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Correlation of Prostate Specific Antigen Immunoactivity (IR-PSA) to Other Prognostic Factors in Female Breast Cancer

JOHN GRINIATSOS[1], ELEFTHERIOS DIAMANTIS[2], JULIA GIOTI[1], IRENE KARYDA[1], PERICLES P. VASSILOPOULOS[1] and NIKI AGNANTI[3]

[1]*Saint-Savvas Anticancer Hospital, Athens, Greece,* [2]*Department of Clinical Biochemistry, University of Toronto, Canada;* [3]*University of Ioannina, Greece*

Abstract. *Recently, using an ultrasensitive time-resolved immuno-fluorometric assay, PSA immunoreactivity (IR-PSA) was found in breast tumor cytosols. We retrospectively studied 219 breast cancer patients, measuring IR-PSA in the tumor cytosols, and classified the breast cancers as either PSA positive or PSA negative based on an IR-PSA cut off level of 1pg/mg. Multivariated analysis showed that IR-PSA is an independent favourable prognostic indicator for postmenopausal, node positive breast cance patients. Additionally, IR-PSA correlates with reduced risk of relapse in ER+ve tumors and is negatively correlated with mutated p53, which increases the risk of relapse.*

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 the many differences in clinical and biological behavior found between breast cancers. The specific set of alterations within the tumor may provide necessary information on its identily and best type of treatment.

PSA is one of the most useful biological markers, and its value in the diagnosis and monitoring of prostate cancer is well established. The PSA protein and its encoding gene have been characterized. PSA has not been detected in any tissue in women except in the periurethral glands, which are androgen responsive and have a similar structure to the male prostate [1].

Recently, using an ultrasensitive time-resolved, immuno-fluorometric assay for PSA [2], from a cohort of more than 1200 female breast cancer patients, PSA immunoreactivity (IR) higher than 0,03 ng/mg of total protein was found in 30% of breast tumor cytosols [3]. Due to the relatively low level of the protein in the breast tumor cytosol, it has not yet been possible to purify sufficient amounts of IR-PSA for protein sequencing [4]. However, the presence of PSA in breast tumors is strongly suggested by compelling evidence namely, a) IR-PSA in breast tumors can be measured not only using one method [2] but also using 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) [2,5]. b) The molecular weight of IR-PSA, determined by ultrasensitive, time-resolved, immunofluorometric assay and Western blot analysis, is identical to the molecular weight of PSA from seminal plasma [6]. c) The receptor-dependent androgenic up-regulation of as well as the antagonizing effect between androgen and estrogen on PSA production in the prostate is also demonstrated in breast cancer cell lines [6] and d) using reverse-transcription-PCR and DNA sequencing techniques, PSA mRNA has been identified in IR-PSA-positive breast tumors but not in IR-PSA-negative breast tumors. The sequence of the generated PCR product is identical to that of the PSA gene [7].

In this paper we examined the ability of breast tumors to produce PSA, and investigated where PSA is produced, whether it has any correlation with other well known prognostic factors, including p53 tumor suppressor gene product, and the risk of recurrence.

Materials and Methods

Two hundred and nineteen (219) patients with primary breast cancer were included in this study. The patients were selected consecutively from the list of patients who underwent curative surgical treatment in our hospital, provided that their tumor tissue was sufficient for analysis. They represented approximately 75% of all new cases of breast cancer diagnosed and treated during the period of January 1992 to December

Correspondence to: Pericles P. Vassilopoulos, M.D., Assoc. Professor of Surgery, Director, 1st Surgical Oncology Clinic, Saint-Savvas Anticancer Hospital, Alexandras Ave 171, Athens 115 22, Greece. Tel.: 0030-1-6459411 6430811, Fax :0030-1- 6421022, 6420146.

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Table I. *Correlation of PSA to known prognostic factors.*

	PSA ≥ 1 pg/mg		PSA < 1 pg/mg		
	No (146)	%	No (73)	%	p value
Age					
Premenopausal	15	10.3	12	16.4	NS
Postmenopausal	131	89.7	61	83.6	
Tumor size					
T_1	39	26.7	20	27.4	
T_2	84	57.5	34	46.6	
T_3	7	4.8	11	15	NS
T_4	16	11	8	11	
Grade					
I	13	9	6	8.2	
II	73	50	38	52	NS
III	60	41	29	39.8	
Axillary lymph nodes					
Negative	52	36.4	41	56.2	p=0.004
Positive	94	63.6	32	43.8	
Stage					
I	24	16.5	17	23.3	
II	70	47.9	34	46.6	NS
III	52	35.6	22	30.1	
ER					
Positive	127	87	53	72.6	p=0.001
Negative	19	13	20	27.4	
PgR					
Positive	104	71.2	49	67.1	NS
Negative	42	28.8	24	32.9	
p53					
Positive	22	15	29	39.7	p<0.001
Negative	124	85	44	60.3	

1994. Exclusion criteria included: (a) inadequate amount of breast cancer tissue, (b) Paget£s disease of the breast or *in situ* tumors, (c) non-curative surgical treatment, due to either advanced age of the patient or the presence of disseminated disease at the time of diagnosis or within two months after surgery, (d) patients who had undergone neo-adjuvant chemotherapy prior to breast operative treatment were also excluded from the study. All patients in this study had undergone modified radical mastectomy or conservative breast surgery plus axillary lymph node dissection followed by postoperative irradiation. The patient age ranged from 21 to 84 years with a median of 62 years. Twenty-seven (27) were premenopausal and the remaining 192 were post-menopausal. Adjuvant systemic treatment was administered to the patients according to the axillary lymph node involvement of the tumor and the menopausal status. All premenopausal, node positive patients, received adjuvant combined chemotherapy in the form of CMF (cytoxan, methotrexate, fluorouracil) or CEF (cytoxan, epirubicin, fluorouracil). All postmenopausal patients with ER positive tumor, irrespectively of nodal status, received adjuvant tamoxifen treatment for 3-4 years. For those who had either ER negative tumor or more than four axillary lymph nodes involved by the tumor, adjuvant systemic chemotherapy similar to the above was administered in 6 to 8 cycles. The follow-up was scheduled once every 3 months during the first 2 years following the treatment, at 6 month intervals for 3 years, and once a year thereafter.

For each patient clinical and pathological information was recorded including clinical stage, size and grade of the primary tumor, axillary lymph node involvement, presence of ER and PgR in tumor cells, as well as the most recent follow-up evaluation. Clinical staging was performed according to the postsurgical International Union Against Cancer Tumor-Node-Metastasis classification [8]. Of the 219 patients, 41 were stage I, 104 stage II and 74 stage III. The size of the tumor recorded was the maximum diameter of the fresh specimen. The histologic grading was performed according to the criteria described by Bloom and Richardson [9]. There were 19 grade I cases, 111 grade II and 89 grade III [13]. Ninety-three patients were axillary lymph node negative and one hundred-twenty six (126) positive. Estrogen and progesterone receptors in the primary tumor were measured with the use of the dextran-coated charcoal method [10,11]. At a cut-off point of 10 mol/mg tumor protein 180 patients (82,19%) were ER+ and 153 (69,86%) were PgR+.

Measurement of PSA and p53. PSA immunoreactivity in cytosol extract

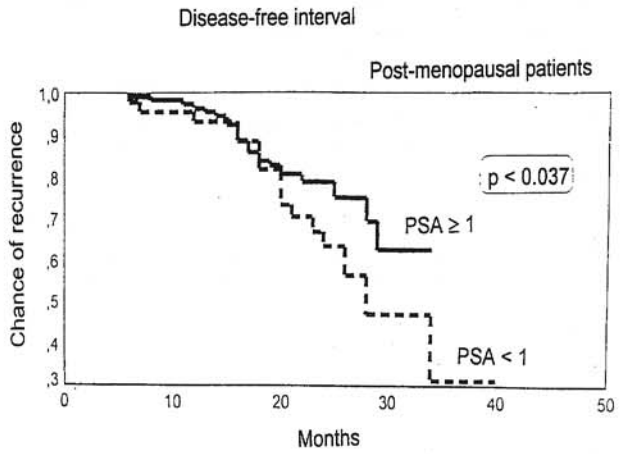

Figure 1. *Disease-free interval-Post-menopausal patients.*

was measured with an ultrasensitive time-resolved immunofluorometric PSA assay [2].

Briefly, the assay incorporated one monoclonal capture anti-PSA antibody and one biotinylated polyclonal detection anti-PSA antibody. Streptavidin conjugated with alkaline phosphatase was used as the label, and the enzymatic activity of alkaline phosphatase was detected through the hydrolysis of the 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.

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 pg of PSA/mg of total protein. Tumors with PSA immunoreactivity ≥1 pg/mg were considered positive for PSA. 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).

For the p53 assay [12] we used goat anti-mouse immunoglobulin coated to polystyrene microtiter wells, a mouse monoclonal anti-p53 capture antibody (mutant specific, PAb 240), a rabbit polyclonal anti-p53 antibody (CM-1, wild-type and mutant specific), and alkaline phosphatase-labeled goat anti-rabbit immunoglobulin (GARIg-ALP). In the assay, 50 µl of sample was incubated along with 100 µl of mouse PAb 240 antibody, for 3 hours, followed by washing × 6. The rabbit polyclonal CM-1 antibody is then added for 2 hours followed by washing × 6. The GARIg-ALP conjugate is then added for 1 h, followed by washing × 6. The activity of ALP is then measured as described for the PSA assay [2].

Results

Correlation of PSA to other prognostic factors. The correlation of PSA to clinical and pathological variables is shown in Table I. PSA-positive patients (146) did not differ significantly from PSA-negative patients (73) in terms of menopausal status, size and grade of the primary tumor and PgR status. Statistically significant differences were found between PSA-positive and PSA-negative patients for axillary node involvement (more PSA-positive patients were axillary node positive), ER (PSA-positivity was associated with ER positive tumors) and p53 expression (PSA is reverse correlated to p53). Multivariate analysis of PSA and known prognostic factors showed independent significance only in the group of postmenopausal breast cancer patients (Table II) and not in

Table II. *Multivariate analysis of known prognostic factors and PSA in postmenopausal breast cancer patients.*

	p value	Relative risk	95% conf. interval
Tumor size			
T_1			
T_2	NS		
T_4			
Grade			
I			
II	NS		
III			
Axillary lymph node			
Negative		1.00	
Positive	p=0.0012	1.02	1.5471-5.8984
Stage			
I			
II	NS		
III			
ER			
Negative		1.00	
Positive	p=0.0133	2.41	1.2016-4.8461
PgR			
Negative	NS		
Positive			
p53			
Negative		1.00	
Positive	p=0.001	0.21	0.1038-0.4234

Table III. *Correlation of PSA to recurrence rate.*

		PSA ≥ 1 pg/mg		PSA < 1 pg/mg		
		No	%	No	%	p value
Postmenopausal		(104)		(43)		
Recurrence	→ No	80	76.9	26	60.5	
	→Yes	24	23.1	17	39.5	p=0.037
Premenopausal		(12)		(10)		
Recurrence	→ No	7	58.3	6	60	
	→Yes	5	41.7	4	40	NS
All patients		(116)		(53)		
Recurrence	→ No	87	75	32	60.4	
	→Yes	29	25	21	39.6	NS

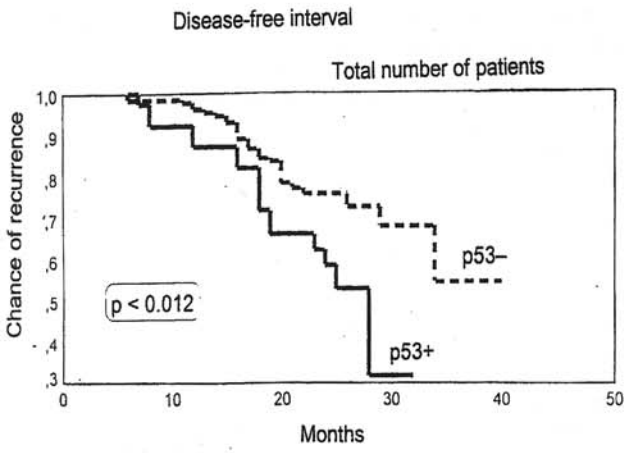

Figure 2. *Disease-free interval-Total number of patients.*

Table VI. *Correlation of PSA and p53 with recurrence rate.*

	PSA ≥1 and p53		PSA < 1 and p53+		
	No (97)	%	No (20)	%	p value
Recurrence → No	77	79.4	11	55	
→ Yes	20	20.6	9	45	p<0 01

the premenopausal group. The number of patients, however, in the two groups were by far different and this might be the explanation for the findings.

Relationship between PSA and recurrence rate. In a median follow-up time of 2.8 years we were able to obtain full information for their disease status from 169 patients (22 premenopausal and 147 postmenopausal). The correlation of PSA to recurrence rate is shown in Table III. Statistically significant difference was observed in postmenopausal patients (the presence of PSA immunoreactivity correlates to reduced recurrence rate (Figure 1)).

The presence of mutant p53 gene was detected in 13 premenopausal and in 26 postmenopausal patients and was correlated with increased recurrence rate (Table IV, Figure. 2).

The multivariate Cox regression model was also used to assess the impact of PSA immunoreactivity on patients£ recurrence risk while controlling for other clinical and pathological variables that may also affect the recurrence rate. The variables included in the model were: menopausal status, tumor size, grade, axillary lymph nodes involvement, presence of steroid hormone receptors and mutated p53 gene. In postmenopausal patients, after adjusting for all the variables studied except stage, PSA-positivity correlated significantly with reduced risk of relapse when compared to

Table V. *Multivariate analysis of known prognostic factors and PSA to recurrence rate in postmenopausal patients.*

	Multivariate Cox Regression Modeling Analysis		
	p value	Relative risk	95% conf. interval
Tumor size			
T_1		1.00	
T_2	p=0.2110 (NS)	0.73	0.4444- 1.1961
T_3	p=0.5886 (NS)	0.79	0.3467 - 1.8240
T_4	p=0.0041	2.40	1.3208 - 4.3763
Grade			
I			
II	NS		
III			
lymph node status			
Negative		1.00	1.2927-2.7963
Positive	p=0.0011	1.90	
PSA			
PSA < 1 (Negative)		1.00	
PSA ≥ 1 (Positive)	p=0.0378	0.69	0.4854-0.9792
ER			
Negative		1.00	
Positive	p=0.0133	0.69	0.4854 - 0.9792
PgR			
Negative	NS		
Positive			
p53			
Negative	NS		
Positive			

Table VI. *Correlation of p53 to recurrence rate.*

	p53+		p53-		
	No	%	No	%	p value
Postmenopausal	(26)		(121)		
Recurrence → No	15	57.7	91	75.2	
→ Yes	11	42.3	30	24.8	NS
Premenopausal	(13)		(9)		
Recurrence → No	6	46.2	7	77.8	
→ Yes	7	53.8	2	22.2	NS
All patients		(39)		(130)	
Recurrence → No	21	53.8	98	75.4	
→ Yes	18	46.2	32	24.6	p=0.012

Table VII. *Prognostic factors in breast cancer.*

Established	
Tumor size	
Axillary lymph nodes	
ER and PR	
Nuclear grade	
Under Investigation	
Markers of proliferation	*Growth factors/receptors*
Thymidine labeling	EGFR
S-phase fraction	Insulin-like growth factors
DNA ploidy	Insulin receptor
Ki-67	Transforming growth factors
PCNA/cyclin	
Topoisomerase 11	*Invasion-related factors*
Histone H3	Cathepsin D
Thymidylate synthetase	uPA/PA-I
	Laminin receptor
Oncogene/tumor suppressor genes	Stromelysin-3
HER-2 neu	Angiogenesis factors
int-2	
c-myc	*Miscellaneous factors*
ras	pS2
p53	NM23
RB	Heat shock proteins

EGFR = epidermal growth factor receptor; ER = estrogen receptor; PCNA = proliferating cell nuclear antigen; PR = progesterone receptor.

PSA-negativity (Table V). In addition IR-PSA was inversely correlated to p53 for the recurrence rate (Table VI).

Discussion

A variety of parameters have been reported to have prognostic significance in patients with breast cancer. Among these markers, some are better established than others, and most are associated with prognostic value for relapse free or overall survival rather than predictive value for response to a specific treatment (Table VII) [13-16].

The presence of IR-PSA in breast tumors is not a random event. It is associated with certain clinically important parameters such as the clinical stage, the presence of steroid hormone receptors, p53 expression and recurrence rate.

In the prostate, PSA production is up-regulated by androgen through the androgen receptor. An adrogen that up-regulates PSA production has also been demonstrated in breast cancer cells culture [6]. 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 [17,18]. Cell culture studies have shown that androgen inhibits the proliferation of breast cancer cells [19] and counteracts the effect of estrogen [20].

An antagonistic interaction between androgen and estrogen on the production of PSA has been observed in a cell culture study [6] and it has been further supported by the observation of PSA production induced by tamoxifen, an antiestrogen agent. These observations indicate that the presence of PSA may suppress or render the estrogenic influence on breast tumors less effective. Consequently, we suspected that PSA may serve as a favorable prognostic indicator for breast cancer patients. In this study, we observed a significantly reduced risk of relapse in postmenopausal breast-cancer patients with PSA-positive tumors, as compared to patients with PSA-negative tumors. This favorable indication was independent of other prognostic factors.

Similarly, IR-PSA was found to be a favorable prognostic indicator for ER-positive tumors. Furthermore, IR-PSA has a negative correlation to mutated p53, which was found to be an indicator of worse prognosis.

Potential future applications of PSA include the visualization of breast tumors, since it is known that no female tissue contains PSA, or in the selection of therapy, since it has been speculated that the presence of PSA might be an indicator of functional estrogen receptors [4].

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