Prognostic Value of Prostate-specific Antigen for Women with Breast Cancer: A Large United States Cohort Study[1]

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ABSTRACT

Prostate-specific antigen (PSA) is a valuable tumor marker used for diagnosis and management of prostate cancer. Recently, PSA has been found in various female tissues and body fluids. Female breasts, both normal and abnormal, including cancerous tissues, can produce PSA, and this production is regulated by androgens and progestins. Preliminary data suggested that patients with breast tumors positive for PSA may have better prognosis compared to those with PSA-negative breast tumors. This study examines the prognostic value of PSA in a large cohort study of United States patients. Using a PSA assay that has a lower detection limit of 0.001 ng/ml, we measured PSA in tumor cytosolic extracts of 953 women with primary breast cancer. Other information available for this study included age, follow-up time, survival outcome, tumor size, nodal status, steroid hormone receptor levels, DNA analysis by flow cytometry, and postoperative treatment. The median follow-up time was 73 months. During the follow-up, 200 patients relapsed and 188 died. PSA presence was found to be significantly associated with smaller tumors, tumors with low S-phase fraction, diploid tumors, younger patient age, and tumors with lower cellularity. Survival analysis indicated that the relative risks (RRs) for relapse and death were both significantly lower [RR = 0.67 (P = 0.01) for relapse; RR = 0.72 (P = 0.05) for death] in PSA-positive patients (levels higher than the 30th percentile of PSA values) than in PSA-negative patients. The reduced risks for relapse and death remained statistically significant after other clinical and pathological variables were adjusted in the multivariate analysis [RR = 0.68 (P = 0.02) for relapse; RR = 0.65 (P = 0.02) for death]. Our results suggest that the measurement of PSA in breast tumor extracts provides additional information on the prognosis of patients with primary breast cancer.

INTRODUCTION

PSA[4] is one of the most valuable tumor markers used for the diagnosis and management of prostate cancer (1, 2). Because of its clinical significance, PSA has been studied extensively over the past 10 years, and as a consequence, the understanding of this molecule has improved substantially. It is now widely accepted that PSA is not a tissue-specific protein. PSA has been found in a variety of female tissues and body fluids (3–16). The expression of PSA in these tissues is regulated by a variety of steroid hormones, including androgens, progestins, glucocorticoids, and estrogens (17, 18). Although the physiological role of PSA in female tissues and fluids remains undetermined, there is evidence suggesting that PSA may be involved in a regulatory pathway of IGFs (19, 20).

A number of studies have demonstrated that the breast is the principal female tissue capable of producing PSA. PSA has been detected in normal, hyperplastic, and cancerous breast tissues, as well as in various breast fluids (3–5, 9, 11). *In vitro* experiments have indicated that PSA production by breast tissue is up-regulated by androgens and progestins at the level of transcription. Estrogens down-regulate expression of PSA stimulated by androgens or progestins (17). Our preliminary clinical studies have indicated that PSA expression in breast cancer is associated with ER, PR, tumor size, and clinical stage, suggesting a possible role of PSA in patient prognosis (21). In a small clinical cohort study, we observed a significantly reduced risk of relapse among patients with PSA-positive breast cancer compared to those whose breast tumors were PSA negative. In that study, the favorable prognostic value of PSA was independent of other clinical and pathological features of the disease (22).

Because the prognostic value of PSA in breast cancer has been examined previously only for a small number of patients who were followed for a relatively short period of time (174 cases with a median follow-up of 33 months), further clinical studies are obviously needed to confirm the association between

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[4] The abbreviations used are: PSA, prostate-specific antigen; IGF, insulin-like growth factor; IGFBP-3, IGF-binding protein 3; ER, estrogen receptor; PR, progesterone receptor; BCTR, Breast Cancer Tissue Resource; NIRL, Nichols Institute Research Laboratories; RR, relative risk.

PSA in breast tumors and patient survival. The study reported here is a large cohort study conducted to validate our previous preliminary findings. In this study, we analyzed PSA along with other markers in breast cancer specimens from more than 900 patients who had been followed for an average of more than 5 years.

MATERIALS AND METHODS

Breast Cancer Specimens and Patients. Tumor specimens from 1000 breast cancer patients were obtained from the BCTR, a collaborative project between the University of Texas Health Science Center at San Antonio and the NIRL. BCTR stores a large collection of frozen breast tissue powders that were leftover tissue specimens. These specimens had been sent from hospitals throughout the United States to NIRL for routine measurement of ER and PR in tissue extracts as well as for flow cytometric analyses of tumor cells. The selection criteria for the specimens were as follows: (*a*) collection prior to 1991; (*b*) the availability of sufficient tissue mass for extraction and assay; and (*c*) the availability of clinical and pathological information, including that related to survival of the patient.

The patients included in the study were from 165 hospitals distributed widely across the country and were operated at ages between 22 and 94 years (mean age, 60 years) and from August 1985 to October 1991. All patients had histologically confirmed diagnosis of primary breast cancer and received no treatment before surgery. Modified radical mastectomy with axillary lymph node dissection was performed on 97% of the patients. Three % of the patients had incisional biopsy (2%) or lumpectomy without axillary lymph node dissection (1%). For the patients who had axillary node dissection, the positivity rate for cancer involvement of lymph nodes was 45%. The sizes of the tumors resected during surgery ranged from 0.1 to 14.5 cm, and the mean and median sizes were 2.6 and 2.3 cm, respectively. Tumor cellularity, defined as the percentage of malignant cells in each tissue specimen, was evaluated for 797 specimens by a single pathologist blinded to all other clinical or pathological information. This assessment revealed that 29% of the specimens had cellularities greater than 70%, 46% had between 31 and 70% tumor cells, 21% had between 11 and 30%, and 4% of the 797 specimens had less than 10% tumor cells. Information on disease stage, histological grade, and histological type was unavailable.

Postoperative treatment information was known for 948 patients. Whereas 39% received no further treatment after tumor resection, 9% had regional radiotherapy alone, 16% were given adjuvant chemotherapy only, 17% were treated with endocrine therapy only, 4% had endocrine therapy and radiotherapy, 7% were given both chemotherapy and radiotherapy, 6% received both systemic adjuvant therapies, and 2% had all three treatment modalities. Disease relapse was defined as the first documented evidence of local or regional axillary recurrence; distant metastasis; or new ipsilateral or contralateral breast cancer that was revealed by clinical, radiological, or histological evaluations.

Follow-up information was available for 997 patients and included survival status (alive or deceased) and disease status (disease-free or recurrence/metastasis) along with the dates of the events and cause of death, if applicable. Such information was updated annually by the institutions from which the specimens originated. The distribution of follow-up times for patients still alive at the time of analysis ranged from 28 to 112 months, with a median of 77 months; only 33 and 9 patients had been followed less than 48 and 36 months, respectively. Follow-up times for the entire cohort, however, ranged from 7 to 121 months and had a median of 73 months. The disease-free survival time in each case was the time interval between the date of surgical removal of the primary cancer and the date of the first documented evidence of relapse. The overall survival time was the time interval between the date of surgery and the date of death due to any cause, or the date of last follow-up for those who were alive at the end of the study.

Preparation of Tissue Cytosols. After surgical resection, the tissue specimens were immediately frozen in liquid nitrogen and sent to NIRL, where they were pulverized and assayed for steroid hormone receptors. The remaining pulverized tissues were frozen and transferred to BCTR, where they were stored at −80°C until cytosolic extraction for PSA analysis. The extraction procedure included treatment of the powders (10–50 mg) in a cell lysis buffer (500 μl) for 30 min on ice and separating cell debris from the cytosols by centrifugation. The cell lysis buffer (pH 8.0) contained 50 mM Tris, 150 mM NaCl, 5 mM EDTA, 10 g/liter NP40 surfactant, and 1 mM phenylmethylsulfonyl fluoride. The supernatant was collected for measuring PSA and total protein after a 30-min centrifugation at 15,000 × *g* and 4°C.

PSA Immunoassay. PSA concentration in tissue cytosols was measured with an in-house PSA method that is a time-resolved immunofluorometric assay (23). This sandwich-type assay uses two monoclonal anti-PSA antibodies and has a lower limit of detection of 0.001 ng/ml. This detection limit is 10-fold lower than the detection limit of our previous assay (24). The analytical performance of this new assay has been evaluated in detail (23). All specimens were measured in duplicate. The values used for statistical analysis were adjusted for total protein content and are expressed as ng of PSA/g of total protein in the cytosolic extracts. The total protein concentration was determined by a commercial kit based on the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL). All of the measurements were performed without knowledge of any clinicopathological information and follow-up outcome of the patients.

ER and PR Quantification. ER and PR measurements were performed at NIRL and provided as a routine service. The method used for cytosol extraction has been described by Dressler *et al.* (25). ER and PR were measured using a dual ligand-binding assay and a dextran-coated charcoal separation method. The ER and PR data were interpreted based on Scatchard analysis (26), and the total protein was determined by the method of Lowry *et al.* (27).

Flow Cytometry. The sample preparation method for DNA flow cytometic analysis has been described elsewhere (25). Briefly, the tumor tissue was gently homogenized, and the cells were separated through filtration and centrifugation with a double cushion of sucrose. The cells were resuspended and counted before being lysed and stained with propidium iodide. Nuclei were analyzed on an Epics V flow cytometer (Coulter Electronics, Hialeah, FL) for diploid content and S-phase fraction. The cell-cycle analyses for the determination of S-phase

Table 1 Distributions of numerical variables in the cohort of breast cancer patients

Variable[a]	No. of patients	Median	Range
PSA (ng/g)	953	6	0–19,629
Age (years)	999	61	22–94
Tumor size (cm)	959	2.3	0.1–14.5
Lymph nodes[b]	959	0	0–46
ER (fmol/mg)	1000	73	0–1786
PR (fmol/mg)	1000	91	0–3093
S-phase fraction (%)	1000	5.8	0.2–65

[a] Of the patients for whom PSA measurements were determined, tumor size was unknown for 38 patients, nodal status was unknown for 36 patients, and tumor cellularity was unknown for 155 patients.
[b] Number of lymph nodes positive for malignancy.

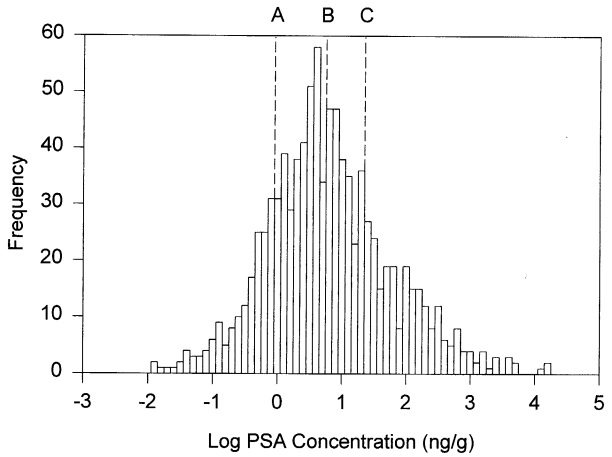

Fig. 1 Frequency distribution of logarithmically transformed PSA concentrations in 953 breast tumor extracts. *Lines A, B,* and *C* indicate the 30th (1 ng/g), 50th (6 ng/g), and 70th (17 ng/g) percentiles of the distribution, respectively.

fraction were performed using the Modfit program (Verity Software House, Inc., Topsham, ME). Debris was modeled as an exponential function, and S phases were modeled as single trapezoids.

Statistical Analysis. The distribution of PSA was skewed, and the correlations between PSA and patient age, number of positive nodes, tumor size, percentage of cells in S phase, and ER and PR status were examined using a nonparametric method, the Spearman correlation coefficient. In this analysis, PSA was used as a continuous variable. PSA values were also classified into two categories (PSA-positive and -negative groups), and associations between PSA status and other variables were analyzed using the χ^2 test. A cutoff point equal to the 30th percentile of the distribution of PSA concentrations was used, because this value effectively discriminates specimens with measurable quantities of PSA (above the assay detection limit) from specimens with undetectable PSA levels. ER and PR values were categorized into positive and negative status using 10 fmol/mg as cutoff points. The cutoff value for tumor size was 2 cm and for S-phase fraction was 6.7%. Lymph node status was either positive (any positive number of nodes) or negative. Tumor cellularity was used as a four-level categorical variable defined as above. Patients were also categorized with respect to the receipt of chemotherapy (yes *versus* no), radiotherapy (yes *versus* no), and hormonal therapy (yes *versus* no). Survival analyses, including disease-free and overall survival, were performed by constructing Kaplan-Meier survival curves (28), as well as by calculating the estimated RR for relapse or death using the Cox proportional hazards regression model (29). The models were developed at both univariate and multivariate levels, and in the univariate analysis, PSA was first analyzed as a continuous variable (log-transformed PSA values) and then as a categorical variable using the 30th percentile cutoff point. Only patients for whom complete information was collected were included in the multivariate regression models, which incorporated PSA and all of the other variables for which the patients were characterized. Interactions between PSA and other prognostic factors were also examined by the inclusion of the interaction terms in the regression models. To evaluate the prognostic value of PSA after eliminating the impact of some crucial prognostic indicators, such as nodal status, ER status, and tumor size, we calculated the RRs for relapse and death in subgroups of patients. The effect of PSA on survival was also examined in subgroups of patients who received or did not receive adjuvant treatment (endocrine therapy or chemotherapy). All statistical analyses were performed using the computer software SAS version 6.03 (SAS Institute, Cary, NC), and the *P*s were derived from two-sided tests of significance.

RESULTS

PSA Distribution and Relationships with Clinical or Pathological Factors. Of the 1,000 patients, 953 were evaluated for PSA content in their tumor specimens; PSA could not be measured in 47 patients due to insufficient amount of tumor. The PSA content (in ng of PSA/g of total protein) among the 953 samples varied widely from 0 to 19,629 ng/g; the median was 6 ng/g (Table 1). Because the mean value (150 ng/g) was substantially larger than the median, the distribution of PSA was extremely skewed toward the lower values. Fig. 1 shows the distribution of logarithmically transformed PSA concentrations. The distributions of other numerical variables, including patient age, tumor size, number of positive lymph nodes, ER and PR levels, and S-phase fraction are shown in Table 1. With the exception of PSA and steroid hormone receptors, these variables are approximately normally distributed.

The relationships between PSA concentrations and the patient ages, tumor sizes, numbers of involved lymph nodes, ER and PR concentrations, and percentages of malignant cells in the specimens were first examined by Spearman correlation analysis, by which no correlations equal to or exceeding 0.20 in magnitude were revealed (data not shown). Associations were observed, however, between PSA status and both tumor size and S-phase fraction (Table 2). PSA levels tended to be higher in smaller tumors or in tumors with a lower S-phase fraction. Another feature of the chromosome analysis, DNA ploidy, was also shown to be significantly associated with PSA status, such that PSA positivity was higher in diploid than in aneuploid tumors (Table 2). PSA positivity was lower with increased tumor cellularity. No associations were found between PSA and either nodal status or steroid hormone receptor expression.

Table 2 Associations between PSA status[a] and other variables

Variable	No. of patients (%)		*P*
	PSA negative	PSA positive	
Age (years)			
<50	66 (25.2)	196 (74.8)	
≥50	160 (23.2)	530 (76.8)	0.517
Tumor size (cm)			
<2	88 (20.7)	337 (79.3)	
≥2	131 (26.8)	358 (73.2)	0.032
Nodal status			
Negative	119 (23.5)	388 (76.5)	
Positive	106 (25.9)	303 (74.1)	0.393
S-phase fraction (%)			
<6.7	137 (20.9)	519 (79.1)	
≥6.7	89 (30.1)	207 (69.9)	0.002
DNA ploidy			
Diploid	86 (19.5)	356 (80.5)	
Aneuploid	140 (27.5)	370 (72.5)	0.004
ER status[b]			
Negative	46 (24.7)	140 (75.3)	
Positive	180 (23.5)	586 (76.5)	0.72
PR status[b]			
Negative	71 (26.1)	201 (73.9)	
Positive	155 (22.8)	525 (77.2)	0.28
Tumor cellularity			
0–10%	4 (14.3)	24 (85.7)	
11–30%	26 (16.1)	135 (83.9)	
31–70%	91 (24.8)	276 (75.2)	
>70%	85 (35.3)	156 (64.7)	<0.001

[a] PSA status was based on a cutoff point equal to the 30th percentile of the distribution of PSA concentrations (1 ng/g).

[b] Steroid hormone receptor status was based on cutoff points equal to 10 fmol/mg.

PSA and Breast Cancer Survival. Of the 953 patients whose tumors were assessed for PSA content, follow-up information was available for 952 of them. During the follow-up period, 200 patients developed cancer relapse, and 188 died. In the univariate survival analysis (Table 3), the risk of relapse or death was not altered when PSA was used as a continuous variable. Also, there was no difference in disease-free or overall survival between PSA-positive and PSA-negative patients when PSA was analyzed as a categorical variable classified dichotomously using the 50th or 70th percentile (data not shown). However, significantly reduced risks for relapse as well as death, associated with PSA positivity, were suggested when the PSA cutoff was at the 30th percentile. These regression models showed that there was an approximately 30% reduction in risk of relapse or death in patients with PSA-positive cancer compared to those with PSA-negative cancer. The Kaplan-Meier survival curves (Fig. 2) also show that PSA-positive patients had both better disease-free and overall survival rates than PSA-negative patients. The difference in survival rate between the two groups was greater for disease-free survival than for overall survival. In the multivariate analysis of PSA, the Cox regression model was adjusted for age; nodal status; tumor size; S-phase fraction; DNA ploidy; ER status; PR status; tumor cellularity; and postoperative treatment, including adjuvant chemotherapy and endocrine treatment, all of which were used as categorical variables defined as above. Similar to the results of the univariate analysis, the risks of relapse and death were both significantly lower in PSA-positive patients than in PSA-negative patients (Table 3). In addition to PSA, other variables that were significantly associated with the RRs of relapse and death in the multivariate models included nodal status and tumor size; patients with node-positive tumors were shown to have a RR for relapse of 1.59 ($P = 0.0011$) and a RR for death of 1.81 ($P = 0.0007$), and patients with tumors equal to or greater than 2 cm in size had RRs for relapse and death equal to 1.48 ($P = 0.024$) and 1.53 ($P = 0.019$), respectively. The other variables in the models (DNA ploidy; ER status; PR status; S-phase fraction; and treatment with chemotherapy, radiotherapy, and endocrine therapy) were not significant factors in the multivariate analysis (data not shown).

When the relationship between PSA and survival was examined separately in node-positive and node-negative patients, patients with PSA-positive tumors in both subgroups were shown to have better disease-free and overall survival than patients with PSA-negative tumors, but none of the differences were statistically significant (data not shown). For patients who were either ER positive or ER negative, the significant associations between PSA and disease-free or overall survival were shown only in ER-positive patients (Fig. 3). Similarly, significant associations between disease-free and overall survival and PSA status were seen for patients with tumors ≥2 cm but not for patients with tumors <2 cm (Fig. 4). We also examined interactions between PSA and the variables used to define the patient subgroups. Statistically significant interactions between PSA status and ER status could not be demonstrated in multivariate Cox regression models (data not shown). However, interaction between PSA and tumor size was shown for disease-free survival (interaction term, $P = 0.013$) but not for overall survival (data not shown).

Of the patients for whom adjuvant treatment information was available, 337 patients received adjuvant therapy. Of those, 159 had adjuvant endocrine therapy and 178 received adjuvant chemotherapy. No significant impact of PSA on disease-free survival or overall survival was shown in any of the compared subgroups (data not shown).

DISCUSSION

This study was conducted in an attempt to confirm that PSA is a favorable prognostic indicator for breast cancer, using a much larger and different patient population from the one studied previously by our group (22). In both our present and previous studies, we consistently found that PSA presence is associated with smaller tumor size and prolonged disease-free survival. Concordant with these data is the finding that PSA is inversely associated with two markers of active cell proliferation, high S-phase fraction and DNA aneuploidy, both of which are indicative of poor prognosis (25). These inverse relationships between PSA and S-phase fraction or DNA aneuploidy have also been found in another study (30).

Although PSA was shown in both of our studies (Ref. 22 and the present study) to be associated with improved survival, there were some differences in the findings between the studies as well. The two studies consistently demonstrated that PSA was significantly associated with disease-free survival and that the impact of PSA on disease-free survival was sustained after

Table 3 Associations between PSA and disease-free and overall survival

PSA status	Disease-free survival			Overall survival		
	RR[a]	(95% CI)[b]	*P*	RR[a]	(95% CI)[b]	*P*
Univariate analysis (n = 952)						
Continuous variable						
0	1.00			1.00		
Log(PSA + 1)	0.99	(0.92–1.07)	0.81	0.93	(0.85–1.01)	0.09
Categorical variable[c]						
Negative	1.00			1.00		
Positive	0.67	(0.49–0.91)	0.01	0.72	(0.52–0.99)	0.048
Multivariate analysis[d] (n = 712)						
Categorical variable[c]						
Negative	1.00			1.00		
Positive	0.68	(0.48–0.94)	0.02	0.65	(0.45–0.93)	0.02

[a] RR estimated from Cox proportional hazard regression model.
[b] Confidence interval of the estimated RR.
[c] PSA status based on a cutoff point equal to the 30th percentile of the distribution of PSA concentrations.
[d] Multivariate models were adjusted for lymph node status; tumor size; patient age; DNA ploidy; tumor cellularity; S-phase fraction; ER and PR expression; and the receipt of chemotherapy, radiotherapy, and endocrine therapy.

patient age, nodal status, tumor size, steroid hormone receptors, and other variables were adjusted in the analysis. For overall survival, a significantly reduced risk for death was suggested in the previous study, but it was demonstrated only in the univariate analysis. In the multivariate analysis, this association was no longer statistically significant. In the present study, the impact of PSA on overall survival was shown at both univariate and multivariate levels. This difference between the two studies could be explained by the differences in follow-up time and sample size. Compared to the present study, the previous one had fewer patients, fewer deaths, and shorter follow-up times, which, in turn, provided less statistical power to detect any given difference. Providing support to this suggestion is the Kaplan-Meier plot demonstrating greater differentiation of the survival curves as the follow-up time increased (Fig. 2).

Another difference between the two studies was the cutoff level used for PSA classification. In the previous study, PSA status was classified using the 70th percentile of the PSA distribution values, whereas in this study, the cutoff for PSA was at the 30th percentile. The lower cutoff used in this study was a result of the availability of a newer, more sensitive assay for PSA (23) compared to the older method (24). With the older method, 60% of specimens had PSA levels below the detection limit of 0.01 ng/ml; with the new method, only 10% of specimens had undetectable PSA (<0.001 ng/ml). Although the advantages of the newer assay have been described previously (23), the direct comparison of the two methods for the assay of PSA in the breast tumor extracts studied here was not performed.

One might speculate that the association between PSA and survival was due to the inverse relationship between PSA and tumor size. Large tumor size is known to be a strong unfavorable prognostic indicator for patients with breast cancer. However, this explanation is not supported by the results of the multivariate analysis in which the impact of PSA on survival remained significant after tumor size was adjusted in the Cox regression model. In further survival analyses of subgroups of patients who were categorized by their tumor size, substantial differences in disease-free survival and overall survival were

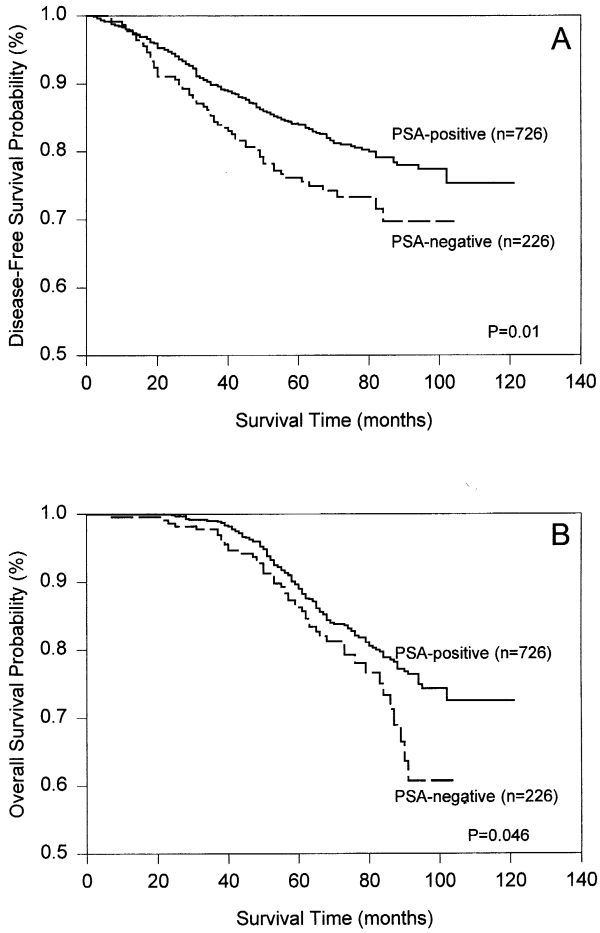

Fig. 2 Disease-free (*A*) and overall (*B*) survival of 952 breast cancer patients with PSA-negative or PSA-positive tumors.

demonstrated between PSA-positive and -negative patients who had relatively large tumors, *i.e.*, ≥2 cm (Figs. 3 and 4).

A brief comparison of patients between the two studies is shown in Table 4. Women in the present study were slightly

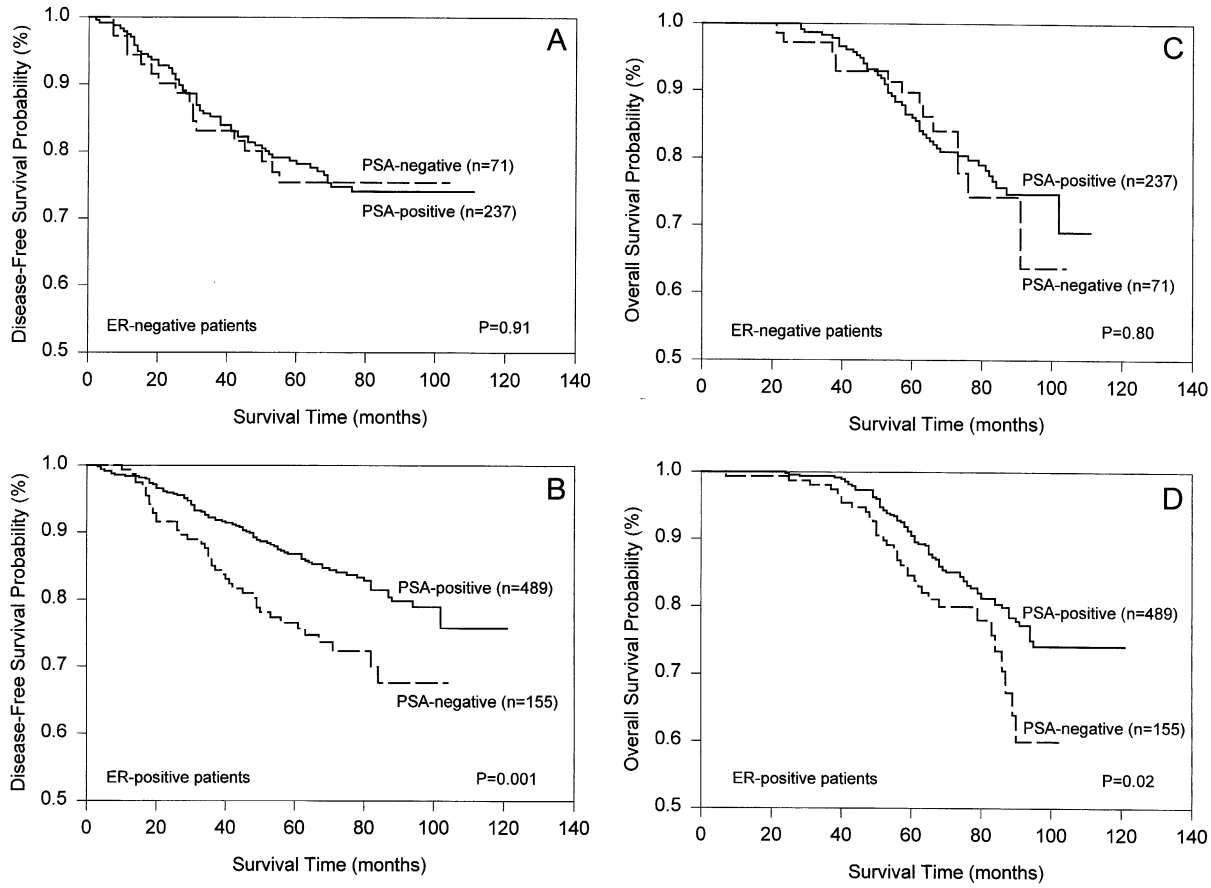

Fig. 3 Disease-free (*A* and *B*) and overall (*C* and *D*) survival of patients with PSA-negative or PSA-positive tumors, stratified by their ER status: 308 ER-negative patients (*A* and *C*); 644 ER-positive patients (*B* and *D*).

older than those in the previous study. Although patients in the two studies were similar in regard to median tumor size (2.4 *versus* 2.3 cm), the percentage of patients with positive lymph node status was slightly higher in the previous study than in the present one (51 *versus* 45%). This difference may have given rise to a higher rate of disease relapse in the previous study than in the current one (24% *versus* 21%). The difference in relapse would be expected to be much greater if the follow-up time in the previous study had been extended from 3 to 6 years. The higher death rate in the present study than in the previous one (20% *versus* 16%) would have been anticipated, because patients in this study had been followed much longer. The different rates of relapse and lymph node involvement between patient populations in the two studies suggest a possible difference in disease prognosis, and this may have contributed to the discrepancies observed. Demographic differences between the populations, as well as differences with respect to disease stage and postoperative treatment, may also have led to the different findings of the two studies, but because these characteristics of the patients were not determined in sufficient detail, we are unable to speculate on their importance.

In Table 4, we also compared the ER and PR positivities between the two studies. Because two different methods were used, the positivity rates were significantly different in spite of the same cutoff levels being used. A positive association between PSA and ER but not PR was observed in the previous study, whereas in this study, PSA was suggested to be associated with PR but not with ER (data not shown). The inconsistency was also noticed when the results of our previous study were compared to the findings of a cross-sectional study (21). In the latter study, PSA status was found to be associated with both ER and PR, and PR appeared to be more strongly associated with PSA than ER when ER and PR were analyzed in combination. This discordance may have resulted from different calibrations, extraction methods, and detection principles used in the methods for measuring the steroid hormone receptors. For example, the method used in this study and the previous study (22) was a ligand-binding assay that measures only the empty receptors. However, the method used in the cross-sectional study (21) was an immunoassay that measures both the unbound and ligand-bound receptors. Therefore, the large variability in results for ER and PR by different methods causes difficulty in establishing a more consistent relationship between ER, PR, and PSA.

On the basis of the observations of our cell culture experiments (17), PSA production in breast cancer cells has been shown to be mediated by PR but not ER. Progestins, but not estrogens, induce PSA production *in vitro*. In this study, we

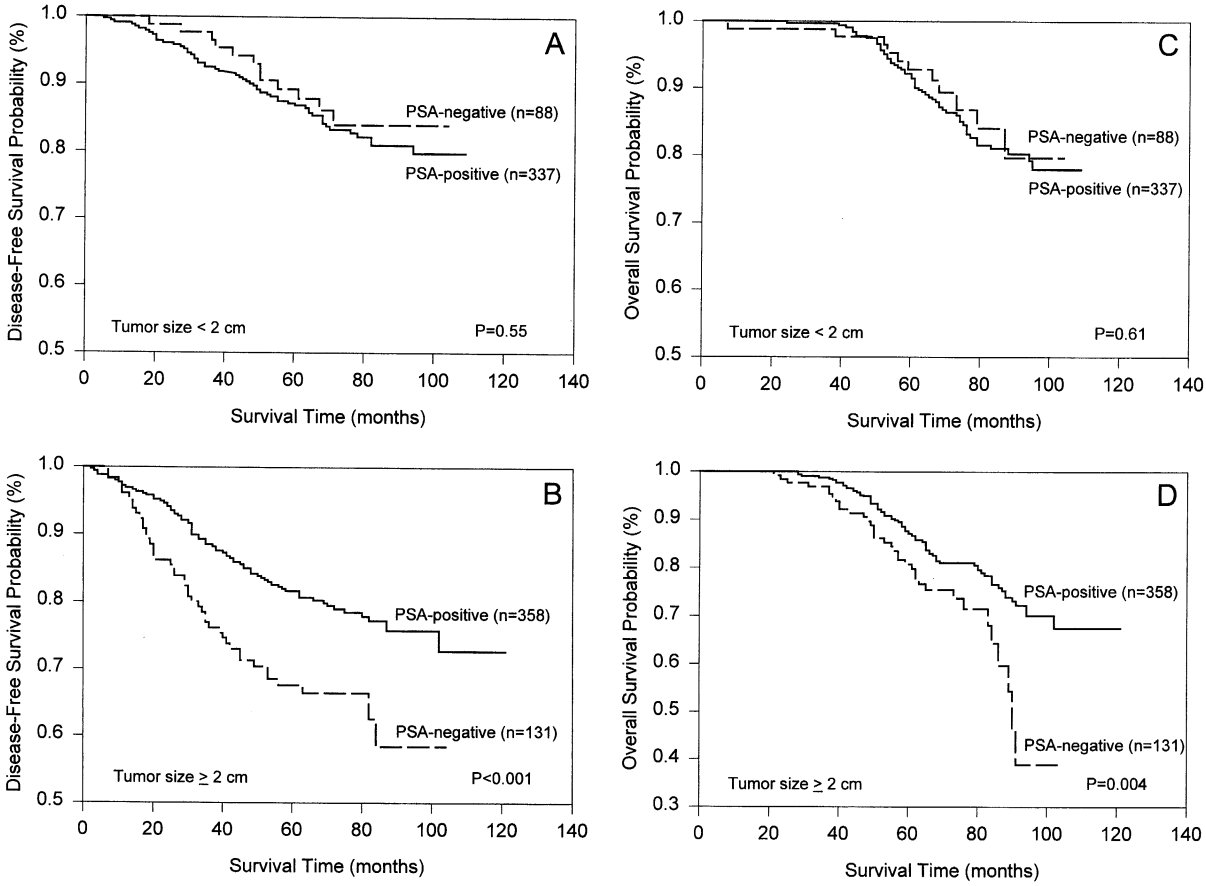

Fig. 4 Disease-free (*A* and *B*) and overall (*C* and *D*) survival of patients with PSA-negative or PSA-positive tumors, stratified by their tumor size: 425 patients with tumors <2 cm (*A* and *C*); 489 patients with tumors ≥2 cm (*B* and *D*).

Table 4 Patient comparison between present and previous study by our group

Parameter	Previous study[a]	Present study
Number of patients	174 Italians	953 Americans
Months of follow-up (median)	7–67 (33)	7–121 (73)
Relapse rate	24%	21%
Death rate	16%	20%
Age, years (median)	25–91 (56)	22–94 (61)
Age ≥50 years	68%	72%
Positive nodes	51%	45%
Tumor size, cm (median)	0.7–6.0 (2.4)	0.1–14.5 (2.3)
Positive ER[b]	66%	80%
Positive PR[b]	57%	71%

[a] Reported in Ref. 22.
[b] The cutoff level for receptor positivity was 10 fmol/mg.

found no association between PSA and ER. We have further examined the relationship between ER and patient survival. We found that positive ER status was not associated with better disease-free survival or overall survival, whereas these associations were shown in our previous study (22). This discrepancy underscores the heterogeneity of both the patient population and the methodology for measuring ER.

The previous observation that PSA may be a stronger prognostic factor in node-positive or ER-negative patients was not confirmed in the present study. When the effect of adjuvant treatment on survival was examined, we found that PSA status had no impact on patient survival for those who received only adjuvant endocrine therapy or chemotherapy. Therefore, it appears that measurement of PSA for predicting response to adjuvant therapy has limited value. Because the information on postoperative treatment was not sufficiently collected, this observation needs further confirmation from studies with well-defined postoperative treatment.

Because many clinicopathological variables were available for our patient population, we have performed statistical analyses at both univariate and multivariate levels to examine which of these have independent value. Our data demonstrated that nodal status, tumor size, and PSA status were independent markers of prognosis. Among these, only PSA confers favorable prognosis. Information regarding the stage classifications and histological grades and types of the tumors in our series was not available, making the adjustments for these variables in the multivariate survival analysis impossible. Our previous study had shown elevated PSA levels to be associated with early-stage disease, but statistically significant relationships between PSA

and tumor grade and histotype were not demonstrated (22). In that study, PSA remained a significant prognostic factor after adjustment for grade and stage in multivariate Cox models.

Because very little is known about the physiological role of PSA in breast tissue, it would be difficult to formulate a hypothesis that could explain the mechanism by which PSA confers a favorable prognostic outcome in breast cancer. On the basis of knowledge derived from studies of prostatic tissue, we have considered three possibilities. The first one relates to the effect of androgen. PSA production in both prostatic and breast tissues is up-regulated by androgens through the androgen receptor. The presence of PSA could indicate the existence of an androgenic effect that might counteract or interfere with the impact of estrogens. Estrogens are essential contributing factors for the growth and progression of breast cancer cells. The second possibility stems from knowledge of breast cancer cell differentiation. Well-differentiated cancer cells could be associated with the presence of PSA, given that PSA is one of the end products of steroid hormone action. Because poorly differentiated cells are frequently independent of steroid hormone regulation, the presence of PSA may be a marker of a functional steroid hormone receptor pathway. PSA could be a better indicator of a functional pathway than the steroid hormone receptors themselves. On the basis of the fact that not all breast cancers with steroid receptors respond to endocrine treatment (31), it has been speculated that the physical existence of the receptors may not necessarily constitute proof of their functionality. Defective receptors have been shown to exist that do not have the ability to form complexes with their ligands or to bind to the hormone response elements in the target genes (32). The third possibility is based on the relationship between PSA and certain growth factors. *In vitro* experiments have demonstrated the proteolysis of IGFBP-3 by PSA (19, 20). IGFBP-3 is one of the six IGF-binding proteins that regulate the bioavailability and binding of IGFs to their receptors on various cell membranes (33). IGFs, a family of peptide hormones including IGF-I and IGF-II, have strong mitogenic effects and other functions in a variety of normal and abnormal cells, including breast cancer cells (34, 35). IGFBP-3 is a major regulator of IGFs that could either enhance or inhibit the activity of IGFs depending on the status of IGFBP-3 and the type of tissue involved (36). Our recent study indicated that IGFBP-3 levels were positively correlated with the IGF-II levels in breast cancer tissues and that these two factors were associated with some poor prognostic markers of breast cancer, such as ER negativity and high S-phase fraction (37).

In summary, we found that PSA was present in most breast cancer tissues at relatively low concentrations. PSA was more frequently present in small tumors and in tumors with lower proliferative activity. PSA levels were not significantly different between node-positive and node-negative patients. The association between PSA and steroid receptors was not consistently observed, and this inconsistency may have been partially due to the differences in the methods for ER and PR measurement. Breast cancer patients with tumors positive for PSA tended to have a 30–40% reduction in risk for relapse or death. This difference in survival remained unchanged after clinical and pathological features, which were also related to survival, were taken into consideration. Therefore, the measurement of PSA in tumor extracts may provide additional information related to breast cancer prognosis.

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