculated BfreePSA/total PSA ratio and the calculated BfreePSA/bound PSA ratio. Significant differences were observed for total PSA ($p=0.018$), but not for free PSA or proPSA.

We compared the ability of different tests to differentiate between prostate cancer and BPH by ROC analysis. Table II shows the areas under the curve obtained for each parameter. The area obtained for PSA was 0.594. The highest areas were found for the free/total PSA ratio (0.705), the calculated Bfree PSA/total PSA ratio (0.719) and the calculated Bfree PSA/bound PSA ratio (0.726).

Using univariate logistic regression analysis (Table II), total PSA, calculated bound PSA, free/total PSA, proPSA/total PSA, the calculated Bfree PSA/total PSA ratio and the calculated Bfree PSA/bound PSA ratio significantly ($p<0.05$) differentiated men with prostate cancer from those without. However, applying stepwise backward multivariate logistic regression analysis, proPSA, proPSA/total PSA ratio and the calculated Bfree PSA/total PSA ratio were retained and generated an ROC curve with an area under the curve of 0.753 (Figure 1).

Discussion

The range of total PSA concentrations in patients with BPH overlaps with the concentrations in prostate cancer patients. The measurement of fPSA and the evaluation of the ratio between fPSA and total PSA enhanced the diagnostic clinical utility of PSA testing by significantly increasing the specificity. Despite the success of fPSA in prostate cancer diagnosis, several limitations remain and better serum markers are needed.

It was recently reported that fPSA is more complex than originally thought. Thus, fPSA includes the subforms BPSA and proPSA. BPSA is associated with pathologic BPH, whereas proPSA is associated with the presence of prostate cancer. In addition, several studies have demonstrated the presence of proPSA forms in serum (20-21) and more recently immunoassays for the proPSA form have been developed.

Using a specific immunoassay for proPSA, it was observed that this fraction is associated with prostate cancer, whereas the calculated Bfree PSA is higher in patients with BPH than in those with prostate cancer, but no statistical differences were found for either forms. Other authors had described similar results, although the non-existence of standardized techniques for the measurement of proPSA and the differences in the characteristics of patients included in these series makes it difficult to establish precise comparisons (15-18).

The relevance of proPSA and Bfree PSA in our study increases when evaluated as a fraction. It was shown that

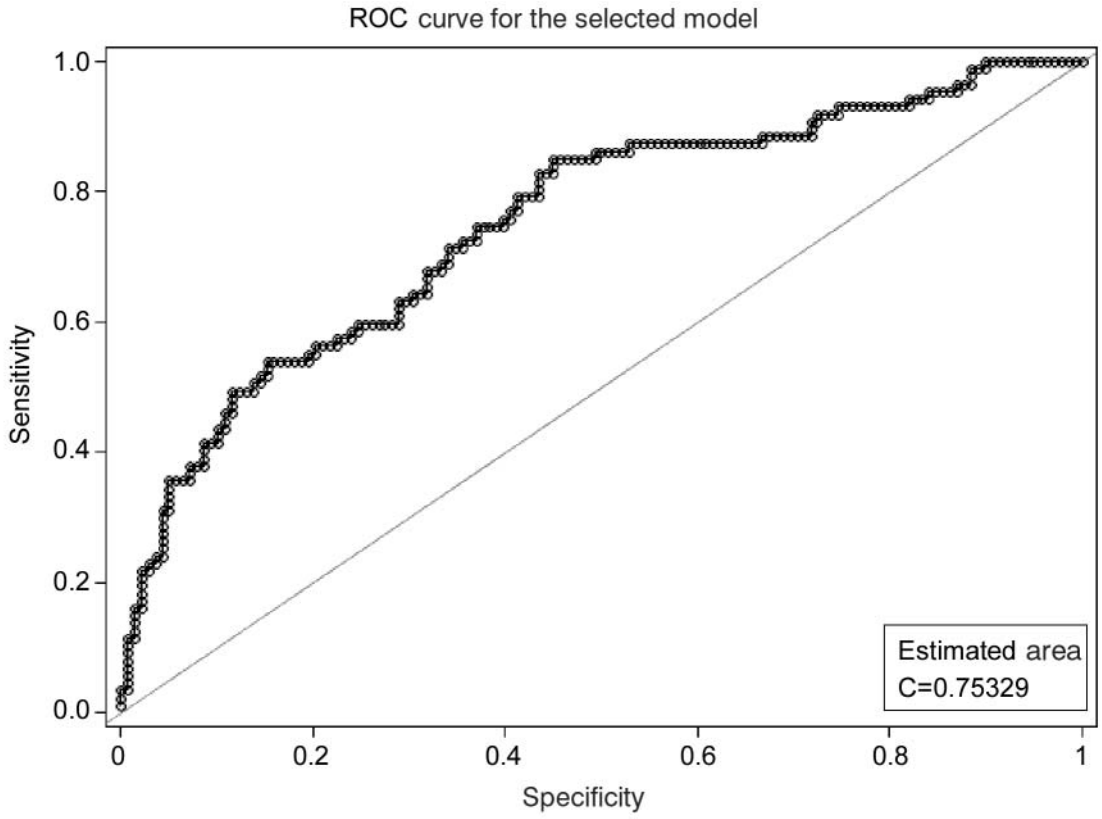

Figure 1. *ROC curve obtained using multivariate logistic regression analysis.*

the proPSA/Bfree PSA ratio in patients with prostate cancer is significantly higher than in patients with BPH ($p=0.038$). On the other hand, significant differences were observed between these groups for the calculated BfreePSA/total PSA ratio and for the calculated Bfree PSA/bound PSA ratio ($p<0.001$). These results are compatible with the expected behavior of the different subforms of fPSA, with Bfree PSA being related to BPH and proPSA to prostate cancer.

In addition to proPSA, several markers for the diagnosis of prostate cancer had been described in the last years, including complexed PSA or interleukin-6, while other newer markers will be described with the development of proteomic techniques. More complicated algorithms incorporating these variables will be used with the aim to predict the presence of prostate cancer. Mikolajczyk *et al.* (22) suggested the interest of the multiparameter approximation for the diagnosis of prostate cancer. In this sense, Khan *et al.* (15) proposed the use of a combination of proPSA, total PSA and the fPSA/total PSA ratio with the objective of improving the specificity of early prostate cancer detection in men with a total PSA between 4 and 10 ng/ml. A similar approach was evaluated in our series by means of logistic regression analysis. Our study has shown that the combination of the proPSA concentration, the proPSA/total PSA ratio and the calculated Bfree/total PSA ratio improves the AUC obtained for individual tests. Although a deeper study is necessary to confirm our preliminary findings, we consider that the proPSA may be a useful test in the diagnosis of prostate cancer.

References

1 Ambruster DA: Prostate-specific antigen: biochemistry, analytical methods, and clinical application. Clin Chem *39*: 181-195, 1993.

2 Filella X, Molina R, and Ballesta AM, Gil MJ, Allepuz C and Rioja LA: Value of PSA (prostate-specific antigen) in the detection of prostate cancer in patients with urological symptoms. Results of a multicentre study. Eur J Cancer *32A*: 1125-1128, 1996.

3 Oesterling JE: Age-specific ranges for serum PSA. N Engl J Med *335*: 345-346, 1996.

4 Carter HB, Pearson JD, and Metter EJ, Brandt LJ, Chan DW, Andres R, Fozard JL and Walsh PC: Longitudinal evaluation of prostate specific antigen levels in men with and without prostate disease. JAMA *267*: 2215-2220, 1991.

5 Lilja H, Christensson A, Dahlén U, Matikainen MT, Nilsson O, Pettersson K and Lovgren T: Prostate-specific antigen in serum occurs predominantly in complex with alpha-1-antichymotrypsin. Clin Chem *37*: 1618-1625, 1991.
6 Stenman UH, Leinonen J, Alfthan H, Ranniko S, Tuhkanen K and Alfthan O: A complex between prostate-specific antigen and alfa-1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res *51*: 222-226, 1991.
7 Brawer MK, Meyer GE, Letran JL, Bankson ER, Morris DL, Yeung KK and Allard WJ: Measurement of complexed PSA improves specificity for early detection of prostate cancer. Urology *52*: 372-378, 1998.
8 Filella X, Alcover J, Molina R, Corral JM, Carretero P and Ballesta AM: Measurement of complexed PSA in the differential diagnosis between prostate cancer and benign prostate hyperplasia. The Prostate *42*: 181-185, 2000.
9 Fischer K, Loertzer H and Fornara P: The use of complexed PSA for the early detection of prostate cancer. Anticancer Res *25*: 1591-1596, 2005.
10 Filella X, Truan D, Alcover J, Gutierrez R, Molina R, Coca F and Ballesta AM: Complexed prostate-specific antigen for the detection of prostate cancer. Anticancer Res *24*: 4181-4186, 2004.
11 Filella X, Alcover J, Molina R, Rodríguez A, Carretero P and Ballesta AM: Free and total PSA in the diagnosis of prostate cancer. Tumor Biology *18*: 332-340, 1997.
12 Yang CR, Su CK, Chiu KY, Ho HC, Ou YC, Cheng CL and Lee H: Free/total prostate specific antigen ratio for prostate cancer detection: a prospective blind study. Anticancer Res *25*: 2430-2443, 2005.
13 Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, Wheeler TM and Slawin KM: A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. Cancer Res *60*: 756-759, 2000.
14 Mikolajczyk SD, Marks LS, Partin AW and Rittenhouse HG. Free prostate-specific antigen in serum is becoming more complex. Urology *59*: 797-802, 2002.
15 Khan MA, Partin AW, Rittenhouse HG, Mikolajczyk SD, Sokoll LJ, Chan DW and Veltri RW: Evaluation of proprostate specific antigen for early detection of prostate cancer in men with a total prostate specific antigen range of 4.0 to 10.0 ng/ml. J Urol *170*: 723-726, 2003.
16 Sokoll LJ, Chan DW, Mikolajczyk SD, Rittenhouse HG, Evans CL, Linton HJ, Mangold LA, Mohr P, Bartsch G, Klocker H, Horninger W and Partin AW: Proenzyme PSA for the early detection of prostate cancer in the 2.5-4.0 ng/ml total PSA range: preliminary analysis. Urology *61*: 274-276, 2003.
17 Catalona WJ, Bartsch G, Rittenhouse HG, Evans CL, Linton HJ, Horninger W, Klocker H and Mikolajczyk SD: Serum pro-prostate specific antigen preferentially detects aggressive prostate cancers in men with 2 to 4 ng/ml prostate specific antigen. J Urol *171*: 2239-2244, 2004.
18 Mikolajczyk SD, Catalona WJ, Evans CL, Linton HJ, Millar LS, Marker KM, Katir D, Amirkhan A and Rittenhouse HG: Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer. Clin Chem *50*: 1017-1025, 2004.
19 Khan MA, Sokoll LJ, Chan DW, Mangold LA, Mohr P, Mikolajczyk SD, Linton HJ, Evans CL, Rittenhouse HG and Partin AW: Clinical utility of proPSA and 'benign' PSA when percent free PSA is less than 15%. Urology *64*: 1160-1164, 2004.
20 Mikolajczyk SD, Grauer LS, Millar LS, Hill TM, Kumar A, Rittenhouse HG, Wolfert RL and Saedi MS: A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. Urology *50*: 710-714, 1997.
21 Peter J, Unverzagt C, Krogh TN, Vorm O and Hoesel W: Identification of precursor forms of free prostate-specific antigen in serum of prostate cancer patients by immunoabsortion and mass spectrometry. Cancer Res *61*: 957-962, 2001.
22 Mikolajczyk SD, Song Y, Wong JR, Matson RS and Rittenhouse HG: Are multiple markers the future of prostate cancer diagnostics? Clin Biochem *37*: 519-528, 2004.

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