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HSP27 diagnostic utility in the fine needle aspirate of breast. Correlation with progesterone and estrogen receptors

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Fine needle aspiration (FNA) is routine diagnostic tool in breast tumors assessment. In some cases, however, limitations of this method do not permit an unequivocal diagnosis. In these, suspected, cases immunocytochemical evaluation of selected biological markers may be of help. The aim of the study was assessment of HSP27 value in diagnosis and discrimination of benign and malignant breast lesions. HSP27 expression was examined by immunocytochemistry in fine needle aspiration smears assessed to C2-C5 categories. In C5 subgroup HSP27 expression was correlated with ER, PR content. Statistically significant differences in HSP27 expression between subsets C2/C5 and C3/C5 were found (p=0.028 and p=0,04, respectively); the differences between C3/C4 categories were not significant. Expression of HSP27 protein in FNA smears can be additional factor, which helps to differentiate benign, and malignant breast lesions, however it is not useful for discrimination of cytological, suspicious lesions.

Keywords: breast cancer, cytology, HSP27, fine needle aspiration

Triple assessment in diagnosis of breast lesions is based on correlation of clinical, radiological and cytological data [1, 2]. Cytological smears obtained by fine needle aspiration (FNA) are prompt, sensitive and specific method of material acquiring for non-operative diagnosis [3, 4].

According to National Health Service Breast Screening Programme (NHSBSP) and National Cancer Institute (NCI) guidelines FNA smears are classified into five categories [4, 5].

C1 – inadequate – scanty or acellular aspirate. This group encompasses also preparation of poor quality, inappropriate for assessment;

C2 – benign;

C3 – atypia probably benign – cells with benign characteristics with some features of atypia;

C4 – suspicious of malignancy;

C5 – malignant.

Categories C3 and C4 include cases in which unequivocal diagnostic decision, whether suspicious change is either benign or malignant, cannot be made [4, 6]. Recent papers on transcriptional profiling, tissue microarray as well as standard immunocytochemistry show utility of some molecular markers for fine needle aspirate assessment and its possible translation to clinical practice [7, 8, 9]. So far diagnostic value has been proven for estrogen and progesterone receptors as well as HER2/neu [9, 10]. Immunocytochemical evaluation of other proteins: bcl-2, p53 or cyclin D1 yielded contradictory results [11, 12, 13].

Significant differences observed in expression level of stress genes, between normal and malignant cells, induced interest in possible use of stress protein (heat shock proteins, HSPs) as diagnostic and/or prognostic markers [14, 15].

HSP27 (low-molecular/small heat shock protein) is a typical stress protein. Diverse physical or pathological stressors, such as: heat shock, γ radiation, UV, ischemia, virus or bacterial infections induce its expression [16, 17]. Due to the presence of estrogen responsive element (ERE) in the promoter region HSP27 can be also activated by estrogen. Ciocca et al. [18] have found that *hsp*27 gene is active in physiological conditions in tissue/organ-specific manner. The highest HSP27 level was observed in oesophageal and endometrial epithelium, as well as in skin. In normal breast epithelium HSP27 expression level was low.

In vitro experimental studies have shown that HSP27 is a potent anti-apoptotic factor, blocking different stages of ap-

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optosis induced by physical and chemical agents [19]. HSP27 hampers apoptosis by blocking mitochondrial cytochrome c outflow or inhibiting activation of procaspases to caspases [20]. Resistance to common chemotherapeutic agents observed in HSP27-overexpressing cells is also a result of its capability to block apoptosis.

O'Neill et al. [21] demonstrated increase in number of HSP27-positive cells during progression from normal throughout benign, nonproliferative disease (apocrine metaplasia, blunt-duct adenosis, fibroadenoma and papilloma), hyperplasia to cancer *in situ*. Shift from carcinoma *in situ* to invasive cancer was not accompanied by further HSP27 expression enhancement, mean optical density of staining was insignificantly different in DCIS and invasive cancer. Authors of this study suggest that HSP27 overexpression occurs early in breast tumorigenesis, even at the stage of the initiation.

Despite the great number of experimental data and obvious presence of HSP27 in advanced cancer, its prognostic value in breast tumors is often contradictory [15].

The aim of this work was verification of HSP27 utility for discrimination between benign and malignant breast cytological samples (as described by Keeling et al., [22]). Additionally, results of HSP27 immunocytochemical reaction were correlated to estrogen and progesterone receptors status.

Patients and methods

We examined 90 cytological smears obtained by FNA from patients diagnosed with benign, suspicious or malignant breast lesion in Pathology Department, MSC Memorial Cancer Center and Institute of Oncology, Gliwice Branch. Samples were fixed in mixture of ether: ethyl alcohol 96% (1:1) and stained routinely with hematoxylin-eosin. Cases were classified into five categories: 30 were classified as C2, 16 as C3, 11 as C4 and 33 as C5. In all cases immunocytochemical detection of HSP27 was performed. In the group C5 immediately after cytological examination expression of progesterone and estrogen receptors was analyzed. In 19 cases (8 classified as C3 and 11 cases classified as C4) HSP27 expression was verified even on operative material. Surgeries were performed in Surgery Department. Postoperative samples were routinely fixed in 10% formalin and embedded in paraffin.

Table I. Comparison of HSP27 frequency in FNA cytological categories.

	Frequency of HSP27-positive cells					
	negative	positive				
	0%	<=25%	>25% <=50%	>50%<=75%	>75%	**Total**
C2	13 (43,3%)	8 (26,7)	6 (20%)	1 (3,3%)	2 (6,7%)	**30**
C3	7 (43,8%)	5 (31,3%)	3 (18,8%)	1 (6,3%)	0	**16**
C4	3 (27,3%)	2 (18,2%)	2 (18,2%)	2 (18,2%)	2 (18,2%)	**11**
C5	5 (15,2%)	6 (18,2%)	12 (36,4%)	3 (9,1%)	7 (21,2%)	**33**
Total	**28**	**21**	**23**	**7**	**11**	**90**

For detection of HSP27 by immunocyto- and immunohistochemistry, one-hour incubation with mouse monoclonal NCL-HSP27 antibody (clone 2B4, Novocastra) was applied. Incubation (30 min) with anti-human estrogen receptor α mouse antibody (clone 1D5, M7047, DAKO) or mouse anti-human progesterone receptor antibody (clone PgR 636, M3569, DAKO) were used for visualization of estrogen and progesterone receptor. Other steps of immunoreaction were performed according to DAKO manufacturer instructions with DAB as chromogen.

Immunoreaction of HSP27 was always localized in cytoplasm, whereas immunoreaction of ER and PR receptors was observed in cell nuclei.

Evaluation of HSP27 immunoreaction was based on number of stained glandular cells and assessed to five categories: negative; <=25% of cells stained; >25% <=50%; >50%<=75% and >75%. Semiquantitative evaluation of ER and PR receptors was based on number and intensity of positive nuclei (negative, +, ++, +++).

X_2 and Fisher exact tests were used for statistical analysis.

Results

HSP27 positive immunoreaction was heterogeneous, detected in both benign and malignant lesions with various staining intensity. It was very rare in normal glandular cells and always negative in myoepithelial and stromal cells.

In benign tumors positive reaction was visible in glandular cells, displaying features of displasia or atypia. Majority of fibroadenomas showed negative or very weak HSP27 staining, whereas all cases of apocrine metaplasia displayed moderate or strong immunoreaction.

In cancerous lesions frequency of HSP27 staining was assessed only in epithelial cells showing cytological features of malignancy. In malignant tumors HSP27-positivity varied, from negative (0%) to more than 75% cells positive.

Comparison of HSP27 frequency in various cytological categories is shown in table I.

In C2 and C3 group proportion of HSP27-negative cells was the highest (43,3% and 43,8%, respectively). The differences in number of HSP27 positive and negative cases between C2/C5 ($p = 0{,}028$) and C3/C5 ($p = 0{,}040$) groups were statistically significant. Although frequency of HSP27-negative cells was higher in C3 (43,8%) than C4 (27,3%) group this difference was not statistically significant.

Histological diagnosis and HSP27 immunohistochemical evaluation was done in 18 (8/16 C3 and 10/11 C4) cases. In 10 cases results of HSP27 analysis in FNA and histopathological samples were concordant, in the 5 of the remaining cases HSP27 immunohistochemistry was assessed as stronger than immunocytochemistry.

Expression of estrogen (ER) and progesterone (PR) receptors was determined in 32 malignant lesions. Relationship between HSP27 and estrogen and progesterone receptor status is given in tables II and III.

Table II. Comparison between HSP27 expression and ER.

	Number of cases (%)
ER+/HSP27+	19 (59,4%)
ER+/HSP27-	1 (3,1%)
ER-/HSP27-	4 (12,5%)
ER-/HSP27+	8 (25%)

Table III. Comparison between HSP27 expression and PR.

	Number of cases (%)
PR+/HSP27+	16 (50%)
PR+/HSP27-	1 (3,1%)
PR-/HSP27-	4 (12,5%)
PR-/HSP27+	11 (34,4%)

The correlation between HSP27 and ER was statistically significant (p=0,03), on the contrary to relationship between HSP27 and PR (p=0,106), which does not show statistical significance.

Discussion

NCI and NHSBSP recommended five categories for the classification of breast cytological aspirates [1, 2, 23]. Both classification systems assume the need for two separate categories: atypical, probably benign and suspicious, probably malignant. This categorization is still matter of debate, with opinions pro [4] and contra [5]. Kanhoush et al. [5] proposed classification into three groups of lesion: benign, malignant and suspicious (equivocal) to describe inconclusive diagnoses. This judgment is based on histological results, which show some proportion of benign lesions in C4 cytological group. In our study benign lesions presented 73,7% of C3 cases and 8,3% of C4 cases, 48% of C3 or C4 patients were subjected to surgical intervention and the removed lesion didn't show histological malignant features.

Moyes and Dunne [24] tested utility of some cytomorphological criteria for atypical and suspicious cases discrimination. They demonstrated the predictive value, with about 80% specificity, of eccentrically placed epithelial cell nuclei and coarse nuclear chromatin for predicting malignancy. The incidence of myoepithelial cells within epithelial groups was not an indicator of a benign diagnosis.

The lack of definite criteria of malignancy shows need for other, than morphological, markers. HSP27, due to its overexpression in early stages of breast carcinogenesis and estrogen responsiveness, is considered as putative preneoplastic and neoplastic marker. Despite the equivocal results of immunohistochemical analysis its prognostic value is questionable. Diagnostic, prognostic and/or predictive value of HSP27 protein expression is the subject of many investigations, especially in the breast. Low expression of HSP27 was demonstrated in normal breast tissue and fibroadenoma, high in apocrine metaplasia, variable in cancer [18, 25]. Numerous studies have correlated HSP27 alterations with clinical outcome in advanced breast cancer[25-32]. These papers show mostly correlation of HSP27 with ER content [25, 26, 27, 28, 31]. However results concerning the influence of HSP27 on patients' outcome are discordant. Thor et al. [25] observed HSP27 overexpression more frequently in node-positive patients and showed HSP27 correlation with shorter disease-free period but not overall survival. HSP27 was shown as an independent prognostic factor only in univariate analysis. In agreement with these results Thanner et al. [32] in long follow-up (mean 177 months) demonstrated shorter overall survival and survival after first recurrence in HSP27-positive cases. On the contrary, Love and King [27] found that HSP27 positively predicted prolonged survival to the relapse. In other studies there was no relationship between HSP27 expression and disease-free period or overall survival [28, 31]. However, Oesterreich et al. [28] in the subgroup of patients characterized by ER status and use of adjuvant treatment (ER-positive/untreated patients) found that high HSP27 level correlated with shorter disease-free survival.

HSP27 was also studied as a possible discrimination (benign vs. malignant) marker in cytological specimens [22]. HSP27 low expression in benign lesions and high in malignant lesions were statistically different and enabled distinction between C3 and C4 group. In our research difference between C3 and C4 groups was much weaker than in C2/C5 groups and did not reach statistical significance. The relationship between HSP27 and estrogen receptor status were only partially concordant and did not enhance power of HSP27 utility as a discrimination marker. On this basis we can assume, that expression of HSP27 protein in FNA smears can be additional factor, which helps to differentiate benign, and malignant breast lesions, however it is not useful for discrimination of cytological, suspicious lesions. Therefore we propose continuation of research on larger group of patients to obtain clear conclusions.

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