

## ORIGINAL PAPER

# Prostate-specific antigen may serve as a pathological predictor in breast cancer

DIANA NARIȚA, A. ANGHEL, MARILENA MOTOC

*Department of Biochemistry,  
"Victor Babeș" University of Medicine and Pharmacy, Timisoara*

### Abstract

PSA (prostate-specific antigen), a serine protease with chymotrypsin-like activity is the most useful tumor marker for prostate cancer screening, diagnosis, prognosis and monitoring. The identification of PSA in normal and tumoral mammary gland was regarded as a curiosity, but the confirmation of PSA expression in the mammary gland by others teams of researchers and the identification of specific mRNA in tumors with PSA immunoexpression initiated new perspectives for studies. The aim of this study was to examine the prevalence of PSA in breast cancers and to evaluate the correlations between PSA expression and some clinicopathological markers. We analyzed the expression of PSA in series of consecutive breast carcinomas by immunohistochemistry and correlated the PSA expression with the histological type and grade, nodal and metastasis status, estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR) and HER2/neu expression. PSA expression was observed in 44.5% of breast cancers, particularly in lobular types of carcinoma ( $p < 0.0001$ ). In univariate analysis, the expression of PSA was statistically correlated with AR ( $p < 0.0001$ ), PR ( $p = 0.01$ ) and inversely correlated with HER2/neu overexpression ( $p = 0.008$ ) and G3 ( $p = 0.02$ ). PSA did not significantly correlate with ER expression, lymph node and metastasis status. In multivariate analysis, PR was a moderate predictor ( $p = 0.024$ ) but the lobular type ( $p = 0.000$ ), AR ( $p = 0.000$ ), HER2/neu ( $p = 0.002$ ) and G3 ( $p = 0.008$ ) were strong predictors for PSA immunoexpression.

**Keywords:** breast cancer, HER2/neu, prostate-specific antigen, steroid hormone receptors.

### Introduction

Breast cancer is a major concern worldwide and is responsible for one of the highest causes of death. The chance that breast cancer will be responsible for a woman's death is about 1 in 35 (about 3%). In 2007, about 40 460 women will die from breast cancer in the United States. In our country, 1% of women are diagnosed every year with breast cancer and 85% are diagnosed with advanced stages of disease. Breast cancer incidence rates showed an increase from year 2000, and about 2500 women die every year from breast cancer. Although there were progresses against breast cancer relieved by the decrease in death rates since 1990 especially for women between 40–49 ages because of earlier diagnose and better treatment, these progresses were insufficient and the rate of survival is still unsatisfactory [1].

Researchers are trying to discover new biomarkers that might be better for diagnose, prognosis, treatment monitoring and to develop new drugs that might work better against breast cancer. An example in this context is the identification of PSA in normal and tumoral mammary gland. PSA (hK3), 240 amino acids 33-kDa single-chain glycoprotein is one of the human kallikreins 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 the most useful tumor marker for prostate cancer screening, diagnosis, prognosis and monitoring [2].

The identification of extraprostatic PSA, initially by Papotti M *et al.* [3] was regarded as a curiosity, but the confirmation of PSA expression in the mammary gland by others teams of researchers [4–6] and the identification of specific mRNA in tumors with PSA immunoexpression [7] initiated new perspectives for studies. Breast PSA was found in 73% of tumor extracts, in milk of lactating women and in nipple aspirates [8–9].

Sauter ER *et al.* [9] demonstrated that PSA expression in nipple aspirate was inversely associated with the presence of breast cancer and PSA levels in nipple aspirates decreased in tumors with more advanced disease stage, larger tumor size, and nodal involvement. 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, PSA production can be induced by androgens, progesterone, mineral corticoids and glucocorticoids, but not estrogens [10].

It has been suggested that PSA may act as a growth factor or a regulator of growth factors and it could be a marker of endogenous hormone balance between androgens, progesterone and estrogens [11]. Elevated levels of PSA in breast tumors have been shown to be a favorable prognostic indicator in breast cancers, and it is raised the question if PSA could play a role in the hormonal therapeutic strategies, but up to now the potential significance of PSA in breast cancer is still not defined.

The aim of this study was to investigate the prevalence of PSA in consecutive series of breast

carcinomas using immunohistochemical techniques in tissue sections from paraffin-embedded material. In addition, the PSA status was matched with the following clinicopathological parameters: histological type and grade, axillary nodal involvement, metastasis status, ER, PR, AR and HER2/neu expression.

## ☐ Material and methods

We studied 236 surgical specimens from female patients with breast cancer, retrieved from the files of the pathology departments at university hospitals of Timisoara. Information about patient axillary nodal involvement and distance metastasis status were obtained from patient records. Ethical approval was obtained and all patients gave informed consent.

The samples were formalin-fixed and paraffin-embedded, according to the routine procedure. Four  $\mu\text{m}$  thick serial sections were mounted on precoated slides. The sections were dewaxed, rehydrated and rinsed in distilled water.

The pathological diagnosis and grading were done on Hematoxylin–Eosin stained samples and were based on the *Standard Recommendations* by WHO [12] and Elston and Ellis modified Scarff–Bloom–Richardson grading system [13].

Immunohistochemistry was performed to study the correlations between AR, PSA, ER, PR, HER2/neu expressions in human breast cancer using standardized automated procedures (Dako, Glostrup, Denmark).

In brief, the slides were dewaxed and rehydrated. Antigen retrieval by microwave treatment (ER, PR) or heating in a water bath (AR, HER2/neu) was performed before applying the antibodies.

The endogenous peroxidase was blocked using 3% hydrogen peroxide in deionized water. We used the following clones: polyclonal PSA, clone AR441 for AR, 1D5 for ER, PgR636 for PR, and HercepTest for HER2/neu, all from Dako.

After incubation with the primary antibody, incubation with a secondary, biotinylated antibody was performed. After washing, sections were incubated with streptavidine-peroxidase. Finally, the enzyme was visualized with 3,3'-diaminobenzidine (DAB) and the nuclei were stained with Lillie's modified Hematoxylin.

For semiquantitatively evaluation of AR, ER, and PR immunoreactivity we considered the percentage of positive nuclei; samples were considered positive when at least 10% of nuclei were immunoreactive, independently of the intensity of the immunostain.

For semiquantitatively evaluation of PSA, we considered cytoplasmic labeling in more than 10% neoplastic cells as the cut-off point for positivity, similar to standardized criteria used for the steroid hormone receptors [14].

For the determination of HER2 overexpression we evaluated only the membrane staining as presence and intensity. The score +2 was interpreted as weakly positive, +3 as strongly positive and the scores 0 and +1 were reported as negative [15].

Positive control included normal breast tissue surrounding the tumors for steroid receptors, cases of

prostate adenocarcinoma and prostate benign hyperplasia for AR and PSA and Dako positive slides for HER2/neu.

As negative controls, we used additional sections incubated without primary antibodies. At least two senior pathologists evaluated microscopic slides of all cases independently.

## ☐ Statistical analysis

Patients were dichotomized in PSA-positive and PSA-negative groups. Associations between eight variables (histological type and grade, ER, PR, AR, HER2/neu, nodal and metastasis status) and prostate-specific antigen in breast cancer were assessed using univariate logistic regression.

Odds Ratios (OR) and their 95% Confidence Intervals (CI) were calculated. The independent variables that were statistically significant were entered into multivariate logistic regression models.

Using backward elimination procedures, the most parsimonious multivariate logistic model was produced for predicting PSA-positive patients.

All *p* values were calculated based on two-sided statistical tests. Statistical analyses were performed using SPSS v.10 software.

## ☐ Results

All 236 cases included 121 invasive ductal carcinomas (51%), 44 invasive lobular carcinomas (18%), and 71 other types of carcinomas (30%) represented by 16 DCIS (6.7%), 14 LCIS (6%), 10 medullary (4%), three neuroendocrine (1.7%), five metaplastic (2%), six mucinous (2.5%), one case of adenoid cystic carcinoma (0.4%), 13 undifferentiated carcinomas (5.5%) and three metastases (1.3%) of mammary carcinoma (skin, lymph node and brain).

The well-differentiated (G1, *n* = 36), moderately differentiated (G2, *n* = 98) and poorly differentiated (G3, *n* = 69) carcinomas constituted 34%, 48.3% and 34% of cases, respectively. Lymph node invasion was present in 98 of these cases (41.5%) and in 17 cases (7.2%) distant metastases were also present.

In breast carcinomas, PSA was found in the cytoplasm of the tumor cells, with granular pattern and focal or diffuse distribution. It was absent from stromal cells. The intensity of PSA immunoreactivity was generally proportionate to the percentage of positive cells. The majority of cases contained normal breast tissue and benign lesions adjacent to the tumors. We noticed that, unlike in the prostate, in the normal mammary gland (Figure 1) only the acini were constantly positive, whereas the ducts were generally negative.

The benign lesions of apocrine metaplasia (Figure 2) adjacent to the carcinomas were constantly PSA positive. When summarizing, PSA was expressed in 35.5% of normal, respectively 42% of benign conditions adjacent to breast cancers. In breast malignancies, PSA was expressed in 105 of 236 of cases (44.5%), in 14/30 cases (46.67%) of noninvasive and in 91/206 (44.17%) of invasive breast cancers, respectively.

ER, PR and AR immunoreactivity was expressed in 105 (44.5%), 118(50%) and respectively 112(72%) of total cases.

Overexpression of HER2/neu was found in 72 out of 210 cases investigated for this parameter (34%). PSA was observed in 40.8% of axillary lymph node positive and in 45% of axillary lymph node negative tumors. Distant metastases displayed PSA immunoreactivity in nine (53%) of 17 cases (Table 1).

**Table 1 – Expression of PSA correlated to the histological grade, expression of AR, ER, PR, HER2/neu**

Parameter	PSA+	PSA-
G1 (36)	20 (55.5%)	16 (44.5%)
G2 (98)	50 (51%)	48 (49%)
G3 (69)	22 (31.88%)	47 (68.12%)
AR+ (170)	92 (54.12%)	78 (45.8%)
AR- (66)	13 (19.7%)	53 (80.3%)
ER+ (105)	51 (48.6%)	54 (51.4%)
ER- (131)	54 (41.2%)	77 (58.8%)
PR+ (118)	62 (52.5%)	56 (47.5%)
PR- (118)	43 (36.4%)	75 (63.6%)
HER2/neu+ (72)	23 (32%)	49 (68%)
HER2/neu- (138)	70 (50.7%)	68 (49.3%)
N+ (98)	40 (40.8%)	58 (59.2%)
N- (111)	50 (45%)	61 (55%)
M+ (17)	9 (53%)	8 (47%)
M- (192)	72 (37.5%)	120 (62.5%)

Distribution of PSA, steroid receptors and HER2/neu in ductal and lobular invasive carcinomas

Among the invasive ductal carcinomas (Figure 3) PSA was expressed in 35.5% of cases (43/121), but the most frequent histopathological type that expressed PSA was the lobular carcinoma (35/44, 79.5%) (Figure 4).

The percentage of PSA positive tumoral cells ranged from 10-70 with a mean of 41% in IDCs and from 10–85%, with a mean of 69.4% in ILCs. The intensity of PSA immunoreaction was also generally stronger for ILCs than for the IDCs.

The immunohistochemical expression of PSA, AR, ER, PR and HER2/neu in the consecutive series of 121 IDC and 44 ILC is represented in Table 2.

**Table 2 – Immunohistochemical results for PSA, AR, ER, PR and HER2/neu in consecutive series of 121 ductal (IDC) and 44 lobular (ILC) invasive carcinomas**

	PSA+	AR+	ER+	PR+	HER2/neu+
<b>IDC</b> (121)	43 (35.5%)	106 (87.6%)	64 (53%)	68 (56.2%)	45 (37%)
<b>ILC</b> (44)	35 (79.5%)	32 (72.7%)	16 (36.36%)	23 (52.3%)	15 (34%)

The lobular invasive carcinomas displayed a better concordance between the PSA and AR immunoreaction than the invasive ductal carcinoma (Figure 5).

G1 tumors expressed PSA (55.5%) somewhat more frequently than G2 (51%) and G3 (46%) tumors. In comparison with the steroid receptors expression, PSA immunoreactivity was detected in 54.12% of AR+ cases whereas 80% of AR-negative cases were also PSA negative; 52.5% of PR respectively 48% of ER positive cases expressed PSA.

Sixty-eight percents of HER2/neu positive cases were PSA negative in comparison with 32% cases that expressed simultaneous PSA and HER2/neu. The expression of PSA correlated to the histological grade and to the expression of AR, ER, PR, and HER2/neu is represented in Table 1.

LCIS expressed PSA in 57.14% of cases; AR, PR, ER and HER2/neu were expressed in 64.3%, 71%, 57.14% and 21.4%, respectively.

From the DCIS cases, 18.75% were PSA positive especially the apocrine and solid types; 90.9% were AR positive, and ER/PR were expressed in 63.63% respectively 72.73% of cases; 36.36% of DCIS overexpressed HER2/neu.

In general, the areas of DCIS and LCIS associated to the invasive carcinomas showed a similar behavior with the invasive component of the tumor, but the proportion of PSA positive tumor cells was usually lower than that observed in invasive areas. All the areas with apocrine differentiations that we found in nine carcinomas showed PSA and AR positivity and did not express ER/PR or HER2/neu.

The medullary carcinomas expressed PSA in 6/10 of cases, 8/10 of these tumors were also AR positive, while ER, PR and HER2/neu were not expressed. All five metaplastic carcinomas did not show PSA or steroid receptors expression, but were HER2/neu positive (3+).

The adenoid cystic carcinoma presented a weak PSA positive expression for tumoral cells, while the secretion from the glandular lumina was strongly positive. The steroid receptors and HER2/neu were negative for this type of carcinoma. All six mucinous carcinomas identified were ER/PR positive, three (50%) were AR and PSA positive and one (16.6%) overexpressed HER2/neu.

The three neuroendocrine carcinomas were identified in postmenopausal women and immunohistochemically confirmed by positive reactions for chromogranin A and synaptophysin. They showed PSA positive reaction in one case (33.33%), ER/PR expression in two cases (66.66%) and no expression for AR and HER2/neu.

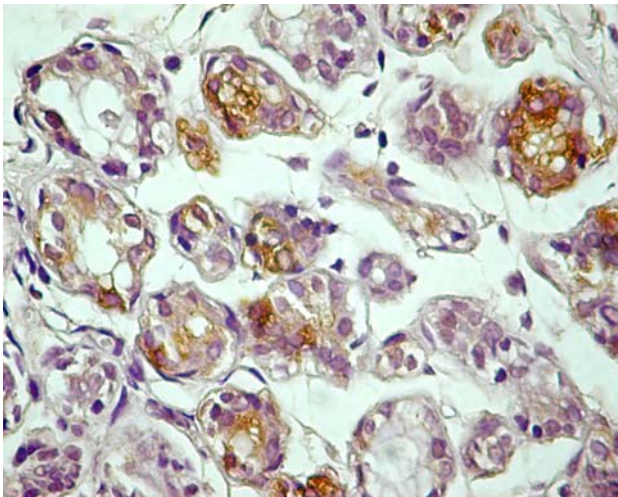
In addition, the metastasis investigated displayed PSA positivity. The brain and lymph node metastasis showed weak PSA immunoreaction, whereas the skin metastasis expressed strongly PSA. In these cases of mammary carcinoma metastases, AR was positive but both ER and PR were negative.

### Statistical analysis

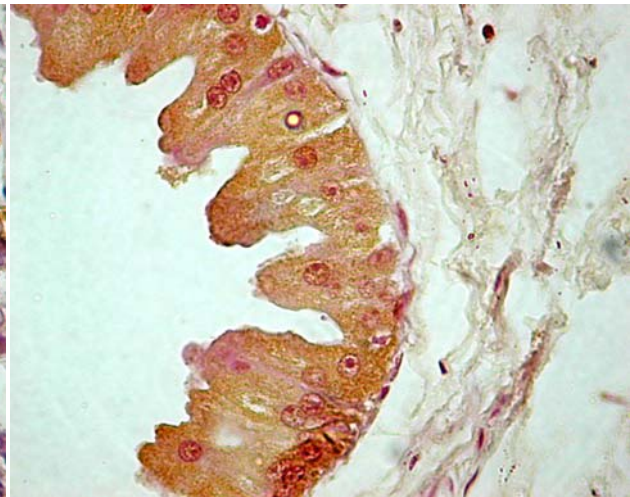
The results of univariate logistic regression are presented in Table 3 and showed a high-significant correlation between PSA and:

1. AR immunoreaction (p<0001);
2. The lobular type of carcinoma (p<0.0001);
3. PR expression (p = 0.01);
4. Inverse correlation between PSA positive cases, HER2 overexpression (p = 0.006) and G3 (p = 0.02).

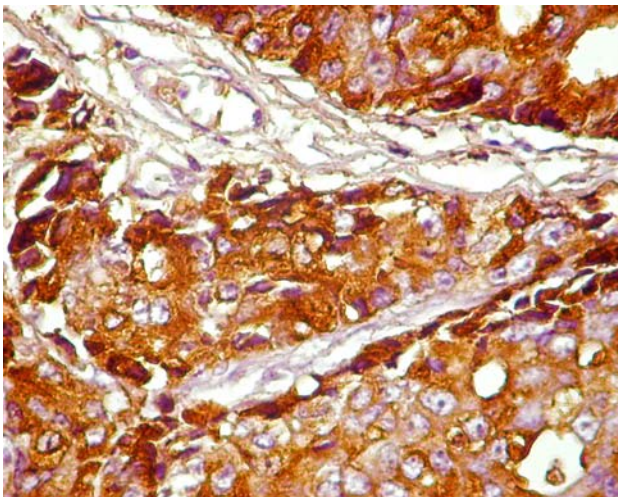
None of other parameters considered, including ER expression, nodal and metastasis status showed any significant correlation with PSA immunoreactivity.



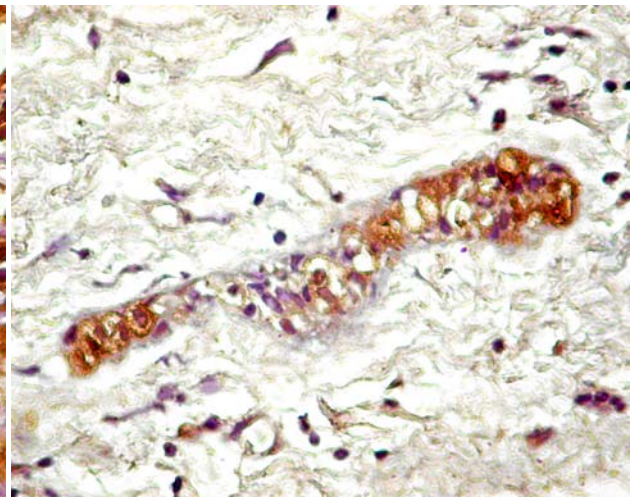
**Figure 1** – Normal breast tissue showing positive, but focally distributed cytoplasmic expression of PSA. Polyclonal DAKO, Working system – EnVision (×400)



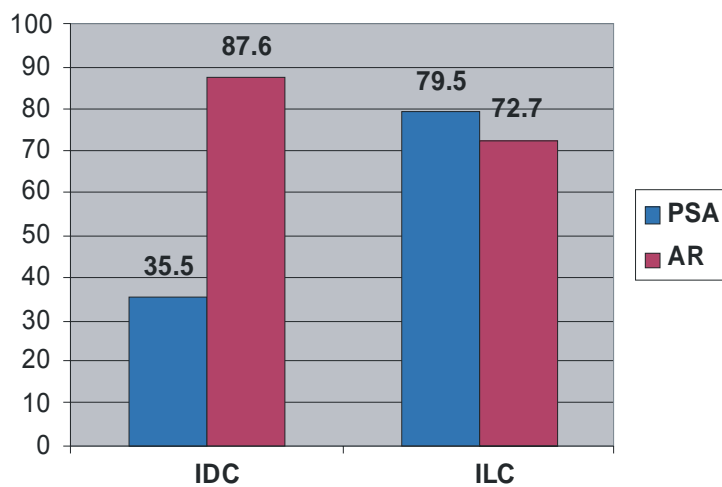
**Figure 2** – Apocrine metaplasia, intense positive immunoreaction for PSA with a granular cytoplasmic pattern. Polyclonal DAKO, Working system – EnVision (×400)



**Figure 3** – Positive immunoreaction for PSA in moderately differentiated invasive ductal carcinoma. Polyclonal DAKO, Working system – EnVision (×400)



**Figure 4** – Intense expression of PSA in invasive lobular carcinoma with typical Indian file pattern of growth. Polyclonal DAKO, Working system – EnVision (×400)



**Figure 5** – Comparison of PSA and AR expression in 121 ductal (IDC) and 44 lobular (ILC) invasive carcinomas

**Table 3 – Results of univariate logistic regression**

Variables	p value	OR	95% CI for OR
Histological type	0.00		
<i>IDC + DCIS</i>		1 (ref)	
<i>ILC + LCIS</i>	0.00*	5.31	2.68–10.54
<i>Other types</i>	0.92	0.96	0.46–2.00
Histological grade	0.03		
<i>G1</i>		1 (ref)	
<i>G2</i>	0.64	0.83	0.39–1.79
<i>G3</i>	0.02*	0.38	0.17–0.88
<i>ER</i>	0.26	1.35	0.80–2.26
<i>PR</i>	0.01*	1.93	1.15–3.25
<i>AR</i>	0.000*	4.80	2.44–9.47
<i>HER2/neu</i>	0.006*	0.45	0.26–0.79
<i>N</i>	0.25	0.74	0.44–1.24
<i>M</i>	0.46	1.43	0.56–3.65

\*p<0.05 statistically significant.

The independent variables that were statistically significant (p<0.25) were entered into multivariate logistic regression models for predicting PSA-positive patients. Multivariate logistic regression data are presented in Table 4.

**Table 4 – Results of multivariate logistic regression**

Variables	df	p value	OR	95% C.I. for OR
Type	2	0.000		
<i>IDC + DCIS</i>			1 (ref)	
<i>ILC + LCIS</i>	1	0.000	13.73	4.84–39.006
<i>Other types</i>	1	0.28	1.76	0.63–4.92
G	2	0.026		
<i>G1</i>			1 (ref)	
<i>G2</i>	1	0.19	0.56	0.23–1.36
<i>G3</i>	1	0.008	0.22	0.07–0.66
<i>PR</i>	1	0.024	2.24	1.11–4.52
<i>AR</i>	1	0.000	5.82	2.43–13.93
<i>HER</i>	1	0.002	0.32	0.15–0.65

p<0.05 statistically significant.

Nagelkerke's  $R^2$  value, an estimate of variations in outcome variables explained by a logistic regression model was calculated. Its value is 0.40, indicating that 40% of the variance in PSA-positive patients was explained by the logistic regression model. Model chi-square test value was 72.24 with seven degrees of freedom (p<0.001). Interactions between covariates were not significant and were not included in the final model. The overall success of the model in predicting PSA-positive cases was 74.8% (for a cut value of 0.5). PR, the lobular type and AR were positively associated with the presence of PSA. G3 and HER2/neu were negatively associated with the presence of PSA. PR was a moderate predictor and the lobular type, G3, AR and HER2/neu were strong predictors for PSA-positive patients.

## Discussion

Several previous studies have demonstrated the expression of PSA in normal or pathological breast tissue, but the functional role and the clinical significance of extraprostatic PSA were not defined.

PSA, like other proteases can digest insulin-like growth factor-binding proteins, leading to the release of these growth factors [16].

Similarly, digestion of the basal membrane and extracellular matrix proteins might facilitate cell migration and invasion. On the other hand, PSA proteolytically cleaves parathyroid hormone related protein (PTHrP) which stimulates breast cancer cell proliferation. A role of PTHrP in breast cancer metastasis has been suggested because PTHrP was detected in most osseous metastases [17–18].

However, PSA inhibit the endothelial cell response to angiogenic stimulation by fibroblast growth factor-2 and vascular endothelial growth factor, suggesting that PSA might be an endogenous anti-angiogenic compound [19].

Moreover, Lai LC *et al.* [20] reported that PSA stimulates the conversion of the potent estradiol to the less potent estrone, inhibiting the growth of certain breast cancer cell lines *in vitro*. This might contribute to the association between PSA and good prognosis observed in some studies [7, 21–25]. In male breast cancer PSA was identified in six (23%) cases of 26 tumors investigated and was not correlated with AR, ER or PR expression. There was no significant association of PSA with positive lymph node status, overall follow-up, and neither was able to predict the clinical behavior in these patients [26]. In male breast, Gatalica Z *et al.* [27] have reported focal strong PSA expression in normal and hyperplastic ductal epithelium in five of 18 cases of gynecomastia.

Previous studies showed that PSA is expressed in a considerable proportion of female breast cancers, ranging from 9.3% to 49% of breast cancers [5, 11, 24, 28–34]. Yu *et al.* [6] detected PSA in 33% of normal, respectively 65% of benign cases. In the present study, PSA was detected in 44.5% of breast cancers, 35.5% of normal tissues and 42% of benign lesions adjacent to breast cancers. In the present study, PSA was expressed in both invasive (91/206, 44.17%) or noninvasive (14/30, 46.67%) breast carcinomas and the PSA expression was correlated with the histopathological type of breast carcinoma. The lobular carcinomas expressed more frequently PSA (79.5%) than ductal carcinoma (51%).

In accordance with other studies [35] we found a strong AR positivity in the majority of lobular carcinomas. We had only a few cases of mucinous, medullary and neuroendocrine carcinomas, but like others authors [36, 32] we found a positive PSA staining in these histological types. We immunohistochemically investigated only three breast carcinoma metastases, but in accordance with the findings of Bayer–Garner and colleagues these three cases displayed AR and loss of ER/PR expression [37].

Moreover, these metastases were PSA positive. In agree with Alanen KA *et al.* [29] most PSA positive carcinomas (51–55%) in the current study were associated with a low or intermediate histological grade (G1, G2).

In concordance with other authors [36] we found PSA in benign lesions adjacent to breast cancer

especially in apocrine foci. The epithelium of the cystic dilated ducts within fibrocystic disease of the breast was negative for PSA.

In the current study, all cases with apocrine differentiation showed PSA and AR positivity, whereas both ER and PR were negative. Our findings add to the literature suggesting that the apocrine feature is associated with the presence of AR immunopositivity and loss of ER/PR expression in DCIS [38–40]. These results correspond with the studies that detected PSA in breast fluid of apocrine cysts [41–42].

One notable finding in the current study was yielded by the comparison of AR and PSA expression. Fifty-four percents of PSA-positive breast carcinomas were simultaneous AR-positive, while 80% of PSA-negative carcinomas did not express AR.

Hall RE *et al.* [43] reported an even stronger association of AR and PSA expression, 98% of breast carcinoma that expressed PSA being AR positive. It has been shown that androgens exhibit growth-inhibitory and apoptotic effects in some, but not in all breast cancer cell lines, suggesting that testosterone may serve as a natural, endogenous protector of the breast. These differences between cell lines appear to be due primarily to the variations in concentrations of specific coregulatory proteins at the receptor level [44–46].

DNA sequencing confirmed that no mutations were present in the coding region of PSA gene in breast tumors, but multiple polymorphisms were detected in the promoter and enhancer region [47–48]. These polymorphisms in proximal ARE (androgen response element), particularly the G/A – A/AA and A/A – AA/AA genotypes were associated with increased transcriptional activity of PSA, and less aggressive forms of breast cancer [25]. In this context, it might be assumed that PSA could be a pathway through which the protective effects of AR operates, at least for a subset of breast carcinomas, but further studies will be needed to verify this supposition.

The literature data concerning the correlation between the expression of PSA and ER/PR showed variable results. There are studies that revealed a significant correlation between the expression of PSA and both ER and PR [6], some studies showed a correlation only between the expression of PSA and PR [29, 49], while other studies reported no correlations [21, 30, 50].

Although in our previous study [34] performed on a smaller sample size we did not find a statistically significant correlation between PSA and ER/PR status, the current study agrees with the studies that reported a correlation only between the expression of PSA and PR [29, 49].

Another noteworthy finding of our study resulted from the comparison of PSA and HER2/neu expression. Sixty-eight percent of carcinoma with HER2/neu overexpression was PSA negative and the correlation was statistically significant. Furthermore, we could suggest a model for predicting PSA-positive cases. In accordance with this model, the presence of AR and PR expression and the lobular type are predictors for PSA-positivity, while HER2/neu overexpression and the

poorly differentiated (G3) carcinomas are highly associated with PSA-negativity.

Previous studies regarding the role of PSA as a prognostic marker for breast carcinoma recurrence and survival reported different results. Some studies showed that PSA is a favorable prognostic indicator for breast cancers [6, 23–26], while others showed that PSA is not useful for prognostic evaluation of breast carcinomas [29, 30–31].

## ☐ Conclusions

Although from this study we could not evaluate the prognostic value of PSA in breast malignancies, PSA was significantly associated with some pathological parameters that are known to be associated with a better prognosis. PSA was directly correlated with the expression of AR, PR and inversely correlated with HER2/neu overexpression and G3. Our findings could partly support the studies that reported PSA as a favorable prognostic indicator; on the other hand, the detection of PSA in breast cancer metastases was in contrast with these results. Further clinical study should be undertaken to investigate the correlation between the PSA expression identified in our present study and the clinical behavior, particularly the prognosis and treatment response of individual breast cancers.

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**Corresponding author**

Diana Narița, MD, PhD, Department of Biochemistry, „Victor Babeș” University of Medicine and Pharmacy Timișoara, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40256–204 476; +40745–471 711, Fax +40256–220 479, E-mail: diana\_narita@yahoo.com

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