ONCOLOGY

Soluble Fragment of Her2/neu Receptor in the Serum of Patients with Breast Cancer with Different Levels of this Protein Expression in the Tumor

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Serum level of soluble HER2/neu in patients with tumors characterized by high expression of this protein (2+/3+ according to immunohistochemical analysis) was significantly higher than in patients with low expression of HER2/neu and in women with benign diseases of the mammary glands. The level of HER2/neu in the serum decreased after removal of the primary tumor in the majority of patients.

Key Words: *soluble HER2/neu; serum; breast cancer*

HER2/neu protein, a product of c-erbB-2 oncogene, belongs to the family of four transmembrane receptor tyrosine kinases, called the epidermal growth factor receptor (EGFR) family (by the best known representative of the family). The main characteristics of receptor tyrosine kinases are transmembrane location and obligatory interaction with an appropriate ligand for the realization of kinase activity and subsequent biological effects (mitogenic, antiapoptotic, *etc.*). EGFR family receptors form homo- and heterodimers upon activation. In many cases, structures containing type 2 receptor HER2/neu, which has no specific ligands, are most active [1,4,10]. Hence, HER2/neu is a unique dispatcher receptor which does not interact with any of the known growth factors activating related receptors, but is a key component in transmission of mitogenic signals of all EGF-like peptides.

According to some authors, hyperexpression or amplification of c-erbB-2 gene has an unfavorable impact for relapse-free survival of patients with breast cancer (BC) without metastases in the lymph nodes, whereas other scientists find no significant relationship between these characteristics [12,13]. There are reports that tumors with amplified HER2/neu gene poorly react to endocrine therapy, but are sensitive to subsequent chemotherapy [6,12]. It is now accepted that patients with HER2/neu-positive tumors should be recommended more intense chemotherapy protocols than patients with tumors without high expression of this oncogene [14]. However, the development of Herceptin drug (humanized antibodies to HER2/neu) raised new interest to the studies of HER2/neu expression, because of the need in defining the individual parameters of treatment [3,9].

A universally acknowledged and most adequate method for evaluating the sensitivity to Herceptin is immunohistochemical staining of tumor tissues for HER2/neu protein (p185) with subsequent evaluation of c-erbB-2 gene amplification by fluorescent *in situ* hybridization (FISH) [15]. On the other hand, one of the main modern trends in re-

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search of biomarkers is the development of less invasive serum tests, which can replace (or supplement) studies at the tissue level. Since HER2/neu consists of intracellular and extracellular parts, dimerization can be accompanied by degradation of the receptor molecule and the release of its external domain into extracellular medium [11]. This feature provided the basis for the development of enzyme immunoassay systems for measurements of soluble HER2/neu in the serum or plasma; preliminary results were published demonstrating good prospects of this trend of research [2,5], though the need for more data and definition of clear-cut quantitative criteria is admitted [7,8].

We compared the levels of soluble HER2/neu in the sera of BC patients with different levels of expression of this protein in tumors.

MATERIALS AND METHODS

The study was carried out in 59 patients with stages I-III BC and 15 women with benign changes in the mammary glands (12 fibroadenomas, 1 benign leaflet tumor, and 2 cases with fibrocystic disease). Clinical diagnosis of mammary tumor was confirmed by the results of morphological analysis of the removed tumor in all patients.

Serum content of soluble HER2/neu was measured by sandwich ELISA using standard HER2/neu ELISA kit (Dako Cytomation). The serum was tested before treatment in all patients and repeatedly 8-38 days after surgery in 10 patients.

The expression of HER2/neu protein in tumor samples from all BC patients was evaluated on paraffin sections by the semiquantitative immunohistochemical method using HercepTest^{R} (Dako Corp).

The data were compared and the relationships between parameters was evaluated using Student's *t* test, nonparametric Wilcoxon test for paired comparison and Mann—Whitney test, Kendall (τ) and Spearman (R) rank correlation tests. The data were statistically processed using Statistical 6.0 software.

RESULTS

The first stage of the study included analysis of HER2/neu protein expression in tumor cells of 171 patients with BC. The level of expression was evaluated using a 4-point scale. High (3+) expression of HER2/neu was detected in tumors of 21 patients (12%), moderate (2+) in tumors of 9 patients (5%), and low in tumors of 48 patients (28%); no HER2/neu protein was detected in tumors of 54% patients. Two groups of BC patients were formed for further analysis of serum levels of soluble HER2/neu: group 1 included 30 patients with high and moderate expression of this protein in the tumor and group 2 consisted of 29 patients with low or null HER2/neu expression, corresponding to group 1 patients by the main clinical morphological characteristics. Serum content of HER2/neu was also measured in 15 women with benign diseases of the mammary glands.

Serum level of soluble HER2/neu was 4.95-39.0 ng/ml (median 9.45 ng/ml) in all BC patients and was somewhat higher than in patients with benign mammary gland diseases (6.28-10.3 ng/ml; median 8.33 ng/ml; $p<0.01$). The range of values was significantly greater in BC patients than in patients with benign mammary gland diseases (Fig. 1). When 95% confidence interval for benign mammary diseases equal to 8.83 ng/ml was taken as a threshold value, we found that 38 (64%) BC patients had higher serum level of soluble HER2/neu, of these 22 patients had high or moderate expression of HER2/neu in the tumor.

Comparison of the values in two groups of BC patients with different expression of HER2/neu showed significant differences (Table 1): serum concentration of this protein in patients with high and moderate expression of HER2/neu in the tumor (HER2/neu-positive) was significantly higher than in patients with low expression of HER2/neu or without it (HER2/neu-negative). The values in group 1 (but not group 2) patients also differed significantly from the level of soluble HER2/neu in women with benign mammary gland diseases (Table 1). Paired comparison of groups with different levels of HER2/neu expression (according to 4-point

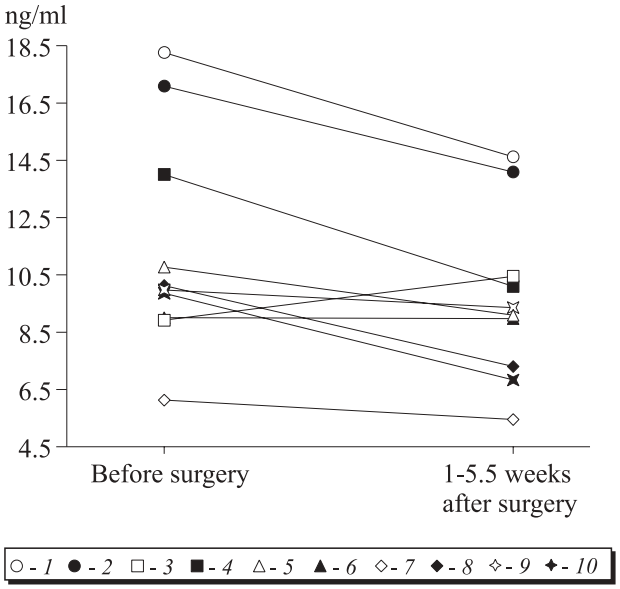

Fig. 1. Postoperative changes in serum concentration of soluble HER2/neu in 10 (*1-10*) BC patients.

TABLE 1. Serum Content of Soluble HER2/neu in BC Patients with Different HER2/neu Status and Women with Benign Changes in the Mammary Glands

Group	HER2/neu expression	*n*	Soluble HER2/neu, ng/mg protein	
			M±*m*	median, range
BC patients	3+	21	12.97±1.65	10.32 (6.13-39.00)
	2+	9	10.00±0.96	10.27 (4.95-14.00)
	2+, 3+	30	11.98±1.16*	10.27+o (4.95-39.0)
	1+	11	8.66±0.43	8.68 (6.16-11.0)
	0	18	9.51±0.46	9.21 (6.22-13.1)
	1+, 0	29	9.19±0.33	8.92 (6.16-13.1)
	Total group	59	10.61±0.63	9.45+ (4.95-39.0)
Women with benign changes in mammary glands	—	15	8.18±0.30	8.33 (6.27-10.3)

Note. *n*: number of patients. *$p<0.05$ compared to patients with benign mammary gland diseases and BC patients with low HER2/neu expression (Student's test); +$p<0.01$ compared to patients with benign mammary gland diseases (Mann—Whitney test); °$p<0.05$ compared to BC patients with low HER2/neu expression (Mann—Whitney test).

scale, Table 1) and analysis of correlations between serum concentrations of HER2/neu and level of its expression in the tumor also revealed no significant differences in these parameters (τ=0.15; p=0.077).

Serum level of HER2/neu did not correlate significantly with the main clinical morphological characteristics of the tumor (stage of the disease, size of primary node, its malignancy, number of metastases in the lymph nodes, status of steroid hormone receptors), but a significant positive correlation with the number of tumor emboli in the lymph vessels was observed (τ=0.32; $p<0.001$).

Serum content of soluble receptor was measured repeatedly in various periods after surgery in 10 patients with high and moderate expression of HER2/neu in the tumor (in 9 of these the preoperative level of HER2/neu surpassed the threshold value: 8.92-18.26; median 10.12 ng/ml, Fig. 1). After removal of the primary tumor, the serum level of HER2/neu decreased by 0.62-3.91 ng/ml (median 2.8 ng/ml), *i.e.* by 6-31% (median 17.5%) of initial level in 8 patients ($p<0.01$; Wilcoxon test); in one patient it virtually did not change, and in one increased. The degree of the decrease in the serum HER2/neu level positively correlated with its initial level (R=0.73; $p<0.05$).

Hence, we showed that BC patients with HER2/neu-positive primary tumors have higher levels of soluble fragment of this receptor in the serum than women with benign mammary diseases or BC patients with HER2/neu-negative tumors. This regularity and the detected highly significant reduction of serum level of soluble HER2/neu in BC patients after surgery indicate that serum values adequately reflect HER2/neu status of the primary tumor. Measurements of serum HER2/neu concentrations can become an adequate noninvasive method for monitoring of the status of HER2/neu during the pre- and postoperative period, which is very important for patients treated with Herceptin.

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