

Serum Total and Free Prostate-specific Antigen for Breast Cancer Diagnosis in Women¹

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ABSTRACT

Prostate-specific antigen (PSA) is a serine protease expressed at high levels in prostate epithelium, and elevated PSA in serum is a well-established marker of prostate cancer. Recently, the relative proportions of free PSA and PSA complexed to the serine protease inhibitor α_1 -antichymotrypsin have become important variables in distinguishing between prostate cancer and benign prostatic hyperplasia. Numerous studies have demonstrated the production of PSA in female tissues such as the breast, and low levels of PSA are present in female sera. The objective of this study was to measure and compare the relative proportions of free PSA and PSA complexed to the serine protease inhibitor α_1 -antichymotrypsin in the serum of women with breast cancer or benign breast disease or women with no known malignancies. PSA was measured with an established immunoassay for total PSA and a novel immunoassay for free PSA, both of which had a detection limit of 0.001 $\mu\text{g/liter}$ (1 ng/liter). The percentage of breast cancer patients with free PSA as the predominant molecular form (>50% of total PSA) in serum was five times higher than that of healthy women or women with benign breast disease, and PSA decreased in the serum of breast cancer patients after surgery. The diagnostic use of free PSA for breast cancer is limited at this point, due to the low diagnostic sensitivity (~20%); however, free PSA as the predominant molecular form shows a high diagnostic specificity (~96%) in comparison to women free of breast cancer or with benign breast

disease. These results suggest that the clinical applicability of free PSA for breast cancer diagnosis and the biological mechanism behind its increase should be further investigated.

INTRODUCTION

PSA³ is a M_r 33,000 single-chain glycoprotein expressed at high levels in the epithelium of the human prostate gland (1). The prostatic function of PSA is to liquefy the sperm-entrapping seminal coagulum after ejaculation (2). The seminal substrates of PSA, a serine protease with chymotrypsin-like specificity, are the major structural constituents of the gel-like coagulum, seminogelin I and II and fibronectin (3).

The name "PSA" reflects the initial widespread belief that expression of the protein was restricted to the prostate gland. Over the past 5 years, however, this notion has clearly been dispelled. Numerous studies have shown that PSA is expressed extraprostatically (4), suggesting that PSA may be functional outside the prostate gland. The periurethral (Skene's) gland was the first female tissue that was suggested to be able to produce PSA (5). This tissue has been referred to as the "female prostate" because its developmental origin is homologous to that of the male prostate (6). Therefore, it may not be surprising that this gland produces PSA in both males and females. It is now clear that hormonally regulated tissues in females, such as the breast, can produce PSA. PSA is detectable in normal and hyperplastic breast tissue (7) and is present in the majority of breast tumors (7–10) and breast cysts (11, 12). PSA is released into breast secretions, such as the milk of lactating women (13) and nipple aspirate fluid (14–16). Mammary PSA is identical in molecular weight and mRNA sequence to seminal PSA (17). PSA gene expression in breast tumors appears to be under hormonal control because the steroid hormone receptor-positive breast cancer cell lines T-47D and BT-474 can be induced by androgens, progestins, mineralocorticoids, and glucocorticoids to produce PSA (18, 19) *in vitro*.

In males, low levels of PSA are normally released into the blood (20). The majority of circulating PSA occurs in a complex with the serine protease inhibitor ACT, whereas the remainder (<30%) occurs in the noncomplexed "free" form (21). The use of serum PSA as a prostatic tumor marker has flourished over the past decade. PSA immunoassays are widely used to detect early-stage prostate cancer, to evaluate disease progression, and to assess therapeutic response (22). In addition to the total serum PSA level, the ratio of free:total PSA has become an important variable for distinguishing between males with benign and ma-

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³ The abbreviations used are: PSA, prostate-specific antigen; ACT, α_1 -antichymotrypsin; HPLC, high-performance liquid chromatography; IGFBP, insulin-like growth factor binding protein; IGF, insulin-like growth factor.

lignant prostatic pathology. The percentage of free serum PSA is lower in males with prostatic carcinoma than in those with benign prostatic hyperplasia or with no apparent prostate pathology (23). Whereas the total PSA level alone is not specific enough for the early diagnosis of prostate cancer, the ratio of free:total PSA may improve specificity without compromising sensitivity (24).

Detectable circulating levels of PSA, likely originating from breast tissue, are present in the serum of women (25). The PSA concentration in female sera is approximately 1000-fold less than that of males ($<0.004 \mu\text{g/liter}$; Ref. 26). Given the variation in the ratio of the molecular forms of PSA in prostate cancer, benign prostatic hyperplasia, and normal males, preliminary investigations of the PSA molecular forms in the sera of females were performed in our laboratory. Using chromatographic techniques and a small number of patients, we demonstrated that the predominant form of PSA in the sera of women without breast cancer is PSA bound to ACT, whereas free PSA constitutes the major form of PSA in the presurgical serum of breast cancer patients (27–30).

The difference in molecular forms of serum PSA between women with and without breast cancer is intriguing. The rationale for this study was that previous studies examining this phenomenon have been performed exclusively with HPLC. HPLC can effectively resolve PSA subfractions, but it is of limited utility as samples become diluted during the separation procedure. Therefore, to obtain immunologically measurable HPLC fractions, the initial serum total PSA concentration must be at least $0.015 \mu\text{g/liter}$ (15 ng/liter ; Ref. 29). This concentration requirement limits the number of samples that can be analyzed because less than 10% of female sera have such a relatively high total PSA concentration (29).

Direct immunoassay for free PSA, in addition to total PSA, is a much more efficient method of analyzing large numbers of samples. Such a PSA immunoassay, suitable for analysis of female serum, must have an extremely low detection limit ($0.001 \mu\text{g/liter}$ or 1 ng/liter). This level of sensitivity is difficult to achieve, and, until now, only one such assay for total PSA has been reported (30). An ultrasensitive immunoassay for free PSA with a detection limit of 1 ng/liter has recently been developed in our laboratory (31). The use of this assay, in conjunction with our total PSA immunoassay, has allowed us to investigate the molecular forms of PSA in the sera of a large number of women with various pathologies.

MATERIALS AND METHODS

Patient Population. Patients were operated on at the Department of Gynecological Oncology (University of Turin, Turin, Italy). Total PSA and free PSA were measured in 118 serum samples from breast cancer patients and in 115 samples from the same breast cancer patients 6 months after surgery. The sera from 46 patients with breast cysts and 116 patients with uterine fibroids were also analyzed for total PSA and free PSA.

Breast cancer patient age ranged from 30–88 years (median age, 56 years). Each tumor was graded histologically according to the criteria described by Bloom and Richardson (32). The tumor grade distribution was 20% grade 1, 50% grade 2, and 30% grade 3. The tumor size, recorded as the maximum

diameter of the fresh surgical specimen, ranged from 0.4 to 8.0 cm, with a median of 2.0 cm. A total of 35% of the patients had nodal involvement, and 65% and 61% of patients showed estrogen and progesterone receptor positivity, respectively. Fourteen percent of the patients had relapsed. A total of 18% of the patients were receiving no adjuvant treatment, 60% were receiving hormonal adjuvant treatment (tamoxifen), 20% were receiving chemotherapy, and 2% were receiving a combination of chemotherapy and hormonal therapy.

Sera from 99 female blood donors were analyzed for total PSA and free PSA. These samples were collected from the Red Cross Society (Toronto, Canada). The blood donors were confirmed by interview to be free of apparent malignancies and were between the ages of 45 and 65 years, with a median age of 51 years. All samples were stored at -80°C until analysis.

PSA Analysis. Sandwich-type ELISAs were used for PSA quantitation. Two different pairs of PSA-specific monoclonal antibodies were used for the total PSA and the free PSA assay. The free PSA assay demonstrates minimal ($<0.5\%$) cross-reactivity with PSA-ACT. The total PSA assay measures free PSA and PSA-ACT with equimolarity. Neither assay shows any cross-reactivity with human glandular kallikrein. The detection limit of both immunoassays is $0.001 \mu\text{g/liter}$ (1 ng/liter ; this unit will be used throughout this report).

The capture antibody and detection antibody were coded 8301 and 8311 (Diagnostic Systems Laboratories, Inc., Webster, TX) for the total PSA assay and F2 and F1 (BiosPacific, Emeryville, CA) for the free PSA assay, respectively. The detection antibodies were biotinylated using Sulfo NHS-LC-Biotin (Pierce, Rockford, IL) at a concentration of $0.2 \mu\text{g}$ biotin/ μg antibody. The capture antibody was immobilized on white polystyrene microtitration wells at a concentration of 500 ng/well in 50 mM Tris buffer (pH 7.8). The wells were washed six times with wash solution (150 mM NaCl, 50 mM Tris, 1 mM NaN_3 , and 0.05% Tween 20) after coating overnight. The following incubations were performed at room temperature, followed by washing: (a) $100 \mu\text{l}$ of sera and $50 \mu\text{l}$ of biotinylated detection antibody diluted 1000-fold from a 0.5 mg/ml stock in assay buffer [50 mM Tris (pH 7.8), 10% goat serum, 6% BSA, 5% mouse serum, 1% bovine γ -globulin, 0.5% Tween 20, and 500 mM KCl] for 1 h; (b) 50 ng/ml alkaline phosphatase-labeled streptavidin diluted in 50 mM Tris (pH 7.8) containing 6% BSA (Jackson ImmunoResearch, West Grove, CA) for 15 min; and (c) diflunilal phosphate (10 mM stock in 10 mM NaOH; prepared in house) diluted 10-fold in substrate buffer [100 mM Tris (pH 9.1), 150 mM NaCl, 1 mM MgCl_2 , and 7.5 mM NaN_3] for 10 min. Developing solution (1 M Tris, 0.4 M NaOH, 3 mM EDTA, and 2 mM Tb^{3+}), was added to the diflunilal phosphate, and the resulting fluorescence was measured on a Cyberfluor 615 Immunoanalyzer (Nordion International, Kanata, Canada) in a time-resolved mode. More details on these time-resolved immunofluorometric procedures can be found elsewhere (30, 33).

Statistical Analysis. Wilcoxon's rank-sum test was used to identify statistically significant differences between the total PSA and free PSA measurements in each patient group. χ^2 analysis was used to identify differences in the percentage of patients with detectable total PSA, detectable free PSA, and free PSA as the major serological form ($>50\%$ of total PSA). Associations between clinicopathological variables and PSA levels

Table 1 Total and free PSA levels in serum of patients
Medians and ranges of values for total PSA (free PSA + PSA-ACT) and free PSA in the sera of patient groups and controls.

A. Patient group	Total PSA (ng/liter)		Free PSA (ng/liter)	
	Median	Range	Median	Range
Controls (<i>n</i> = 99)	0	0–55	0	0–6
Presurg BreastCa (<i>n</i> = 118) ^a	1.3	0–8153	0	0–2451
Postsurg BreastCa (<i>n</i> = 115)	0	0–359	0	0–22
Breast cysts (<i>n</i> = 46)	3.5	0–221	0	0–25
Uterine fibroids (<i>n</i> = 107)	1.0	0–81	0	0–17
B. Statistically significant differences between groups				
Controls vs.				
Presurg BreastCa		<i>P</i> = 0.0001 ^b		<i>P</i> = 0.0001 ^b
Postsurg BreastCa		<i>P</i> = 0.02 ^b		<i>P</i> = 0.81
Breast cysts		<i>P</i> = 0.0001 ^b		<i>P</i> = 0.01 ^b
Uterine fibroids		<i>P</i> = 0.0045 ^b		<i>P</i> = 0.19
Presurg BreastCa vs. Postsurg BreastCa		<i>P</i> = 0.047 ^b		<i>P</i> = 0.0001 ^b
Presurg BreastCa vs. Breast cysts		<i>P</i> = 0.001 ^b		<i>P</i> = 0.28

^a Presurg BreastCa, presurgical serum from breast cancer patients; Postsurg BreastCa, postsurgical serum from breast cancer patients.

^b Statistically significant difference (Wilcoxon's rank-sum test).

were assessed using χ^2 analysis. Diagnostic sensitivity and specificity were calculated as follows: (a) diagnostic sensitivity = $tp/(tp + fn)$; and (b) diagnostic specificity = $tn/(tn + fp)$; where *tp* = the number of true positives, *tn* = the number of true negatives, *fp* = the number of false positives, and *fn* = the number of false negatives. All PSA measurements below the detection limit of the methods (1 ng/liter) were adjusted to 0.

RESULTS

Total PSA. The medians and ranges of values for total PSA (free PSA + PSA-ACT) are shown in Table 1. Wilcoxon's rank-sum test was used to determine any significant differences between the groups. All patient groups had significantly higher total PSA values compared to controls (*P* < 0.025). Breast cancer patients had higher total PSA levels before surgery than after surgery (*P* = 0.047), and patients with breast cysts had significantly higher levels of total PSA than presurgical breast cancer patients (*P* = 0.001).

The percentage of patients in each group with detectable serum total PSA is shown in Table 2 and Fig. 1, with the detection limit being 1 ng/liter. The χ^2 test was used to determine differences between the proportions of patients with detectable total PSA. A higher proportion of patients in every patient group had detectable total PSA compared with the control group (*P* < 0.015). A significantly higher percentage of breast cancer patients had detectable total PSA before surgery than after surgery (*P* = 0.016). A greater percentage of patients with breast cysts had detectable total serum PSA in comparison to breast cancer patients (*P* = 0.003).

Free PSA. The medians and ranges of values for free PSA are also shown in Table 1. Wilcoxon's rank-sum test was used to determine any significant differences between the groups. Patients with breast cancer (presurgical) had significantly

higher free PSA compared to patients without breast disease and controls (*P* = 0.0001). Breast cancer patients had higher levels of serum free PSA before surgery than after surgery (*P* = 0.0001). Unlike total PSA, there was no significant difference in serum free PSA levels between patients with breast cancer and patients with benign breast disease (*P* = 0.28).

The percentage of patients in each group with detectable serum free PSA is shown in Table 2 and Fig. 1, with the detection limit being 1 ng/liter. The χ^2 test was used to determine differences between the proportions of patients with detectable free PSA. A significantly greater percentage of patients with breast cancer had detectable free PSA in comparison to breast cancer patients after surgery, uterine fibroid patients, or controls (*P* < 0.0006). However, this parameter did not differ significantly between benign and malignant breast disease (*P* = 0.32). A greater proportion of breast cancer patients had detectable free PSA before surgery than after surgery (*P* < 0.0001).

Free PSA as the Predominant Molecular Form. The percentage of patients in each group with free PSA as the predominant molecular form, which was defined as free PSA > 50% of total PSA, is shown in Table 2 and Fig. 1. A significant percentage of breast cancer patients have free PSA as the predominant molecular form in serum, in comparison to all other groups (*P* < 0.02). Thus, although there is no significant difference between the percentage of breast cancer patients and benign breast disease patients with detectable free PSA (*P* = 0.32), the proportion of breast cancer patients with free PSA as the predominant molecular form in serum is significantly higher in comparison to patients with benign breast disease (*P* = 0.012). The specificity of this parameter for breast cancer is further illustrated by its decrease in breast cancer patients after surgery (*P* < 0.0001).

Correlation of PSA Levels with Clinicopathological Variables. Detectable total serum PSA (>1 ng/liter) in breast cancer patients before surgery was significantly associated with younger patient age (*P* = 0.023 using χ^2 analysis with a cutoff of 58 years). Free serum PSA levels and free PSA as the predominant molecular form were not significantly associated with breast cancer patient age.

Serological total PSA was significantly associated with larger breast tumor size (*P* = 0.027 using χ^2 analysis with a tumor diameter cutoff of 2.0 cm). A similar trend was observed with free PSA levels, but the difference did not reach statistical significance (*P* = 0.097). We found no association between tumor size and free PSA as the predominant molecular form.

Serum free PSA was significantly associated with higher histological grade in breast cancer patients. A higher proportion of patients with detectable free PSA in serum had tumors classified as grade 3, whereas patients with no detectable free PSA tended to have tumors classified as grade 1 or 2 (*P* = 0.014). No association was found between histological grade and either total PSA or free PSA as the predominant molecular form.

We could not establish any statistically significant association between any form of serum PSA and steroid hormone receptor status, nodal involvement, or relapse.

Diagnostic Specificity and Sensitivity. Table 3 summarizes the diagnostic sensitivity and specificity of serum total PSA, free PSA, and free PSA as the predominant molecular

Table 2 Detectable total and free PSA in serum of patients^a

The percentage of patients with detectable total PSA (free PSA + PSA-ACT), detectable free PSA, and free PSA as the predominant molecular form (>50% of total PSA) in serum.

A. Patient group	Patients with detectable total PSA (%)	Patients with detectable free PSA (%)	Patients with >50% free PSA (%)
Controls (<i>n</i> = 99)	28 (29)	10 (10)	3 (3)
Presurg BreastCa (<i>n</i> = 118) ^b	67 (57)	37 (36)	24 (20)
Postsurg BreastCa (<i>n</i> = 115)	51 (44)	10 (11)	4 (3)
Breast cysts (<i>n</i> = 46)	36 (75)	12 (28)	2 (4)
Uterine fibroids (<i>n</i> = 107)	56 (50)	11 (16)	6 (6)
B.	Statistically significant differences between groups		
Controls vs.			
Presurg BreastCa	<i>P</i> < 0.0001 ^c	<i>P</i> < 0.0001 ^c	<i>P</i> = 0.0001 ^c
Postsurg BreastCa	<i>P</i> = 0.012 ^c	<i>P</i> = 0.79	<i>P</i> = 0.85
Breast cysts	<i>P</i> < 0.0001 ^c	<i>P</i> = 0.007 ^c	<i>P</i> = 0.63
Uterine fibroids	<i>P</i> = 0.012 ^c	<i>P</i> = 0.20	<i>P</i> = 0.37
Presurg BreastCa vs. Postsurg Breast Ca	<i>P</i> = 0.016 ^c	<i>P</i> < 0.0001 ^c	<i>P</i> < 0.0001 ^c
Presurg BreastCa vs. breast cysts	<i>P</i> = 0.003 ^c	<i>P</i> = 0.32	<i>P</i> = 0.012 ^c

^a PSA ≥ 1 ng/liter is considered detectable.

^b Presurg BreastCa, presurgical serum from breast cancer patients; Postsurg BreastCa, postsurgical serum from breast cancer patients.

^c Statistically significant difference (χ^2 test).

form for breast cancer. Total PSA has the highest sensitivity for breast cancer; however, it lacks the specificity to distinguish breast cancer patients from those with benign breast disease or those with no breast pathology. Free PSA demonstrates higher specificity for breast cancer, but sensitivity is compromised. Although free PSA as the predominant molecular form lacks sensitivity for breast cancer, it is highly specific in differentiating between patients with breast cancer and patients with benign breast disease or with no malignancy.

DISCUSSION

PSA is one of the most valuable serum tumor markers, having been used successfully for diagnosis and postsurgical management of prostate cancer (34). The routine use of PSA immunoassays for the management of prostatic carcinoma has resulted in a marked rise in the number of prostate cancers detected (34). To further improve the effectiveness of distinguishing prostate cancer from benign prostatic hyperplasia, measurement of various molecular forms of PSA has been introduced into clinical practice (35).

The purpose of this study was to measure the relative proportions of the molecular forms of PSA in the serum of women. Sera from women with breast cancer, benign breast disease, and uterine fibroids and from women free of malignancies were analyzed for free PSA and PSA-ACT using an established total PSA immunoassay (30) and a novel free PSA assay (31), both with sufficient sensitivity (1 ng/liter) for PSA quantitation in a large proportion of female sera. This study has shown that the predominant molecular form of PSA (>50% of total PSA) in a significant proportion of females with breast cancer is free PSA, and free PSA levels decrease in breast cancer patients after surgery. Whereas a significant proportion of women with breast disease (malignant or benign) have detectable serum free PSA, free PSA as the predominant molecular form in serum is highly specific for breast cancer. The vast majority of healthy women or women with other endocrinopa-

thies, including benign breast disease, have free PSA as the minor molecular form and PSA-ACT as the predominant molecular form.

Although free PSA as the predominant molecular form appears to be unique to breast carcinoma, we observed an increase in total PSA levels in the serum of all patients in comparison to the control group. We speculate that the slight increase in total PSA in the serum of women with breast cancer, benign breast disease, or uterine fibroids is the result of a disrupted hormonal balance in these women, triggering the aberrant expression of hormone-dependent genes such as PSA. The observation that total PSA is only slightly decreased in breast cancer patients after surgery is an indication that a major component of total PSA (in this case PSA-ACT) is not produced by the tumor cells but more likely by normal breast tissue. Alternatively, free PSA decreases more dramatically after surgery, strongly indicating that this fraction is produced by breast tumors. It has been reported that the majority of breast tumor PSA occurs in the free form (8, 27), whereas free PSA and PSA-ACT appear to exist in approximately equal proportions in breast cyst fluid (11). It is likely that the tumor is producing free PSA that is incapable of binding to serine protease inhibitors such as ACT, given the large molar excess of serum ACT in comparison to PSA (28). Alternatively, breast tumors may produce an endopeptidase which causes a posttranslational modification (internal cleavage) of PSA produced by the breast, thus preventing complex formation with ACT and increasing the proportion of free PSA (28).

It has been established that prostate cancer patients have a greater fraction of PSA complexed to ACT (21), whereas we report that a significant proportion of breast cancer patients have a higher percentage of free PSA. The mechanism behind this apparent discrepancy is unknown at present, mainly because the differential generation of PSA molecular forms in benign or malignant prostatic or mammary tissue is not yet clear. However, it must be kept in mind that although breast and prostate

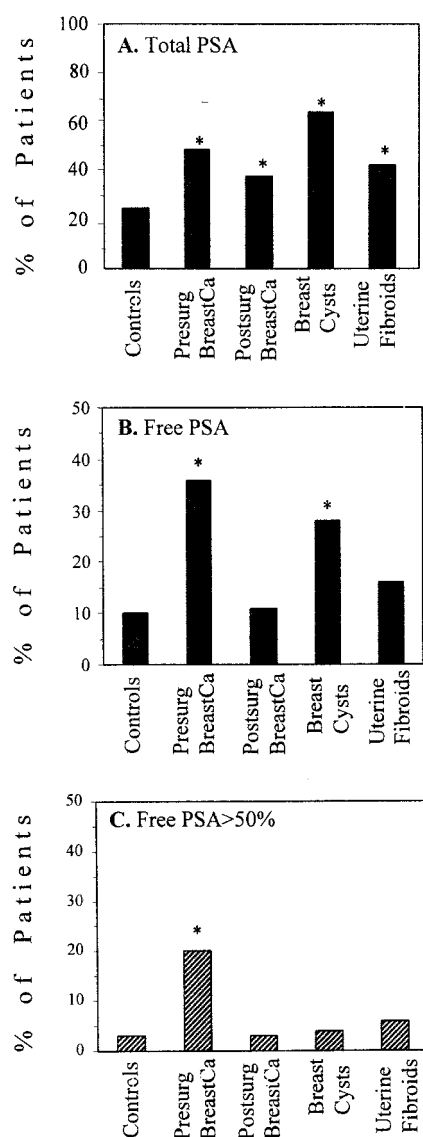


Fig. 1 Percentage of patients in each group with (A) detectable total PSA in serum; (B) detectable free PSA in serum; and (C) free PSA as the predominant molecular form. Detectable PSA means PSA ≥ 1 ng/liter. *, patient groups that are statistically different from controls (χ^2 test).

cells both produce PSA, the two tissues differ in a multitude of ways. For example, prostatic and mammary tissue (healthy, hyperplastic, or malignant) respond differently to hormonal proliferation and differentiation signals (e.g., estrogen *versus* androgen effects).

Some significant associations between serum PSA and clinicopathological variables were observed. Total PSA levels in serum were lower in older women, whereas free PSA levels did not vary with age. The age independence of free PSA may enhance its applicability as a diagnostic tool. Total PSA levels in serum were associated with larger tumor size, but no significant relationship was established between serum free PSA and tumor size. However, serum free PSA was significantly associated with higher histological grade.

Table 3 Diagnostic specificity and sensitivity of PSA

Diagnostic sensitivity and specificity of serum total PSA (free PSA + PSA-ACT), free PSA, and free PSA as the predominant molecular form (>50% of total PSA).

Parameter	Diagnostic sensitivity (%) ^a	Diagnostic specificity (%) ^a
Total PSA		
Presurg BreastCa vs. controls ^b	57	69
Presurg BreastCa vs. breast cysts	57	25
Free PSA		
Presurg BreastCa vs. controls	36	90
Presurg BreastCa vs. breast cysts	36	72
>50% Free PSA		
Presurg BreastCa vs. controls	20	97
Presurg BreastCa vs. breast cysts	20	96

^a Diagnostic sensitivity and specificity calculations are described in "Materials and Methods."

^b Presurg BreastCa, presurgical serum from breast cancer patients; Postsurg BreastCa, postsurgical serum from breast cancer patients.

Breast cancer is a leading cause of morbidity and mortality in females of developed countries and is the most common malignancy among North American women (36). It is estimated that by the year 2000, 500,000 women worldwide will die from breast cancer (36). Currently, the most effective way to minimize morbidity and mortality from breast cancer is by early diagnosis and administration of therapy (37). It is thus highly desirable to devise new methods of early diagnosis. Mammography is the most sensitive and specific screening modality for breast cancer; however, data presently available do not warrant a universal recommendation for mammography for all women (38). Numerous attempts are presently being made to identify serological markers of breast tumors. Prospective markers include carcinoembryonic antigen, carbohydrate antigen 15.3, tissue polypeptide-specific antigen, and mammary serum antigen (39–41). However, the diagnostic sensitivity of these markers is very limited and does not exceed 32% (39–41). Similarly, the results obtained here indicate that the diagnostic sensitivity of free PSA as the predominant molecular form is approximately 20%. However, free PSA as the predominant form is highly specific for breast cancer in comparison with benign breast disease and normal tissue. The high degree of specificity suggests that free PSA may have potential clinical applicability either alone or in combination with other markers.

The physiological role of PSA in breast tissue, normal or hyperplastic, remains unknown. As outlined above, before any speculation can be made regarding the role of mammary PSA, it must first be determined whether the protease has enzymatic activity. No model examining the enzymatic activity of PSA in breast cancer cells has been developed. It is clear from previous studies that PSA may have a number of potential roles in breast cancer. PSA has been reported to induce uncontrollable proliferation in osteoblast and fibroblast cell lines (42). PSA was also shown to stimulate cell detachment, suggesting a role for PSA in tumor progression or metastasis (43). It has been demonstrated that PSA degrades the extracellular matrix proteins fibronectin and laminin (42). Because the destruction of the epithelial basement membrane facilitates local invasion, this study identified PSA as a potential modulator of matrix degradation.

Furthermore, PSA is an IGFBP-3 protease (44). The proteolysis of IGFBP-3 by PSA consequently decreases the affinity of this binding protein for IGF-1, causing a concomitant elevation in the concentration of serum IGFs (45), which are known mitogens for breast cancer cells. Furthermore, it has been proposed that IGFBP-3 may be able to interact with breast cancer cells in a manner that is independent of IGFs through specific receptors for the binding protein (46). Antiproliferative effects of IGFBP-3 on several breast cancer cell lines have been reported (47), and it has been suggested that IGFBP-3 may induce apoptosis in breast cancer cells (48). The ramifications of IGFBP-3 degradation on tumor progression are therefore numerous.

In summary, we have demonstrated that free PSA is the predominant molecular form of serological PSA in a significant proportion of breast cancer patients and that free PSA levels decrease significantly in the serum of women with breast cancer after surgery. Although free PSA as the predominant molecular form is highly specific for breast cancer, its clinical utility is limited at this time due to low sensitivity. Given the heterogeneity of the population of breast cancer patients, additional studies with larger cohorts of patients are required for the examination of molecular forms of PSA with regard to individual parameters such as risk of relapse or metastasis. Finally, the physiological mechanism behind the free PSA increase in breast cancer and its ramifications with respect to tumor progression should be further investigated.

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REFERENCES

- Wang, M. C., Papsidero, L. C., Kuriyama, M., Valenzuela, L. A., Murphy, G. P., and Chu, T. M. Prostate antigen: a new potential marker for prostatic cancer. *Prostate*, 2: 89–96, 1981.
- Lilja, H., Oldbring, J., Rannevik, G., and Laurell, C. B. Seminal vesicle-secreted proteins and their reactions during gelation and liquefaction of human semen. *J. Clin. Investig.*, 80: 281–285, 1987.
- Lilja, H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *J. Clin. Investig.*, 76: 1899–1903, 1985.
- Diamandis, E. P., and Yu, H. Nonprostatic sources of prostate-specific antigen. *Urol. Clin. N. Am.*, 24: 275–282, 1997.
- Pollen, J. J., and Dreilinger, A. Immunohistochemical identification of prostatic acid phosphatase and prostate specific antigen in female periurethral glands. *Urology*, 23: 303–304, 1984.
- Tepper, S. L., Jagirdar, J., Heath, D., and Geller, S. A. Homology between the female paraurethral (Skene's) glands and the prostate. Immunohistochemical demonstration. *Arch. Pathol. Lab. Med.*, 108: 423–425, 1984.
- Yu, H., Diamandis, E. P., Levesque, M., Giai, M., Roagna, R., Ponzzone, R., Sismondi, P., Monne, M., and Croce, C. M. Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. *Breast Cancer Res. Treat.*, 40: 171–178, 1996.
- Diamandis, E. P., Yu, H., and Sutherland, D. J. A. Detection of prostate-specific antigen immunoreactivity in breast tumors. *Breast Cancer Res. Treat.*, 32: 301–310, 1994.
- Yu, H., Diamandis, E. P., and Sutherland, D. J. A. Immunoreactive prostate-specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. *Clin. Biochem.*, 27: 75–79, 1994.
- Yu, H., Giai, M., Diamandis, E. P., Katsaros, D., Sutherland, D. J. A., Levesque, M. A., Roagna, R., Ponzzone, R., and Sismondi, P. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. *Cancer Res.*, 55: 2104–2110, 1995.
- Diamandis, E. P., Yu, H., and Lopez-Otin, C. Prostate specific antigen: a new constituent of breast cyst fluid. *Breast Cancer Res. Treat.*, 38: 259–264, 1996.
- Mannello, F., Bocchiotti, G., Bianchi, G., Marcheggiani, F., and Gazzanelli, G. Quantification of prostate-specific antigen immunoreactivity in human breast cyst fluids. *Breast Cancer Res. Treat.*, 38: 247–252, 1996.
- Yu, H., and Diamandis, E. P. Prostate-specific antigen in milk of lactating women. *Clin. Chem.*, 41: 54–58, 1995.
- Sauter, E. R., Daly, M., Lenahan, K., Ehya, H., Engstrom, P. F., Sorling, A., Bonney, G., Yu, H., and Diamandis, E. P. Prostate-specific antigen levels in nipple aspirate fluid correlate with breast cancer risk. *Cancer Epidemiol. Biomark. Prev.*, 5: 967–970, 1996.
- Foretova, L., Garber, J. E., Sadowski, N. L., Verselis, S. J., and Li, F. P. Prostate-specific antigen in nipple aspirate. *Lancet*, 347: 1631, 1996.
- Sauter, E. R., Babb, J., Daly, M., Engstrom, P. F., Ehya, H., Malick, J., and Diamandis, E. P. Prostate-specific antigen production in the female breast: association with progesterone. *Cancer Epidemiol. Biomark. Prev.*, 7: 315–320, 1998.
- Monne, M., Croce, C. M., and Diamandis, E. P. Molecular characterization of prostate-specific antigen messenger RNA expressed in breast tumors. *Cancer Res.*, 54: 6344–6347, 1994.
- Yu, H., Diamandis, E. P., Zarghami, N., and Grass, L. Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. *Breast Cancer Res. Treat.*, 32: 291–300, 1994.
- Zarghami, N., Grass, L., and Diamandis, E. P. Steroid hormone regulation of prostate-specific antigen gene expression in breast cancer. *Br. J. Cancer*, 75: 579–588, 1997.
- Kuriyama, M., Wang, M. C., Papsidero, L. D., Killian, C. S., Shimano, T., Valenzuela, L., Nishiura, T., Murphy, G. P., and Chu, T. M. Quantification of prostate-specific antigen in serum by a sensitive enzyme immunoassay. *Cancer Res.*, 40: 4568–4662, 1980.
- Lilja, H., Christensson, A., Dahlen, U., Matikainen, M. T., Nilsson, O., Pettersson, K., and Lovgren, T. Prostate-specific antigen in human serum occurs predominantly in a complex with α_1 -antichymotrypsin. *Clin. Chem.*, 37: 1618–1625, 1991.
- Armbruster, D. A. Prostate-specific antigen: biochemistry, analytical methods, and clinical application. *Clin. Chem.*, 39: 181–195, 1993.
- Stenman, U.-H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K., and Alfthan, O. A complex between prostate-specific antigen and α_1 -antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostate cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res.*, 51: 222–226, 1991.
- Woodrum, D. L., Brawer, M. K., Partin, A. W., Catalona, W. J., and Southwick, P. C. Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. *J. Urol.*, 159: 5–12, 1998.
- Yu, H., and Diamandis, E. P. Measurement of serum prostate specific antigen levels in women and in prostatectomized men with an ultrasensitive immunoassay technique. *J. Urol.*, 153: 1004–1008, 1995.
- Melegos, D. M., and Diamandis, E. P. Is prostate-specific antigen present in female serum? *Clin. Chem.*, 44: 691–692, 1998.
- Giai, M., Yu, H., Roagna, R., Ponzzone, R., Katsaros, D., Levesque, M. A., and Diamandis, E. P. Prostate-specific antigen in serum of women with breast cancer. *Br. J. Cancer*, 72: 728–731, 1995.
- Melegos, D. N., and Diamandis, E. P. Diagnostic value of molecular forms of prostate-specific antigen for female breast cancer. *Clin. Biochem.*, 29: 193–200, 1996.
- Borchert, G. H., Melegos, D. N., Tomlinson, G., Giai, M., Roagna, M., Ponzzone, R., Sgro, L., and Diamandis, E. P. Molecular forms of prostate-specific antigen in the serum of women with benign and malignant breast diseases. *Br. J. Cancer*, 16: 1087–1094, 1997.

30. Ferguson, R. A., Yu, H., Kalyvas, M., Zammit, S., and Diamandis, E. P. Ultrasensitive detection of prostate-specific antigen by a time-resolved immunofluorometric assay and the Immulite[®] immunochemiluminescent third-generation assay: potential applications in prostate and breast cancers. *Clin. Chem.*, 42: 675–684, 1996.
31. Black, M. H., Grass, C. L., Leinonen, J., Stenman, U-H., and Diamandis, E. P. Characterization of monoclonal antibodies for prostate-specific antigen and development of highly sensitive free prostate-specific antigen assays. *Clin. Chem.*, 45: 347–354, 1999.
32. Bloom, H. J. G., and Richardson, W. W. Histological grading and prognosis in breast cancer. *Br. J. Cancer*, 11: 359–377, 1957.
33. Christopoulos, T. K., and Diamandis, E. P. Enzymatically amplified time-resolved fluorescence immunoassay with terbium chelates. *Anal. Chem.*, 64: 342–346, 1992.
34. Oesterling, J. E. Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. *J. Urol.*, 145: 907–923, 1991.
35. Akdas, A., Cevik, I., Tarcan, T., Turkeri, L., Dalaman, G., and Emerk, K. The role of free prostate-specific antigen in the diagnosis of prostate cancer. *Br. J. Urol.*, 79: 920–923, 1997.
36. Forbes, J. F. The incidence of breast cancer: the global burden, public health considerations. *Semin. Oncol.*, 24: S120–S135, 1997.
37. Morrison, A. S. Screening for cancer of the breast. *Epidemiol. Rev.*, 15: 244–255, 1993.
38. Ferguson, J. H. National Institutes of Health consensus development conference statement: breast cancer screening for women ages 40–49, January 21–23, 1997. *J. Natl. Cancer Inst.*, 89: 1015–1020, 1997.
39. Devine, P. L., Duroux, M. A., Quin, R. J., McGuckin, M. A., Joy, G. J., Ward, B. G., and Pollard, C. W. CA 15-3, CASA, MSA, and TPS as diagnostic serum markers in breast cancer. *Breast Cancer Res. Treat.*, 34: 245–251, 1995.
40. Eskelinen, M., Kataja, V., Hamalainen, E., Kosma, V. M., Penttila, I., and Alhava, E. Serum tumour markers CEA, AFP, CA 15-3, TPS, and Neu in diagnosis of breast cancer. *Anticancer Res.*, 17: 1231–1234, 1997.
41. Heinze, T., Schurenkamper, P., Minguillon, C., and Lichtenegger, W. Mammary serum antigen (MSA), Ca 549, CA 15-3 and CEA in breast cancer preoperative sensitivity and correlation to prognostic factors. *Anticancer Res.*, 17: 2953–2954, 1997.
42. Killian, C. S., Corral, D. A., Kawinski, E., and Constantine, R. I. Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF- β and a proteolytic modulation of cell adhesion receptors. *Biochem. Biophys. Res. Commun.*, 192: 940–947, 1993.
43. Webber, M. M., Waghay, A., and Bello, D. Prostate-specific antigen, a serine protease, facilitates human prostate cancer cell invasion. *Clin. Cancer Res.*, 1: 1089–1094, 1995.
44. Cohen, P., Graves, H. C. B., Peehl, D. M., Kamerei, M., Giudice, L. C., and Rosenfeld, R. G. Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *J. Clin. Endocrinol. Metab.*, 75: 1046–1053, 1992.
45. Cohen, P., Peehl, D. M., Graves, H. C. B., and Rosenfeld, R. G. Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. *J. Endocrinol.*, 142: 407–415, 1994.
46. Oh, Y., Muller, H. L., Lamson, G., and Rosenfeld, R. G. Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. *J. Biol. Chem.*, 268: 14964–14971, 1993.
47. Oh, Y., Gucev, Z., Ng, L., Muller, H. L., and Rosenfeld, R. G. Antiproliferative actions of insulin-like growth factor binding protein (IGFBP)-3 on human breast cancer cells. *Prog. Growth Factor Res.*, 6: 503–512, 1995.
48. Gill, Z. P., Perks, C. M., Newcomb, P. V., and Holly, J. M. P. Insulin-like growth factor-binding protein (IGFBP-3) predisposes breast cancer cells to programmed cell death in a non-IGF-dependent manner. *J. Biol. Chem.*, 272: 25602–25607, 1997.