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Prostate-specific Antigen Is a New Favorable Prognostic Indicator for Women with Breast Cancer[1]

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ABSTRACT

Prostate-specific antigen (PSA) is thought to be produced exclusively by prostatic epithelial cells and is currently used as a tumor marker of prostatic adenocarcinoma. We recently found that 30% of breast cancers contain PSA immunoreactivity (IR-PSA). To examine the prognostic value of PSA in female breast cancer, we measured IR-PSA in tumor cytosols of 174 breast cancer patients and classified the breast cancers as either PSA positive or PSA negative based on an IR-PSA cutoff level of 0.03 ng/mg. IR-PSA was present in 27% of the patients. IR-PSA presence was associated with early disease stage, small tumors, and estrogen receptor-positive tumors. We used the Cox proportional hazards regression model to analyze survival of patients in association with PSA status and found that patients with IR-PSA-positive tumors had a reduced risk for relapse and death in univariate analysis (P = 0.02 and 0.06, respectively) and a reduced risk for relapse in multivariate analysis (P = 0.03). Further analysis indicated that the effect of IR-PSA on relapse-free survival was evident in node-positive or estrogen receptor-negative patients. Our study suggests that IR-PSA is an independent favorable prognostic marker for breast cancer and may be used to identify a subgroup of estrogen receptor-negative and/or node-positive patients who have good prognoses.

INTRODUCTION

Decisions on whether and how to treat breast cancer patients after local surgery have a significant impact on survival of patients and health care cost. It is important, therefore, that these decisions be based on a rational review of prognostic parameters (1). Currently used prognostic and predictive markers are a mixture of host factors (*e.g.*, age, menopausal status, and inflammatory response) and tumor features (*e.g.*, tumor size, histological grade, nodal involvement, vascular invasion, hormone receptors, growth factors and their receptors, cell proliferation and angiogenesis markers, and various DNA or genetic alterations; Refs. 2–6). Although a variety of markers are available, physicians still have difficulty in identifying patients who need adjuvant treatment and who will benefit from the treatment because the available markers are not appreciably sensitive and specific. The ultimate goal of using prognostic and predictive markers is to allow physicians to accurately differentiate patients who need postsurgical treatment and to appropriately tailor therapy to their specific needs.

PSA[3] is a M_r 33,000 single chain glycoprotein with 240 amino acids (7, 8). PSA is encoded by a gene localized on chromosome 19 which has 80% homology with the kallikrein gene (9). Androgenic hormones up-regulate the production of PSA by increasing its transcription (10). PSA is a member of the serine protease family and has trypsin-like and chymotrypsin-like enzymatic activity (11). Initially, PSA was believed to be produced exclusively by the epithelial cells of the prostate gland (12). Recent studies suggest that a few other tissues may also produce PSA (13–16). PSA concentration in serum is a valuable biological marker for diagnosis, prognosis, and management of patients with prostate cancer (12, 17).

Using an ultrasensitive time-resolved immunofluorometric assay for PSA (18), we recently found that 30% of female breast tumor cytosols, from a cohort of more than 1200 breast cancer patients, contained PSA immunoreactivity higher than 0.03 ng/mg of total protein (19). Such immunoreactivity was also detected by three commercially available PSA kits. The molecular weight of the PSA immunoreactivity in breast tumors, measured by HPLC and Western blot techniques, was identical to the molecular weight of PSA in seminal plasma (20).

Furthermore, we found that the presence of PSA immunoreactivity in breast cancer cells was associated with the presence of ER and PR, as well as with early clinical stage (19, 20). Finally, our cell culture studies demonstrated that the breast cancer cell lines T-47D and MCF-7 could be induced by androgens or progestins to produce PSA immunoreactivity, and that this induction was suppressed by estrogen. Tamoxifen, an antiestrogen agent, was also able to induce the production of PSA in these cell lines (21). The production of PSA immunoreactivity was observed only in breast cancer cell lines which contain steroid hormone receptors (*e.g.*, the T-47D and MCF-7 cells). No production was seen in the breast cancer cell line BT-20, which does not possess steroid hormone receptors.

On the basis of these preliminary observations and the evidence that PSA production is up-regulated by androgen in the prostate through the androgen receptor, and that androgen antagonizes the effect of estrogen, we speculate that the presence of PSA immunoreactivity in breast cancer cells may be a marker of functional steroid hormone receptors, as well as an indicator of endogenous hormone balance between estrogen and androgen/progestin. Thus, PSA immunoreactivity in breast tumors may be valuable in predicting the prognosis of breast cancer patients and/or their response to adjuvant treatment. In order to examine this hypothesis, we investigated the PSA immunoreactivity in breast cancer in association with the relapse-free and overall survival of breast cancer patients.

MATERIALS AND METHODS

Breast Cancer Patients. One hundred seventy-four patients with primary breast cancer were included in the study. These patients were collected consecutively, provided that their tumor tissue was sufficient for tumor analysis. They represented about 70% of all new cases of breast cancer diagnosed and treated in the Department of Gynecologic Oncology at the University of Turin during the period January 1988 to December 1991. Ages of the 174 patients ranged from 25 to 91 years with a median of 56 years. Thirty-two % of the patients were under the age of 50 years, and 68% were at the age of 50 years or over.

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[3] PSA, prostate-specific antigen; IR-PSA, PSA immunoreactivity; ER, estrogen receptor; PR, progesterone receptor.

To be eligible for the study, patients must have a disease at unilateral T, N_0 or N_1, and M_0 clinical stages. Patients with bilateral lesions, Paget's disease of the breast, or disseminated disease at the time of diagnosis or within 2 months after surgery, and patients who received only palliative treatment, were excluded from the study. All patients in the study had their breast cancers removed by modified mastectomy or conservative surgery plus postoperative irradiation of the breast. Axillary lymph node evaluation was performed on all patients except for 11 cases who did not undergo axillary node dissection because of their age (>75 years). The mean number of axillary lymph nodes examined was 15 (±6 SD). The follow-up was scheduled once every 3 months during the first 2 years after the surgery, followed by examination at 6-month intervals for 3 years, and annually thereafter.

Clinical and pathological information, including clinical stage, histological cell type and grade, axillary node involvement, tumor size, presence of ER and PR in tumor cells, and adjuvant treatment after surgery, was recorded for each patient. Clinical staging was performed according to the Postsurgical International Union Against Cancer Tumor-Node-Metastasis classification (22). Of the 174 patients, 45, 47, and 8% had stage I, II, and III or IV, respectively. Each breast cancer specimen was also graded histologically and typed according to the criteria described by Bloom and Richardson (23) and Hopton *et al.* (24). Thirty-nine % of patients had low (I), 42% had moderate (II), and 19% had high (III) grades. Seventy % of patients had ductal carcinomas. The rest had lobular (13%), lobular *in situ* (2%), medullary (5%), papillary (2%), tubular (2%), tubulolobular (3%), or other types (3%). In the data analysis, because of the small number of patients who had types other than ductal carcinomas, we grouped histological type into two categories (ductal *versus* nonductal).

The size of tumor, recorded as the maximum diameter of the fresh mastectomy specimen, ranged from 0.7 to 6 cm. The median and mean sizes were identical, 2.4 cm. Fifty-one % of the patients had tumor invading the axillary lymph nodes. ERs were present in 66% of the tumor specimens, and PRs were present in 57% of the samples (10 fmol/mg of total protein used as cutoff level for both receptors).

Adjuvant tamoxifen treatment (30 mg/day for 5 years) was given to node-positive postmenopausal patients, and adjuvant chemotherapy CM (600 mg/mq cyclophosphamide-40 mg/mq methotrexate-600 mg/mq 5-fluorouracil, *i.v.*, every 21 days for 8 courses) was given to node-positive premenopausal patients. Node-negative patients received no further treatment after surgery. None of the patients received any therapy preoperatively. Of the 174 patients, 56% were treated with adjuvant therapy, including tamoxifen (37%), chemotherapy (15%), and both (4%).

Preparation of Cytosolic Extracts. Tumor specimens were snap frozen in liquid nitrogen immediately after surgical removal and subsequently were stored at −80°C until extraction. About 200 mg of tumor tissue, containing at least 70% of tumor cells as judged by pathological examination, were pulverized to a fine powder at −80°C; the cells were lysed for 30 min on ice with 2 ml of lysis buffer [50 mM Tris buffer (pH 8.0) containing 150 mmol of NaCl, 5 mmol of EDTA, 10 g of NP40 surfactant, and 1 mmol of phenylmethylsulfonyl fluoride/liter]. The lysate was centrifuged at 15,000 × *g* at 4°C for 30 min, and the supernatant was collected for measurement of PSA and total protein.

Measurement of PSA and Other Markers. PSA immunoreactivity in tumor extracts was measured with an ultrasensitive time-resolved immunofluorometric PSA assay. This method and its evaluation have been described in detail elsewhere (18). Briefly, the assay incorporates one monoclonal capture anti-PSA antibody and one biotinylated polyclonal detection anti-PSA antibody. Streptavidin conjugated with alkaline phosphatase is used as label, and the enzymatic activity of alkaline phosphatase is detected through the hydrolysis of a substrate, diflunisal phosphate, the dephosphorylated form of which further reacts with Tb3+-EDTA to form a fluorescent complex. The fluorescence of the complex is measured with time-resolved fluorometry following laser excitation. The detection limit of this assay is 0.01 ng/ml.

All tumor extracts were measured in duplicate for PSA immunoreactivity. PSA immunoreactivity higher than 0.01 ng/ml was divided by the total protein of the extract (mg/ml) to adjust for the amount of tumor tissue extracted. All values were expressed as ng of PSA/mg of total protein. Tumors with PSA immunoreactivity >0.03 ng/mg were considered positive for PSA, as described elsewhere (19). Total protein in the tumor extracts was measured with the use of a commercial kit based on the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL). Estrogen and progesterone receptors were measured with the use of the dextran-coated charcoal method (25, 26).

Statistical Analysis. The distributions of demographic, clinical, and pathological variables, including age, clinical stage, histological grade and type, nodal status, tumor size, ER, PR, and adjuvant treatment were compared between PSA-positive and PSA-negative patients with the use of the contingency table and χ^2 test. The relationship between each of the study variables and relapse-free or overall survival was evaluated by the hazards ratio (a relative risk for relapse or death) and its 95% confidence interval, which was calculated univariately with the use of the Cox proportional hazards regression model (27). The multivariate Cox regression model was also used to assess the impact of PSA immunoreactivity on patient survival while controlling for other clinical and pathological variables that may also affect the survival, such as clinical stage (I, II, or III/IV), nodal status (positive or negative), tumor size (greater or less than 1.5 cm), steroid hormone receptors (presence or absence), and adjuvant treatment (none, tamoxifen, or chemotherapy with or without tamoxifen). In addition to the analysis of all patients together, the analysis was performed separately for each subgroup of patients classified by the status of nodal involvement or estrogen receptor. Kaplan-Meier relapse-free and overall survival curves (28) were constructed to demonstrate the survival difference between PSA-positive and PSA-negative patients. The log rank test (29) was used to examined the significance of the differences among survival curves.

RESULTS

Follow-up Information and Distribution of PSA Immunoreactivity. Of the 174 patients in the study, 42 had cancer relapse and 27 died. The overall follow-up time for these patients ranged between 7 and 67 months, with a median follow-up of 33 months. PSA immunoreactivity higher than 0.03 ng/mg was detected in 27% of the patients (47 of 174). The positive PSA values ranged between 0.031 and 6.61 ng/mg, and the median was 0.12 ng/mg. For convenience, in the following text we will use the term PSA instead of PSA immunoreactivity. The difference between the two terms will be addressed in "Discussion."

Relationship between PSA and Other Variables. The distributions of demographic, clinical, and pathological variables among PSA-positive and PSA-negative tumors are shown in Table 1. PSA-positive patients did not differ significantly from PSA-negative patients in terms of age, nodal status, histological type and grade, PR status, and the adjuvant treatment administered. Patients with PSA-positive tumors tended to have earlier stage disease and smaller and ER-positive tumors. In addition, without considering the follow-up time, PSA-positive patients were less likely to relapse or die than were PSA-negative patients (11 *versus* 29% for cancer relapse and 6 *versus* 19% for death).

Univariate Survival Analysis. The risk for cancer relapse was significantly lower in patients with PSA-positive than in patients with PSA-negative tumors (Table 2), and the hazards ratio (0.32) indicated a more than 60% reduction in risk for relapse. A similar hazards ratio for overall survival was also observed (Table 3). The Kaplan-Meier survival curves are shown in Fig. 1. The probabilities for relapse-free or overall survival were significantly higher in the PSA-positive than in the PSA-negative patients.

Significantly high risks for relapse and death were demonstrated also in patients with advanced stage disease, high histological grade tumor, large size tumor, or tumor that metastasized to the axillary lymph nodes (Tables 2 and 3). Younger patients (under 50 years of age) tended to have higher risk for relapse in comparison to older ones (50 years of age or older; Table 2). Patients with ER-positive tumors had a significantly low risk for relapse (Table 2) and death (Table 3) when compared with ER-negative patients, but a similar tendency was not seen for PR-positive tumors (Tables 2 and 3).

Multivariate Survival Analysis. Clinical stage, tumor size, and presence of ER were all associated with both PSA status and cancer

Table 1 *Association between PSA and clinicopathological variables*

Variable	PSA-positive patients (%)	PSA-negative patients (%)	*P* value
Age (years)			
<50	17 (36.2)	38 (29.9)	
50+	30 (63.8)	89 (70.1)	0.32
Stage[a]			
I	28 (59.6)	50 (39.7)	
II	17 (36.2)	64 (50.8)	
III–IV	2 (4.3)	12 (9.5)	0.06
Nodal status[b]			
Negative	24 (53.3)	56 (47.5)	
Positive	21 (46.7)	62 (52.5)	0.50
Tumor size[c] (cm)			
<1.5	14 (30.4)	10 (8.1)	
1.5+	32 (69.6)	114 (91.9)	<0.01
Histological type			
Ductal	31 (66.0)	91 (71.7)	
Others	16 (34.0)	36 (28.4)	0.47
Histological grade			
I	21 (44.7)	47 (37.0)	
II	19 (40.4)	54 (42.5)	
III	7 (14.9)	26 (20.5)	0.56
Estrogen receptors[d]			
Negative	9 (20.0)	48 (38.7)	
Positive	36 (80.0)	76 (61.3)	0.02
Progesterone receptors[e]			
Negative	17 (37.8)	52 (42.3)	
Positive	28 (62.2)	71 (57.7)	0.60
Adjuvant treatment			
None	21 (44.7)	55 (43.3)	
Tamoxifen	17 (36.2)	47 (37.0)	
Chemotherapy ± tamoxifen	9 (19.2)	25 (19.7)	0.37
Relapse			
No	42 (89.4)	90 (70.9)	
Yes	5 (10.6)	37 (29.1)	0.01
Death			
No	44 (93.6)	103 (81.1)	
Yes	3 (6.4)	24 (18.9)	0.04

[a] Clinical stage unknown for 1 patient.
[b] Nodal status unknown for 11 patients.
[c] Tumor size unknown for 4 patients.
[d] ER status unknown for 5 patients.
[e] PR status unknown for 6 patients.

relapse or death, and possible confounding effects might be present in the relationship between PSA and survival. Therefore, these variables should be controlled in the analysis. Results from the multivariate analysis are shown in Table 4. After adjusting for most of the variables studied, *i.e.*, age, clinical stage, tumor size, histological grade, nodal status, ER, and PR, PSA-positive patients still have a significantly reduced risk for relapse when compared with PSA-negative patients. In addition, the stepwise analysis with the use of the same Cox model in Table 4 indicated that PSA contributed significantly to the change of risk for relapse in the model along with histological grade, nodal status, and ER (data not shown). However, the PSA effect on overall survival was not statistically significant after a similar adjustment.

In the same Cox model, it was also demonstrated that advanced clinical stage, large tumor size, positive lymph nodes, and high histological grade were all independent markers for poor prognosis because the risks for relapse or death were increased significantly in these patients. Among these unfavorable predictors, histological grade had the relatively strongest predictive value, and clinical stage was the weakest predictor if all these variables were included in one model (data not shown). Like PSA, ER was an independent marker for good prognosis because the risks for relapse or death were reduced significantly in patients with ER-positive tumors. PR effect on survival was not observed in the analysis.

PSA Effect on Relapse-free Survival in Patients Classified by Nodal Status. Since node-positive patients are substantially different from the node-negative patients in terms of their prognoses and treatment administered after the surgery, two sets (univariate and multivariate) of the Cox regression models were developed to evaluate the effect of PSA on relapse-free survival for each of the two groups. The results are shown in Table 5. In node-positive patients, a substantial reduction in risk for relapse was observed for PSA-positive patients, and this effect was sustained after adjusting for other variables, including the adjuvant treatment which was administered mainly for node-positive patients. However, in node-negative patients, the effect of PSA on relapse-free survival was not evident, especially when the ER variable was adjusted in the model.

PSA Effect on Relapse-free Survival in Patients Classified by ER Status. It was shown in the multivariate analysis that PSA and ER were both independent markers for good prognosis of breast cancer patients, but PSA presence was also associated with the presence of ER (Table 1). In order to examine whether there is any difference in prognostic significance of PSA between ER-positive and ER-negative patients, the hazards ratio between PSA-positive and PSA-negative patients was calculated separately in the ER-positive and ER-negative subgroups. The analysis was also done at two cutoff levels of the receptors, *i.e.*, 10 or 20 fmol/mg, because with the receptor assays used, levels between 10 and 20 fmol/mg were considered equivocal. The results of the analysis are shown in Table 6. In the ER-positive group, the risks for relapse were almost identical between PSA-positive and PSA-negative patients. However, in the ER-negative group, the risk for relapse was substantially reduced when the tumors were PSA positive (the hazards ratios were between

Table 2 *Association between relapse-free survival and PSA or other clinicopathological variables*

Variable	Hazards ratio[a]	95% confidence interval	*P* value
PSA (*n* = 174)			
Negative	1.00		
Positive	0.32	0.12–0.81	0.02
Age (*n* = 174)			
<50 yr	1.00		
50+ yr	0.52	0.28–0.96	0.04
Stage (*n* = 173)			
I	1.00		
II	2.67	1.28–5.56	0.01
III–IV	4.81	1.83–12.66	<0.01
Nodal status (*n* = 163)			
Negative	1.00		
Positive	3.87	1.84–8.13	<0.01
Tumor size (*n* = 170)			
<1.5 cm	1.00		
1.5+ cm	7.40	1.02–53.9	0.05
Histological grade (*n* = 174)			
I	1.00		
II	2.08	0.90–4.83	0.09
III	7.14	3.04–16.75	<0.01
Estrogen receptors (*n* = 169)			
Negative	1.00		
Positive	0.34	0.18–0.63	<0.01
Progesterone receptors (*n* = 168)			
Negative	1.00		
Positive	1.00	0.53–1.89	0.99

[a] Relative risk for relapse.

0.13 and 0.20). The difference was statistically significant when the cutoff level of the receptors was 20 fmol/mg. The hazards ratio in the ER-negative subgroup remained very low when nodal status, clinical stage, and histological type were controlled in the analysis.

Adjuvant Treatment. In order to examine whether PSA-positive patients had a better response to adjuvant treatment than PSA-negative patients, we analyzed the association between PSA status and cancer relapse in 98 patients who had been given adjuvant treatment, including tamoxifen, chemotherapy, or both. Of the 72 patients with PSA-negative tumors, 38% (27 of 72 patients) developed relapse. In the patients with PSA-positive tumors the relapse rate was 19% (5 of 26 patients). There was a 50% difference in relapse between the two groups, but it did not reach statistical significance ($P = 0.09$). The hazards ratio after adjustment for other clinicopathological variables was 0.58, with a 95% confidence interval between 0.24 and 1.42 ($P = 0.24$). With respect to death, the rates were 25 and 12%, respectively ($P = 0.15$), and the adjusted hazards ratio was 0.64 with a 95% confidence interval from 0.12 to 1.42 ($P = 0.16$).

DISCUSSION

Over the last decade, molecular biological and immunological techniques have revealed an increasing number of biological parameters involved in the regulation of malignant transformation. Breast cancer is a complex but increasingly understood heterogeneous disease. Clearly, multiple alterations of normal mammary cells are required to achieve a transformed phenotype. This explains why there are so many differences in clinical and biological behaviors between breast cancers. The specific set of alterations within the cancer may provide necessary information on how it is unique and how it may be best treated. A variety of parameters have been reported to have prognostic significance in patients with primary breast cancer. Among these markers, some are better established than others, and most of them are associated with prognostic value for relapse-free or overall survival rather than predictive value for response to a specific treatment (2–6).

Table 3 *Associations between overall survival and PSA or other clinicopathological variables*

Variable	Hazards ratio[a]	95% confidence interval	*P* value
PSA (n = 174)			
Negative	1.00		
Positive	0.31	0.09–1.05	0.06
Age (n = 174)			
<50 yr	1.00		
50+ yr	0.76	0.34–1.69	0.50
Stage (n = 173)			
I	1.00		
II	1.72	0.68–4.32	0.25
III–IV	4.48	1.42–14.13	0.01
Nodal status (n = 163)			
Negative	1.00		
Positive	4.31	1.62–11.50	<0.01
Tumor size (n = 170)			
<1.5 cm	1.00		
1.5+ cm	4.09	0.55–30.30	0.17
Histological grade (n = 174)			
I	1.00		
II	2.62	0.71–9.70	0.15
III	15.80	4.35–57.34	<0.01
Estrogen receptors (n = 169)			
Negative	1.00		
Positive	0.21	0.09–0.50	<0.01
Progesterone receptors (n = 168)			
Negative	1.00		
Positive	0.85	0.39–1.88	0.69

[a] Relative risk for death.

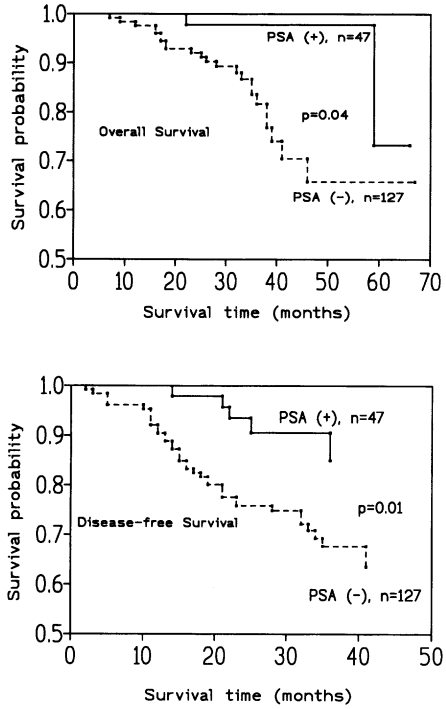

Fig. 1. Kaplan-Meier survival curves. *Top*, overall survival; *bottom*, disease-free survival.

PSA is one of the most useful biological markers, and its value for diagnosis and monitoring of prostate cancer is well established (12, 30, 31). The PSA protein and its encoding gene have been characterized (9, 32, 33). PSA has not been found in any tissue in women except in the periurethral glands, which are androgen responsive and have structures similar to the male prostate (16). Recently, we discovered IR-PSA in about 30% of breast cancer extracts. Although we have not been able to purify sufficient amounts of IR-PSA from breast tumors for protein sequencing yet, due to the relatively low level of the protein in the tissue, the presence of PSA in breast tumors is strongly suggested by compelling evidence: (*a*) IR-PSA in breast tumors could be measured not only by our method (18) but also by three widely used commercial PSA assays, namely the Tandem-E and Tandem-R kits (Hybritech, Inc., San Diego, CA), the IRMA-Count PSA kit (Diagnostic Products Corp., Los Angeles, CA), and the IMx automated PSA kit (Abbott Laboratories, Chicago, IL; Refs. 19, 20); (*b*) the molecular weight of IR-PSA, determined by HPLC and Western blot analysis, was identical to the molecular weight of PSA from seminal plasma (21); (*c*) the receptor-dependent androgenic up-regulation of, and the antagonizing effect between androgen and estrogen on, PSA production in the prostate was also demonstrated in breast cancer cell lines (21); and (*d*) recent work (34) has further strengthened our suggestion of PSA production by breast tumors. Using reverse transcription-PCR and DNA sequencing techniques, we identified PSA mRNA in IR-PSA positive breast tumors but not in IR-PSA-negative breast tumors. The sequence of the generated PCR product was identical to the sequence of the *PSA* gene.

Our finding of IR-PSA in about 30% of breast tumors was based initially on a large number of specimens taken from more than 1200 newly diagnosed breast cancer patients in Canada (19). This study, using frozen tumors from Italy, further confirmed our earlier observation. Furthermore, the presence of IR-PSA in breast tumors is not a random event. It is associated with some clinically important param-

Table 4 *Associations between PSA and relapse-free or overall survival after adjusting for other clinicopathological variables*[a]

Variable	Hazards ratio[b]	95% confidence interval	P value
Relapse-free survival (n = 153)			
PSA negative	1.00		
PSA positive	0.34	0.13–0.91	0.03
Overall survival (n = 153)			
PSA negative	1.00		
PSA positive	0.46	0.12–1.70	0.24

[a] Adjusted for age, ER, PR, clinical stage, nodal status, tumor size, and histological grade.
[b] Relative risk for relapse or death.

Table 5 *Associations between PSA and relapse-free survival stratified by nodal status*

Variable	Hazards ratio[a]	95% confidence interval	P value
Node-positive patients			
Univariate analysis (n = 83)			
PSA negative	1.00		
PSA positive	0.36	0.13–1.03	0.06
Multivariate[b] analysis (n = 77)			
PSA negative	1.00	0.10–0.98	0.05
PSA positive	0.32		
Node-negative patients			
Univariate analysis (n = 80)			
PSA negative	1.00		
PSA positive	0.27	0.03–2.15	0.22
Multivariate[c] analysis (n = 76)			
PSA negative	1.00		
PSA positive	1.02	0.09–11.15	0.99

[a] Relative risk of relapse.
[b] Adjusted for age, ER, PR, clinical stage, tumor size, histological grade, and adjuvant therapy.
[c] Adjusted for age, ER, PR, and histological grade.

eters such as clinical stage, presence of steroid hormone receptors, and, as suggested by this study, survival. These associations have significant potential for clinical applications, especially when the favorable prognostic value was indicated in patients with node-positive and/or ER-negative patients.

IR-PSA was not found frequently in cancer of the ovary, an organ which, like the breast, is under hormonal regulation. We have measured PSA in tumor extracts of 98 patients with primary ovarian cancer and found only 4 patients with IR-PSA levels higher than 0.03 ng/mg of total protein (35). We also measured PSA in three ovarian tumors which metastasized from breast cancer and found one patient with very high levels of IR-PSA (1.4 ng/mg), suggesting that primary breast cancer producing PSA could also do so at the metastatic site (35).

In the prostate, PSA production is up-regulated by androgen through the androgen receptor. Androgen that has up-regulated PSA production in breast cancer cells has also been demonstrated in our cell culture study (21). It is known that androgen receptors are present in breast cancer cells, and their presence is closely related to the presence of estrogen and progesterone receptors (36–39). Cell culture studies have shown that androgen inhibits the proliferation of breast cancer cells (40) and counteracts the effects of estrogen (41). Androgen has been used effectively in the treatment of some patients with breast cancer (42).

An antagonistic interaction between androgen and estrogen on the production of PSA was observed in our cell culture study (21), and it was further supported by the observation of PSA production induced by tamoxifen, an antiestrogen agent. Estrogen is believed to play an essential role in the development of breast cancer (43–45). Depleting, suppressing, or antagonizing the impact of estrogen has been shown to be effective in the treatment of some breast cancer patients. These observations prompted us to speculate that the presence of PSA in breast cancer cells may indicate a suppressed or less effective estrogenic influence. Consequently, we expected that PSA may serve as a favorable prognostic indicator for breast cancer patients. In this study, we observed a significantly reduced risk for relapse in patients with PSA-positive tumors when compared with patients with PSA-negative tumors.

Steroid hormone receptors are favorable prognostic indicators in breast cancer, and receptor-positive patients respond favorably to endocrine treatment (6). It is also known that among the steroid hormone receptor-negative patients, some of them could have good prognoses and respond well to endocrine treatment. In this study, we found that in the steroid hormone receptor-positive patients, the presence or absence of PSA has no additional prognostic significance. However, in the steroid hormone receptor-negative patients, those with PSA-positive tumors tended to have a reduced risk for relapse. This finding is also supported by an observation in which we described two patients with ovarian cancer metastatic from a breast primary. One of the patients whose ovarian tumor was steroid hormone receptor negative but PSA positive had a good response to adjuvant treatment and excellent disease-free survival. The other, whose ovarian tumor was receptor positive and PSA negative, died soon after the administration of adjuvant therapy (35).

A large number of cell biological parameters are available currently to predict the prognosis of patients with breast cancer, but not many markers are available to predict the response of patients to adjuvant treatment. A valuable prognostic marker can be a worthless predictive indicator for response to treatment. Because of the relatively small number of patients in the study, it was not clear whether the PSA-positive patients belong to a subgroup that responds better to endocrine treatment. However, we did observe a 40% reduction in the risk for relapse in PSA-positive patients who received the adjuvant treat-

Table 6 *Associations between PSA and relapse-free survival stratified by the status of estrogen receptors*

Variable	Hazards ratio[a]	95% confidence interval	P value
ER-positive patients (cut off at 10 fmol/mg)			
Univariate analysis (n = 112)			
PSA negative	1.00		
PSA positive	0.98	0.37–2.61	0.97
Multivariate[b] analysis (n = 103)			
PSA negative	1.00		
PSA positive	0.80	0.27–2.32	0.68
ER-negative patients (cut off at 10 fmol/mg)			
Univariate analysis (n = 57)			
PSA negative	1.00		
PSA positive	0.16	0.02–1.22	0.08
Multivariate[b] analysis (n = 50)			
PSA negative	1.00		
PSA positive	0.13	0.02–1.15	0.07
ER-positive patients (cut off at 20 fmol/mg)			
Univariate analysis (n = 95)			
PSA negative	1.00		
PSA positive	1.42	0.46–4.34	0.54
Multivariate[b] analysis (n = 88)			
PSA negative	1.00		
PSA positive	0.96	0.27–3.33	0.94
ER-negative patients (cut off at 20 fmol/mg)			
Univariate analysis (n = 74)			
PSA negative	1.00		
PSA positive	0.18	0.04–0.76	0.02
Multivariate[b] analysis (n = 65)			
PSA negative	1.00		
PSA positive	0.20	0.04–0.93	0.04

[a] Relative risk for relapse.
[b] Adjusted for age, clinical stage, nodal status, tumor size, and histological grade.

ment, although the difference did not reach statistical significance. Furthermore, the reduced risks for relapse observed in node-positive patients who are PSA positive may also indicate the effectiveness of the adjuvant treatment in relation to PSA status because this treatment was administered mainly in node-positive patients.

Steroid hormones are known to play an important role in breast cancer initiation and progression. It is not clear whether there are subsets of breast tumors which are "estrogen dependent" and "androgen/progestin dependent," and if the latter group could be identified by their ability to produce PSA. If this could be demonstrated, therapeutic interventions could be examined based on this subclassification. For example, antiestrogen regimens may be more effective in estrogen-dependent tumors and antiandrogens or antiprogestins may be more effective in the androgen/progestin-dependent tumors. These suggestions must be examined in prospective clinical trials.

In recent literature (46), it was commented that although many new prognostic markers have emerged lately, most of them have limited clinical utility because they are correlated highly with already existing markers or they are difficult and expensive to measure. New prognostic markers, in order to be practically useful, must provide additional information, and their measurement should be easy and inexpensive. IR-PSA may meet all these criteria: (*a*) our finding indicated that the prognostic value of IR-PSA on relapse-free survival was more prominent in node-positive or ER-negative patients. It is known that patients classified by ER or nodal status are still not homogeneous (3, 5), and markers for identifying further differences in these subgroups of patients are not readily available; and (*b*) PSA is, biochemically, a very stable protein (12), and can be easily, objectively, reliably, and economically quantified by immunoassay in the same tumor extracts prepared for steroid hormone receptor assays. Thus, this assay fulfills the criteria of being a practical tool for breast cancer prognosis.

Our study suggested that the presence of IR-PSA in breast tumors may be used as a new biological marker to distinguish a subgroup of patients, probably among the steroid hormone receptor-negative and/or node-positive patients, who could have improved prognosis and respond better to endocrine treatment. However, caution is still needed in interpreting the results because of the relatively small number of patients in the study. Further confirmation of this observation should be based on studies with a larger number of patients and longer follow-up times.

In conclusion, we demonstrated that a traditional and highly specific tumor marker for prostate cancer may also be used as a prognostic marker for women with breast cancer. Further basic and clinical studies are needed to help understand the mechanism of the *PSA* gene expression in breast cancer and its role, if any, in cancer initiation and progression.

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