Prostate-specific antigen value as a marker in breast cancer*

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The immunoexpression of prostate-specific antigen in breast cancers has been well established, but the role of this extra-prostate PSA appears to be a complex, poorly understood and of doubtful prognostic value. In this context, our aim was to evaluate PSA in breast carcinomas and to compare the results with several established prognostic markers of breast cancer: estrogen and progesterone receptors status, HER2/neu status, histological type of tumor, grade of differentiation, stage, tumor size, nodal and menopausal status. We have immunohistochemically assessed 53 breast carcinomas for PSA, ER, PR and oncoprotein HER2/neu status. The relationship between the clinical and histopathological markers was analyzed by chi-square test. In the present study PSA was expressed in 60.3% of cases, and we have found a significant correlation with the histological type and HER2/neu negative status in premenopausal women. No statistically significant difference was found between PSA positivity and menopausal status of the patients, nodal status, estrogen and progesterone receptors, HER2/neu status in postmenopausal patients, tumor size or histological grade. We conclude that in our study PSA can not be considered as a valuable independent prognostic factor in breast carcinoma. As long as the majorities of PSA positive carcinomas were small in size, early stage, better and moderately differentiated, HER2/neu negative and 70% of ER/PR positive carcinomas expressed PSA, it might be useful as a marker for a subset of breast cancers with better prognosis, which could respond to endocrine therapy, in correlation with other prognostic markers.

Key words: breast cancer, immunohistochemistry, prostate-specific antigen, prognosis

Breast cancer is a major health problem worldwide and is responsible for the highest causes of death in women. According to the World Health Organization, more than 1.2 million new cases will be diagnosed with breast cancer in 2005 worldwide. The American Cancer Society estimates that in 2005, approximately 211.240 women in the United States will be diagnosed with invasive breast cancer (Stages I–IV). Another 58.490 women will be diagnosed with *in situ* breast cancer. The chance of developing invasive breast cancer during the woman's lifetime is approximately 1 in 7 (13.4%). In addition, 1,600 men will be diagnosed with breast cancer and 400 will die this year. It is estimated that 40.410 women and 460 men will die from breast cancer in the United States this year. If detected early, the five-year survival rate exceeds 95% [1].

One of the new directions in cancer research is to identify new biomarkers used in diagnosis, prognosis and pharmacodiagnosis. Tissue kallikreins are encoded by 15 structurally similar steroid hormone-regulated genes that colocalize to chromosome 19q13.4, and are representing the largest cluster of contiguous protease genes in the entire genome. Recent data suggest that kallikreins may be involved in carcinogenesis, particularly in the tumor invasion and thus, may represent important drug targets [2–4]. One of these kallikreins, prostate specific antigen (PSA) or human tissue kallikrein 3 (hK3) is a 240 amino acids 33-kDa single-chain glycoprotein expressed at high levels in the epithelium of the human prostate gland. It is a serine protease with chymotrypsin-like activity and the main biological role of this protease is to liquefy the seminal fluid, increasing the sperm motility. PSA is useful in the detection, staging and monitoring of prostate cancer [5]. It was originally identified as a tissue-specific protein expressed exclusively by the epithelial cells of the prostate gland [6, 7]. Improved ultrasensitive methods and RNA analysis have shown that PSA is not exclusively synthesized by the human prostate gland, but it is

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also produced by the breast, ovary, liver, kidney, pancreas, lung, adrenal and parotid glands [8–11].

mRNA expression in breast cancer was identified by MONNE et al in 1998 [12]. Breast PSA is identical in molecular weight and mRNA sequence to seminal PSA and PSA gene expression in breast tumors appears to be under hormonal control, because in the steroid hormone receptor-positive breast cell lines T-47D and BT-474 the PSA production can be induced by androgens, progesterone, mineral corticoids and glucocorticoids [13]. Although DNA sequencing confirmed that no mutations were present in the coding region of PSA gene in breast tumors, multiple mutations/polymorphisms were detected in the core promoter and enhancer region [14]. These mutations/polymorphisms may alter the steroid hormones regulation of the gene, affecting the PSA expression level [15].

On the other hand, although the presence of PSA within breast tissue, both benign as well as malignant has been well established, the clinical significance of PSA expression in breast carcinomas is far to be clear.

The purpose of the present study is to analyze by immunohistochemistry the characteristics of PSA in breast carcinomas and to compare the staining results of PSA with several established prognostic markers of breast cancer: estrogen and progesterone receptors status, HER2/neu overexpression status, histological type of tumor, stage, differentiation grade, tumor size, nodal status and menopausal status.

Material and methods

Breast cancer specimens. We have studied 53 surgical specimens from patients with breast cancer, during the year 2004. Clinical features of the patients were collected from the archives of the hospitals. Mean age of patients was 54.41 and the median was 53, ranging between 23 and 88. Clinical Ethical approval was obtained, and all patients gave informed consent.

The samples were formalin-fixed and paraffin-embedded, according to the routine procedure. The pathological diagnosis and grading were done on hematoxylin-eosin samples and were based on the Standard recommendations by AFIP in 2004 and ELSTON and ELLIS modified Scarff-Bloom-Richardson grading system [16]. Tumor size was defined as the maximum tumor diameter measured preoperatively or, in cases of non-palpable tumor, during the histological examination.

PSA immunohistochemistry. Additional sections from every case were immunohistochemically stained for PSA, using polyclonal anti-human prostate-specific antigen in the EPOS working system, provided by DakoCytomation, Denmark. After the sections were dewaxed and rehydrated, the next steps of the technique were: antigen retrieval by microwave heating in Dako target retrieval solution pH=6.6 for 5–10 minutes; blocking of endogenous peroxidase; washing with distillated water and Tris; incubation with anti PSA antibody for 30 minutes at room temperature; washing with TBS; incubation with chromogenic substrate solution (DAB) for 10 minutes; washing with distillated water and then, counterstain of nuclei with Lillie's modified hematoxylin and mount with coverslip; the negative control was the Dako negative control reagent. As outer positive control we used sections from cases of prostate adenocarcinoma. The cellular staining pattern for PSA was granular, cytoplasmic.

We elaborated a quantification system for the PSA immunohistochemical expression in the breast tissue that was used as an internal standard for all our samples. Quantification of the results was performed according to a histoscore modified after ALANEN [17] calculated from the estimated percentage of the PSA-positive cancer cells multiplied by the staining intensity category (weak positive = +1, moderate positive = +2 and strong immunoreaction = +3). The staining intensity of human prostate epithelium used as a positive control was always stronger (+4 = very strong immunoreaction) than that of the breast carcinoma cells. For example, if 5% of the tumor cells showed moderate immunoreaction, the histoscore value was 0.05x2=0.1. The results were estimated as negative (-), weak positive (+1), moderate positive (+2) and strong positive (+3) in the following manner: 0–0.04 = negative; 0.05–0.74 = (+1); 0.75–1.4 = (+2); 1.5–3 = (+3).

Estrogen and progesterone receptors were evaluated by immunohistochemistry using ready-to-use mouse monoclonal anti-human estrogen receptor (clone 1D5) and mouse monoclonal anti-human progesterone receptor (clone PgR 636), in the LSAB2 working system provided by Dako. After the blocking of endogenous peroxidase with 3% hydrogen peroxide, the antigen retrieval was performed by microwave heating at 90–99 °C for 20 minutes. The primary antibody reaction was carried out at room temperature for 30 minutes. Following this, the secondary antibody was applied and then, incubation with chromogenic solution (DAB) and nuclei counterstain with Lillie's modified hematoxylin. The cellular pattern of immunostaining was nuclear and ER and PR receptors were evaluated by both the intensity of the immunoexpression and the ratio of the positive cells, according to the Allred score [18].

HER2/neu oncoprotein overexpression was evaluated using the ready-to-use polyclonal antibody Rabbit Anti-Human HER2 Protein (HercepTest, Dako). After the epitope retrieval (microwave heating at 90–99 °C for 40 minutes) we performed the autostain procedure. The control slides were provided by Dako. For the determination of HER2 protein overexpression, only the membrane staining intensity and pattern was evaluated. We adopted the evaluation system according to that the score +2 is interpreted as weakly positive, +3 as strongly positive and the scores 0 and +1 were reported as negative [19].

Statistical analysis. The frequency distribution and menopausal status, lymph node status, tumor size, histological type and grade, estrogen, progesterone and HER2/neu status in PSA-positive and PSA-negative groups were compared using chi-square test with odds ratio and 95% confidence intervals. A p-value of less than 0.05 was considered to be significant.

Results

Patients and tumors characteristics. All the patients were female with the age range of 23–88 years (median 53 years). Regarding the menopausal status, 18 patients (median 43.5 years) were premenopausal and 35 postmenopausal (median 61). The commonest histological type was ductal NOS (not otherwise specified), comprising 62.26% of cases and lobular type (13%). Other histological types were ductal in situ (solid, apocrine and cribriform) (5.66%), medullary (3.77%), papillary (7.54%), squamous cell (5.66%) and mucinous (1.88%) carcinomas. The majority of cases were graded as G2 (64%), 18% were G1 and 18% were G3. 37.7% of the tumors were ≤2 cm in size, 50.9% were between 2 and 5 cm and 11.32% were more than 5 cm in size. Concerning the lymph node status 50.9% were negative. Regarding the stage grouping, 5.66% were stage 0, 24.5% of the cases were stage I, most of the cases (54.7%) were stage IIA+B, 13.2% were IIIA+B and 1.8% was stage IV.

PSA immunohistochemistry. For the estimation of PSA immunohistochemical expression we studied first the outer positive control samples, represented by cases of prostate adenocarcinoma with Gleason score less than 8. We estimated the presence, distribution and pattern of the reaction's final product and the reproducibility of the method in the same technical conditions. The PSA immunoreaction was highly positive (+4) in all cases of prostate carcinomas, the great majority of malignant cells being strongly stained.

We noticed that, unlike the prostate, in the normal mammary gland only the acini were constantly positive, whereas the ducts were generally negative (Fig. 1). This finding may have a high significance in the evaluation of the reaction for the malignant tumors of the breast, because in these conditions we expect a higher positive reaction for the lobular invasive carcinomas than for the ductal invasive carcinomas.

In breast malignancies, PSA was found in cytoplasm of tumor cells, with granular pattern and focal or diffuse distribution. It was absent in stromal cells. PSA positive immunoreaction was also seen in normal mammary gland and some benign lesions, especially apocrine metaplasia adjacent to the carcinomas.

We compared PSA immunostaining histoscores with general clinicopathological parameters of studied breast carcinomas (Tab. 1). When summarizing, PSA was expressed in 32 (60.3%) of 53 breast carcinomas, 11 (61.1%) cases of premenopausal patients and 21 (60%) cases of postmenopausal patients. Among the positive cases, 9.375% were weak positive (+1), 50% were moderate positive (+2) and 40.62% were strong positive (+3). Concerning the size of the tumor, PSA positive were 80% of the tumors ≤2 cm, 55.5% of the tumors between 2 and 5 cm and 66.6% of the tumors more than 5 cm in size. Regarding the grade of differentiation, PSA positive were 77.7% of G1 carcinomas, 62.5% of G2 and 55% of the G3 breast carcinomas. 63% of the lymph node negative tumors were PSA positive. Regarding the stage of the tumor, 68.75% of the PSA-positive tumors were stage 0 or I and 56.75% were stage II, III and IV. The histological type that expressed most frequent PSA was the lobular type (all our 7 cases being positive) (Fig. 2). The medullary (Fig. 3) and mucinous types of carcinomas were moderate positive. The majorities of our cases were represented by the ductal invasive type (Fig. 4). 60.6% (20/33) from ductal invasive carcinomas were positive (9 cases were +3, 9 cases +2 and 2 cases were +1). We had 4 cases of papillary carcinoma and 2 were moderate positive. One (solid type with apocrine differentiation) from the 3 cases of ductal *in situ* carcinomas was positive (+2). The carcinoma with squamous cell differentiation did not express PSA.

ER, PR and HER2/neu immunohistochemistry. Of the total cases, 37.73% were ER positive [7.54% were (+1), 15.09% (+2) and 15.09% were (+3)]. 41.4% of cases were PR positive [7.54% (+1), 18.86% (+2) and 15.86% (+3)].

24.5% [9 cases (+2) and 4 cases were (+3)] of the cases overexpressed HER2/neu oncoprotein, 16.6% of the premenopausal females and 28.57% of the postmenopausal females. In Table 2, we have compared the PSA immunoexpression with ER/PR and HER2/neu status.

Statistical analysis results. When the material was divided into PSA-negative (histoscore ≤0.04) and positive (histoscore >0.04) groups, we have found a statistically significant association with the histological type of the tumor (p=0.03)

Table 1. Comparison of PSA immunoexpression with general clinicopathological parameters in breast carcinomas

Parameter	Total positive cases	PSA Immunoexpression			
	+1,+2,+3	–	+1	+2	+3
All cases	32	21	3	16	13
Premenopausal (18 patients)	11	7	1	7	3
Postmenopausal (35 patients)	21	14	2	9	10
Tumor size (T) (cm)					
≤ 2 (20 cases)	16	4	2	8	6
≥ 2 < 5 (27 cases)	12	12	1	8	3
> 5 (5 cases)	4	2	0	1	3
Histological grade					
G1 (9 cases)	7	2	1	4	2
G2 (32 cases)	20	12	1	8	8
G3 (9 cases)	5	4	1	4	0
Histopathological type					
Ductal in situ (3 cases)	1	2	0	1	0
Invasive ductal (33 cases)	20	13	2	9	9
Invasive lobular (7 cases)	7	0	1	2	4
Medullary (2 cases)	2	0	0	1	1
Mucinous (1 case)	1	0	0	1	0
Papillary (4 cases)	2	2	0	2	0
Squamous cell (3 cases)	0	3	0	0	0
Nodal metastasis					
Negative (27 cases)	17	10	3	8	6
Positive (26 cases)	14	12	0	8	6

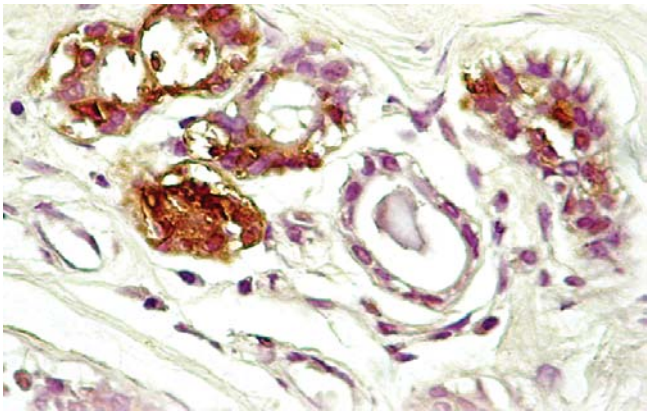

Figure 1. Normal mammary gland. Only the acini are positive, whereas the ducts are negative (PSA, EPOS, DAB, x 200).

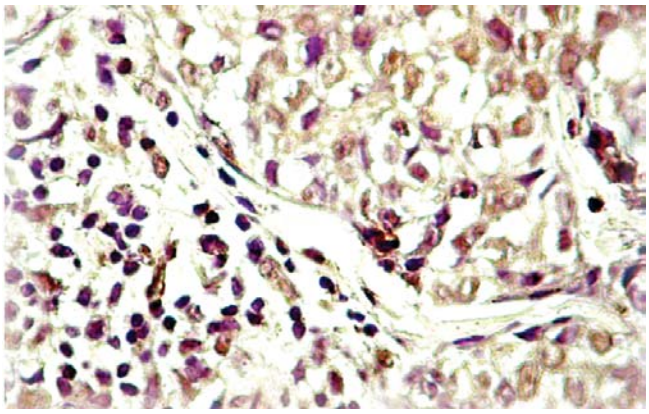

Figure 3. Medullary carcinoma. Moderate positive immunoreaction for tumor cells and negative for the surrounding lymphocytes and connective tissue (PSA, EPOS, DAB, x 200).

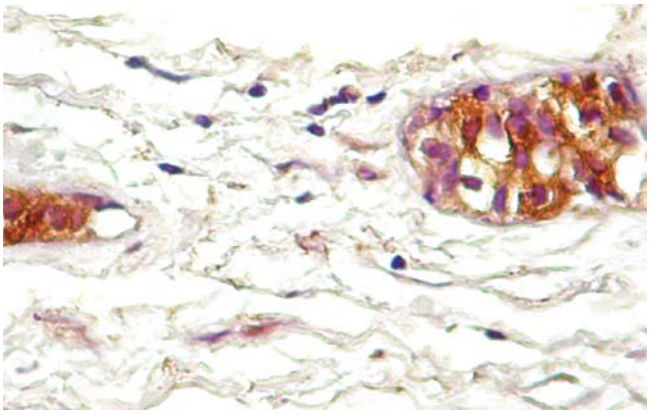

Figure 2. *In situ* and invasive lobular carcinoma. Strongly positive PSA immunoreaction (PSA, EPOS, DAB, x 200).

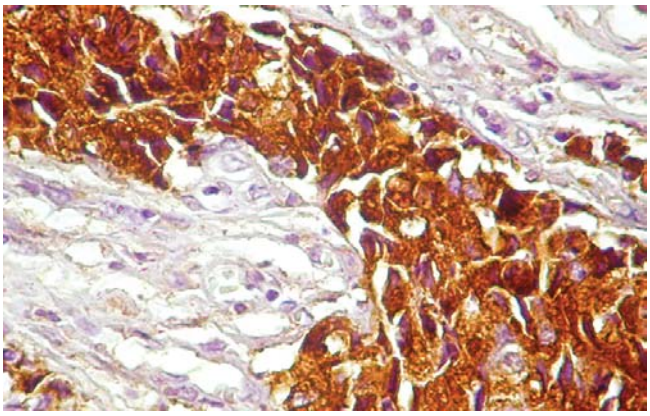

Figure 4. Ductal carcinoma. Focal, strong positive immunoreaction in malignant cells (PSA, EPOS, DAB, x 200).

Table 2. Correlations between PSA immunoexpression, hormone receptors (ER, PR) expression and HER2/neu overexpression in the breast carcinomas

Parameter	Total PSA positive cases	PSA Immunoexpression			
	+1,+2,+3	–	+1	+2	+3
ER status (37,7% ER+)					
– (33 cases)	16	15	3	9	6
+ (4 cases)	1	3	0	0	1
++ (8 cases)	7	1	1	4	2
+++ (8 cases)	6	2	1	4	1
PR status (41.5% PR+)					
– (31 cases)	16	15	1	6	9
+ (4 cases)	3	1	0	2	1
++ (10 cases)	8	2	0	5	3
+++ (8 cases)	5	3	2	3	0
HER2/neu					
– (0,+1) (40 cases)	26	14	3	14	9
+2 (9 cases)	3	6	0	1	2
+3 (4 cases)	3	1	0	1	2

and HER2/neu status in premenopausal females (p=0.03), 75% of the premenopausal patients with PSA positive staining being HER2/neu negative; no statistically significant association was found with the menopausal status of the patients at the time of diagnosis, nodal status, tumor size, differentiation grade, estrogen receptors, progesterone receptors status; no statistically correlation was found also with the HER2/neu status in all cases or postmenopausal patients (Tab. 3).

Due to relatively small number of cases investigated, further studies will be needed.

Discussion

In the present study we have investigated the potential link between the PSA immunoexpression in breast carcinomas and some clinical and histopathological parameters established to be prognostic markers of breast cancer: estrogen and progesterone receptors status, HER2/neu overexpression

Table 3. Association between immunohistochemical PSA-labeling and the established prognostic factors of breast carcinomas in the whole material of 53 cases: pre-and postmenopausal status, nodal status, histopathological type, differentiation grade, hormone receptors status and HER2/neu overexpression

Feature	PSA positive (+1,+2,+3)	PSA –	PSA (+1,+2,+3) (%)	p-value	Odds ratio	95% Confidence interval
Premenopausal	11	7	61.11 %			
Postmenopausal	21	14	60 %	0.9376	1.04	0.33 – 3.35
Nodal status						
Negative	17	10	62.9 %			
Positive	14	12	53.84 %	0.5007	1.45	0.5 – 4.3
ER/PR status						
Estrogen +	14	6	70 %			
Estrogen –	18	15	54.54 %	0.2648	1.94	0.6 – 6.3
Progesterone +	16	6	72.72 %			
Progesterone –	16	15	51.61 %	0.1215	2.50	0.7 – 8.08
HER 2/neu status (all cases)						
negative	26	14	65 %			
positive	6	7	46.15 %	0.2275	2.16	0.6 – 7.7
HER 2/neu status (premenopausal)						
negative	12	4	75 %			
positive	0	2	0	0.0339*	13.88	0.5 – 348.28
HER 2/neu status (postmenopausal)						
negative	15	9	62.5 %			
positive	6	5	54.54 %	0.6556	1.38	0.33 – 5.9
Histological grade						
G1 – G2	27	14	66%			
G3	5	4	55%	0.5	1.5	0.35 – 6.6
Histological type						
Ductal type**	21	18	53.8%			
Other types	12	2	85.7%	0.03*	0.19	0.04 – 0.9
Tumor size						
≤ 2	16	4	80 %			
> 2	19	14	57.57 %	0.09	2.94	0.8 – 10.7
Stage						
0, I	11	5	68.75 %			
II, III, IV	21	16	56.75 %	0.41	1.67	0.4 – 5.7

*statistical significance, **DCIS and carcinomas with squamous cell differentiation are included.

status, histological type of tumor, histological grade, tumor size, axillary nodal status, stage, menopausal status.

By immunohistochemistry we have found PSA positivity in 60.3% cases of breast cancers [9.38% were weak positive (+1), 50% were moderate positive (+2) and 40.62% were strong positive (+3)]. In the literature, the results concerning PSA immunoexpression in breast carcinomas are ambiguous. So, PSA has been found immunohistochemically in 28% cases [11], 100% [20], in 49% cases [21], 32% [17], and 22.3% [22]. By immunofluorometry, PSA was found in 30–40% of female breast cancers [4] or in 73% of tumor extracts [23]. By RT-PCR, 40% patients had PSA mRNA expression [14].

Performing a statistical analysis, we have found a significant correlation between the immunohistochemically determined PSA level and the histopathological type of the tumor. As we have shown in a previous study [24] the lobular, mucinous and medullary types expressed more frequently PSA. It is known that lobular, medullary and mucinous carcinomas are associated with a low percent of positive staining for HER2/neu and these types are correlated with a favorable prognostic. HOWARTH et al [25] also found PSA staining in mucinous carcinoma and YAO et al [22] noticed that 55% of breast carcinomas with neuroendocrine cell differentiation were PSA positive.

We have observed statistically significant correlation between the HER2/neu negativity in premenopausal women and PSA positivity (75% of cases were PSA positive and HER2 negative), that could suggest a subset of malignancies with better prognosis, which could respond to adjuvant therapy. It is well established that HER2/neu or c-erb B2 is an oncoprotein overexpressed in breast carcinomas with poor prognosis and actually, the HER2/neu immunohistochemical expression has a great value for the prediction of response to trastuzumab (Herceptin), a monoclonal antibody against this oncoprotein.

In breast carcinomas, ER and PR receptor status is assessed for the selection of patients which could benefit from endocrine therapy. Regarding the ER and PR immunohistochemical expression, in our current study 70% (14/20) were concomitant PSA and ER positive and 72.7% (16/22) were PR-positive cases. The correlation was not statistically significant. We have found no correlation between PSA expression, menopausal, lymph node status, histological grade and stage of the cancer. Concerning the tumor size, we did not find a statistical significance (our sample of cases was not enough large and indicates the need of further investigation), but 80% of the breast carcinomas with a tumor size ≤2 cm were PSA positive.

ALANEN et al [17] found a strong association of PSA immunoexpression with progesterone receptor positivity and histological degree, but no correlation between survival and positive PSA immunohistochemistry, even though the conventional prognostic factors such as mitotic index, nodal status and tumor size correlated well with the outcome of the disease. Other authors found PSA-positive more frequently in younger patients [10]. MITCHELL et al [26] found no significant association between PSA in nipple aspiration fluid and serum steroid hormones and did not show repeated cyclical variability during the ovarian cycle. It was assumed that the expression of PSA gene in the female breast may be different

in pre- and postmenopausal women because the expression is under control of androgens and progestins [27], and the PSA content of nipple aspirates is higher in premenopausal females [28].

The literature data concerning the role of PSA as a prognostic marker for breast carcinoma recurrence and survival are controversial. Some studies showed that PSA is a favorable prognostic indicator [20, 29–33], while others showed that PSA is not useful for prognostic evaluation of breast carcinomas [21, 34]. BLACK et al [29] have found a significant correlation of PSA concentrations in cytosolic extracts of breast carcinomas and the status of ER and PR and a negative correlation with age of patients. It was found [31, 35] an association between the presence of a polymorphism at the PSA promoter region and less aggressive forms of breast cancers that could be looked on as a favorable prognostic factor. HEYL et al [21] and MILLER et al [26] have found no significant correlations. BODEY et al [20] found no correlation with ER status and suggested that PSA significance in breast cancers may lay in the identification of a subset of estrogen receptor negative breast cancer patients with a good prognosis. YU et al [32, 33] found no correlation with histological type and grade, but reported an association with progesterone receptor status, incipient stage of the lesion, small tumors, reduced risk of relapse and longer survival, making PSA as a favorable prognostic indicator for women with breast cancer. Furthermore, it was concluded that PSA is a marker of the endogenous hormone balance between androgens, progesterone and estrogen and tumor PSA values might identify a subset of estrogen-negative tumors but which would respond to endocrine therapy [36, 33]. According to FOEKENS et al [37], high tumor PSA levels are associated with a reduced risk of relapse and death and better prognosis, whereas high PSA levels after hormonotherapy are associated with poor response to treatment.

In the present study, there was a statistical significance only between the histological type of the tumor and HER2/neu status in premenopausal females. We conclude that in our study PSA can not be considered as a valuable independent prognostic factor for breast carcinomas. The fact that the majorities of our PSA positive carcinomas were of small size, early stage, better and moderately differentiated, HER2/neu negative and 70% of ER/PR positive carcinomas expressed PSA suggests that it might be useful as a marker for a subset of breast cancers with better prognosis, which could respond to endocrine therapy, in correlation with other prognostic markers.

References

[1] JEMAL A, MURRAY T, WARD E, SAMUELS A, THUN MJ. Cancer Statistics, 2005. CA Cancer J Clin 2005; 55: 10–30.

[2] BORGONO CA, MICHAEL IP, DIAMANDIS EP. Human tissue kallikreins: physiologic role and applications in cancer. Mol Cancer Res 2004; 2(5): 257–280.

[3] CLEMENTS JA, WILLEMSEN NM, MYERS SA, DONG Y. The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. Crit Rev Clin Lab Sci 2004; 41(3): 265–312.

[4] DIAMANDIS EP, YOUSEF GM, LUO LY, MAGKLARA A, OBIEZU CV. The new human kallikrein gene family-implications in carcinogenesis. Trends Endocrinol Metab 2000; 11: 54–60.

[5] PARTIN AW, OESTERLING JE. The clinical usefulness of prostate specific antigen: update 1994. J Urol 1994; 152: 1358–1368.

[6] STAMEY TA, YANG N, HAY AR. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987; 317: 909–916.

[7] WANG MC, VALENZUELA LA, MURPHY GP, CHU TM. Purification of a human prostate specific antigen. Investigative Urology 1979; 17: 154–163.

[8] PAPOTTI M, PATIES C, PEVERI V. Immunocytochemical detection of prostate-specific antigen in skin adnexae and breast tissues and tumors. Basic Appl Histochem 1989; 33: 25–29.

[9] DIAMANDIS EP, YU H, SUTHERLAND DJ. Detection of prostate-specific antigen immunoreactivity in breast tumors. Breast Cancer Res Treat 1994; 32: 301–310.

[10] DIAMANDIS PE, YU H. Nonprostatic sources of prostate-specific antigen. Urol Clin North Am 1997; 24: 275–282.

[11] YU H, DIAMANDIS EP, LEVESQUE M. Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue. Breast Cancer Res Treat 1996; 40: 171–178.

[12] MONNE M, CROCE CM, YU H, DIAMANDIS EP. Molecular characterization of prostate-specific antigen messenger RNA expressed in breast tumors. Cancer Res 1994; 54: 6344–6347.

[13] MAGKLARA A, BROWN TJ, DIAMANDIS EP. Characterization of androgen receptor and nuclear receptor co-regulator expression in human breast cancer cell lines exhibiting differential regulation of kallikreins 2 and 3. Int J Cancer 2002; 100(5): 507–514.

[14] YANG QF, SAKURAI T, SHAN L, YU Z, YOSHIMURA G et al. Novel polymorphism of prostate-specific antigen (PSA) gene associated with PSA mRNA expression in breast cancer. J Hum Genet 2000; 45: 363–366.

[15] MAJUMDAR S, DIAMANDIS EP. The promoter and the enhancer region of the prostate-specific antigen of the KLK3 (prostate-specific antigen) gene is frequently mutated in breast tumors and in breast carcinoma cell lines. Br J Cancer 1999; 79: 1594–1602.

[16] ELSTON CW, ELLIS IO. Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19(5): 403–410.

[17] ALANEN KA, KNOPIO T, COLLAN YU, KRONQVIST P, JUNTTI L NEVALAINEN TJ. Immunohistochemical labeling for prostate-specific antigen in breast carcinomas. Breast Cancer Res Treat 1999; 56: 169–176.

[18] REINER A, NEUMEISTER B, SPONA J. Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. Cancer Res 1990; 50: 1057–1061.

[19] ELLIS I O, DOWSETT M, BARTLETT J, WALKER R, COOKE T et al. Recommendations for HER2 testing in the UK. J Clin Pathol 2000; 53: 890–892.

[20] BODEY B, BODEY B JR, KAISER HE. Immunocytochemical detection of prostate specific antigen expression in human breast carcinoma cells. Anticancer Res 1997; 17: 2577–2581.

[21] HEYL W, WOLFF JM, BIESTERFELD S. Immunohistochemical analysis of prostate-specific antigen does not correlate to other prognostic factors in breast cancer. Anticancer Res 1999; 19; 2563 -2565.

[22] YAO GY, ZHOU JL, ZHAO ZS. Biological characteristics of breast carcinomas with neuroendocrine cell differentiation. Chin Med J 2004; 117(10): 1536–1540.

[23] BLACK MH, MAGKLARA A, OBIEZU C, LEVESQUE MA, SUTHERLAND DJ et al. Expression of a prostate-associated protein, human glandular kallikrein (hK2) in breast tumors and in normal breast secretions. Br J Cancer 2000; 82(2): 361–367.

[24] NARITA D, RAICA M, ANGHEL A, SUCIU C, CIMPEAN AM. Immunohistochemical localization of prostate-specific antigen in benign and malignant breast conditions. Romanian J of Morphology and Embryology 2005; 46(1): 41–45.

[25] HOWARTH DJ, ARONSON IB, DIAMANDIS EP. Immunohistochemical localization of prostate-specific antigen in benign and malignant breast tissues. Br J Cancer 1997; 75: 1646–1651.

[26] MITCHELL G, SIBLEY PE, WILSON AP, SAUTER E, A'HERN R, EELES RA. Prostate-specific antigen in nipple aspiration fluid: menstrual cycle variability and correlation with serum prostate-specific antigen. Tumor Biol 2002; 23: 287–297.

[27] ZARGHAMI N, GRASS L, SAUTER ER, DIAMANDIS EP. Prostate-specific antigen levels in serum during the menstrual cycle. Clin Chem 1997; 43: 1862–1867.

[28] SAUTER ER, TICHANSKY DS, CHERVONEVA I, DIAMANDIS EP. Circulating testosterone and prostate-specific antigen in nipple aspirate fluid and tissue are associated with breast cancer. Environ Health Perspect 2002; 110(3): 241–246.

[29] BLACK MH, DIAMANDIS EP. The diagnostic and prognostic utility of prostate-specific antigen for diseases of the breast. Breast Cancer Res Treat 2000; 59: 1–14.

[30] MANNELLO F, GAZZANELLI G. Prostate-specific antigen (PSA/hK3): a further player in the field of breast cancer diagnostics? Breast Cancer Res 2001; 3: 238–243.

[31] YANG Q, NAKAMURA M, NAKAMURA Y, YOSHIMURA G, SUZUMA T et al. Correlation of prostate-specific antigen promoter polymorphism with clinicopathological characteristics in breast cancer. Anticancer Res 2002; 22(3): 1825–1828.

[32] YU H, GIAI M, DIAMANDIS EP. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 1995; 55: 2104–2110.

[33] YU H, LEVESQUE MS, CLARK GM, DIAMANDIS EP. Prognostic value of prostate-specific antigen for women with breast cancer. A large United States cohort study. Clin Cancer Res 1998; 4: 1489–1497.

[34] MILLER MK, UNGER PD, BLEIWEISS IJ. Immunohistochemical analysis of prostate specific antigen in breast cancer. Breast cancer Res Treat 2001; 68(2): 111–116.

[35] BHARAJ B, SCORILAS A, DIAMANDIS EP, GIAI M, LEVESQUE MA. Breast cancer prognostic significance of a single nucleotide polymorphism in the proximal androgen response element of the prostate specific antigen gene promoter. Breast Cancer Res Treat 2000; 61(2): 111–119.

[36] TANAKA A, KAMIAKITO T, TAKAYASHIKI N, SAKURAI S, SAITO K. Fibroblast growth factor 8 expression in breast carcinoma: associations with androgen receptor and prostate specific antigen expressions. Virchows Arch 2002; 441(4): 380–384.

[37] FOEKENS JA, DIAMANDIS EP, YU H, LOOK MP, MEIJER-VAN GELDER ME et al. Expression of prostate-specific antigen (PSA) correlates with poor response to tamoxifen therapy in recurrent breast cancer. Br J Cancer 1999; 79: 888–894.