Lateral differences in Ki-67 in breast cancer

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Abstract. Morphological features of right- and left-sided breast cancer (BC) have been rarely studied. Ki-67 is an independent predictive marker for a local relapse, and for overall and disease-free survival. In previous literature, lateral differences in Ki-67 in BC have not been reported. Using immunohistochemical analysis, the biopsy material of 500 patients was investigated. Four BC parameters were investigated: The expression levels of the estrogen receptor (ER), progesterone receptor (PR), Ki-67 and Her-2-neu. A comparative analysis of Ki-67 in groups with right- and left-sided BC was performed. Lateral differences in Ki-67 in three age groups, ≤49, 50-59 and ≥60 years, were assessed. A higher expression of Ki-67 corresponded with right-sided BC (r=-0.08; P=0.046). Correlation of Ki-67 with the age of the patient was characteristic only for right-sided BC (r=-0.28; P<0.001). The comparative analysis revealed that tumors from patients with right-sided BC aged ≥60 years exhibited a lower Ki-67 index compared with tumors from patients aged ≤49 (P=0.003) and 50-59 years (P=0.000013). In left-sided BC, the Ki-67 in the different age intervals revealed no significant difference. On performing an assessment of lateral differences for the different age intervals, it was established that the Ki-67 index of right-sided BC was significantly higher compared with the left-sided BC in two of the age groups, ≤49 (P=0.038) and 50-59 years (P=0.047). In the ≥60 years group, lateral differences in Ki-67 were not revealed (P=0.4). The analysis of lateral differences in Ki-67 in tumors of various receptor profiles revealed significant differences in Ki-67 of the right- and left-sided BC under ER$^+$ PR$^-$ (P=0.007) and ER$^-$ PR$^-$ (P=0.016) conditions. The reasons behind lateral and age differences of Ki-67 are discussed.

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Introduction

Features of right- and left-sided breast cancer (BC) have been poorly studied to date. Publications on lateral differences and the incidence of BC are few, and only a single study has been published on lateral differences and survival following BC (1-3). Results from population studies have indicated that the frequency of occurrence of left-sided BC is higher compared with right-sided BC (4-6). The distinction is not great (4-8%), although the difference is not considered to be trivial, and may be associated with unidentified etiological factors (7).

The laterality of BC does not belong to recognized predictive factors. In a study performed by the Israeli Cancer Register, it was suggested that the survival of patients with BC is not dependent on tumor laterality (8). However, the results of other previous studies showed that the survival of patients with left-sided BC is higher compared with patients with the right-sided BC (9,10).

Studies on morphological differences of right-and left-sided BC are also few. Erendeeva *et al* (9) performed a comparative analysis of right- and left-sided BC on 45 clinico-morphological parameters. Results from this analysis revealed a number of statistically significant characteristics, including size of the primary center, histological type of tumor, existence of metastasis in regional lymph nodes and background pathology. This study established that primary tumors localized on the right side were significantly more in size compared with tumors of the left side, and had a significant tendency to metastasize in regional lymph nodes. Among the various forms of a mastopathy (background pathology), a particular emphasis was placed on the existence of mazoplasia, the significant processes of proliferation in breast tissue. It was noted that mazoplasia associated with right-side tumors was observed five times more often compared with left-side tumors. The authors considered that asymmetry of hormonal and immune influences in a human body could be the predominant reason for asymmetry of the clinico-morphological parameters of BC. Nouh *et al* (11) showed that metastases in regional lymph nodes of left-sided BC were observed less frequently compared with right-sided BC. The authors hypothesized that this may be associated with the greater activity of the right hand.

Proliferative activity of a tumor defines its aggression, and is an indicator of the prognosis (12). A number of indicators of proliferative activity are known. The greatest interest in recent years has focused on the biomarker, Ki-67, as an index

of proliferative activity. Numerous previous studies have shown that Ki-67 is an independent predictive marker for a local relapse, and for overall and disease-free survival (13-23). No previous studies, to the best of our knowledge, have shown lateral differences in Ki-67 in BC. Research in this area may enrich our knowledge of the natural history of BC and assist in creating personalized treatment for this disease.

Materials and methods

Following the protocols of immunohistochemical research of the Rostov Regional Morphological Bureau (2008-2010), an analysis of biopsy material from 500 patients was performed. Four BC parameters were investigated: The expression levels of the estrogen receptors (ERs), of the progesterone receptors (PRs), of Ki-67 and the epidermal growth factor receptor, C-erbB-2 (Her-2-neu). From these 500 BC cases, right-sided localization of the BC was observed in 238 (47.6%) patients; that of left-sided BC was observed in 262 (52.4%) patients. The present study aimed to do the following: i) Perform comparative analysis of Ki-67 levels in groups of patients with right- or left-sided BC; ii) to define lateral differences of Ki-67 levels in three different age groups (≤49, 50-59 and ≥60 years); and iii) to estimate changes in Ki-67 levels, depending on other parameters (ER, PR, Her-2-neu).

The statistical analysis was performed with the use of Statistics 8.0 software (StatSoft, Inc., Tulsa, OK, USA). To define interrelations, correlation matrixes were created. Comparison of different groups was performed using Student's t-test. In the case of the small size of groups, Fisher's exact test was used. Comparison of averages was performed by analysis of variance. The data are shown as percentages, with 95% confidence intervals (95% CI) ± the standard error of the mean. P<0.05 was considered to indicate a statistically significant difference.

Production of immunohistochemical reactions and interpretation of the results were performed, according to a previous study (24). For the light optical and immunohistochemical experiments, biopsy material was fixed in 10% buffering (neutral) formalin (BlikMedicalProduction, Taganrog, Russia) within 24 h at room temperature. Following treatment with paraffin (BlikMedicalProduction), serial cuts 4 μm-thick were performed. The cut sections were mounted onto the subject glasses and were loaded or covered with poly-L-lysine (Sigma-Aldrich, St. Louis, MO, USA). For the light optical experiments, the cut sections were stained with hematoxylin and eosin (DakoCytomation, Hamburg, Germany). For the immunohistochemical experiments, the cut sections made from paraffin blocks were placed in the thermostat for 30 min at 56˚C. Paraffin was removed from the sections by incubating in xylol (BlikMedicalProduction) five times. The duration of one incubation was between 5 and 10 min. The sections were subsequently dehydrated in three changes of absolute ethanol for 3 min, prior to placement in distilled water. To visualize the proteins, the sections were incubated with buffered solution (DakoCytomation) of primary antibodies (DakoCytomation, Hamburg, Germany) in a water bath for 20 min at 95˚C. The tissue sections were subsequently washed in the buffer. Endogenous peroxidase activity was suppressed using a hydrogen peroxide-based blocking solution (DakoCytomation) for 5-7 min at room temperature.

Immunohistochemical reactions were performed in an automatic robotized device (Autostainer Plus; DakoCytomation) according to the following protocol: Incubation with primary antibodies (15-30 min, according to antibody protocol), incubation with secondary antibodies (30 min), incubation with a substratum chromogen (8 min) and staining of kernels with Mayer's hematoxylin (5 min). A Dako EnVision system and peroxidase (antirabbit, antimouse) was used. The sections were washed using Tris-HCl, containing Triton-X, to reduce background coloring and promote penetration of solutions into the tissue. For each studied gene, a negative control was used instead of primary antibodies, and also a positive control, the fabric which containing this antigen. The following mouse monoclonal antibodies were used: Anti-progesterone receptor (Clone PgR 636; Target Retrieval S 1700 pH 6.0; dilution 2:100), anti-Human Ki-67 (Clone MIB-1; Target Retrieval S 1700 pH 6.0; dilution 1:100), all from DakoCytomation. HER-2/neu protein expression was performed using the HercepTest, according to the manufacturers instructions (DakoCytomation).

Results

The proliferative activity of Ki-67 significantly correlated with the BC laterality (r=-0.08; P=0.046). A higher Ki-67 index corresponded with right-side localization of the BC. The Ki-67 median for the right-sided BC was 18.1% (95% CI=15.3-21.0) and for the left-sided BC was 14.5% (95% CI=12.2-16.8). A t-test revealed the significance of differences (P=0.046).

Correlation of Ki-67 with the age of the patients was characteristic only for right-sided BC (r=-0.28; P<0.001). The maximum Ki-67 values for right-sided BC were observed in the age interval of 50-59 years (25.3%; 95% CI=19.4-31.3) and the minimum Ki-67 values were observed in the age interval ≥60 years (12.0%; 95% CI = 9.1-14.8; Fig. 1). The comparative analysis revealed that tumors of patients with right-sided BC of an age interval ≥60 years had a Ki-67 index significantly lower compared with tumors of patients at the age of ≤49 (P=0.003) and 50-59 years (P=0.000013). In the left-sided BC, the Ki-67 index in age intervals did not significantly differ. An assessment of the lateral differences in age intervals established that the Ki-67 index of right-sided BC was significantly higher compared with left-sided BC in two age groups, ≤49 (P=0.038) and 50-59 (P=0.047) years. For the ≥60 years age group, lateral differences in Ki-67 were not observed (P=0.4; Fig. 2).

The analysis of lateral differences of Ki-67 in tumors of different receptor profiles revealed significant differences in Ki-67 of the right- and left-sided BC under ER⁺ PR⁻ (P=0.007) and ER⁻ PR⁻ (P=0.016) conditions, therefore, when there is no expression of PR. In these cases, the Ki-67 index was higher in right-sided BC (Table I). In general, the maximum Ki-67 index was found in the group of right-sided tumors with the ER⁻ PR⁻ receptor profile, where the Ki-67 index reached 33.7±5.1%.

In tumors with the ER⁺ PR⁺ receptor profile, lateral differences of the BC on the Ki-67 index were absent (r=0.61). The assessment of the ER⁻ PR⁺ receptor profile was not performed due to insignificant numbers of samples.

Table I. Ki-67 expression in right- and left-sided BC in different receptor profiles.

	Right-side		Left-side		
Receptor profile	n	Ki-67 (%)	n	Ki-67 (%)	P-value
ER$^+$ PR$^+$	136	11.4±3.2	155	12.3±3.0	0.610
ER$^+$ PR$^-$	39	21.4±5.9	37	9.5±6.1	0.007
ER$^-$ PR$^+$	10	-	1	-	-
ER$^-$ PR$^-$	53	33.7±5.1	69	22.2±4.5	0.016

- indicates when comparative analysis was not performed due to small case numbers. BC, breast cancer; ER, estrogen receptors; PR, progesterone receptors.
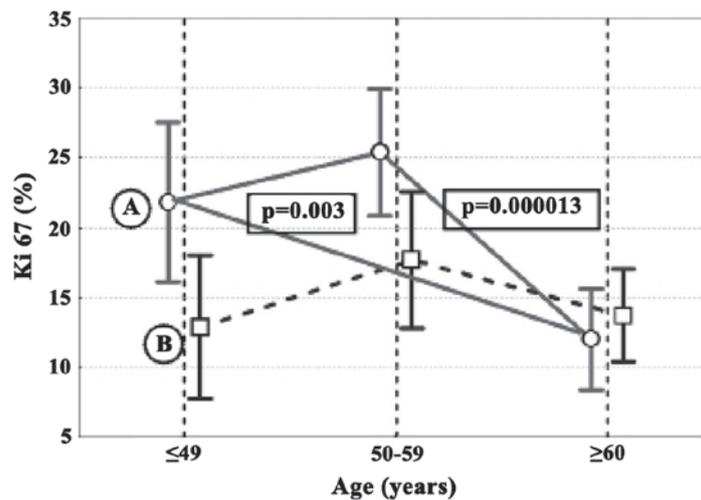

Figure 1. Comparative analysis of Ki-67 in breast cancer from patients at various ages in (A) right- and (B) left-sided breast cancer.
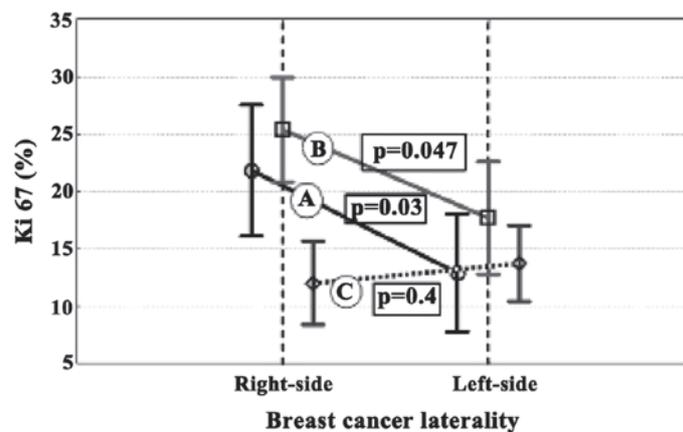

Figure 2. Comparative analysis of Ki-67 in right- and left-side breast cancer from patients aged (A) ≤49, (B) 50-59 and (C)≥60 years.

Expression of the epidermal growth factor receptor, C-erbB-2 (Her-2-neu), positively correlated with the Ki-67 index (right-side, r=0.27; P<0.001; left-side, r=0.17; P=0.006).

Discussion

Features of right-sided BC, including the bigger size of primary tumor and more frequent metastasis in regional lymph nodes (9,11), as revealed by previous studies, can be partly explained by the results of the present research. It is known that the Ki-67 index is directly connected with certain biological characteristics, including aggressive growth and metastatic potential.

Notably, the significantly higher rate of Ki-67 for the right-sided BC, in comparison with the left-sided BC, was observed only up until 60 years. In women who developed

BC at ≥60 years, lateral differences in Ki-67 index were not observed. It is also necessary to note that, in patients with right-sided BC, those women who became ill at ≥60 years were characterized by significantly lower Ki-67 levels compared with patients of the two other age groups. This suggested a dependence of the proliferative activity of the BC on the functioning mechanisms and the cessation of the menstrual cycle. In the ≥60 years group, Ki-67 was at a markedly low level and exhibited no lateral differences.

Results of the present study demonstrated the differences in the expression of Ki-67 of right- and left-side tumors of the breast. Age dynamics of lateral differences of Ki-67 in BC is a reflection of age dynamics of structural-functional asymmetry of a human body (25-27). In turn, asymmetry of neurohumoral regulation of the organism can be directly connected with mechanisms of the menstrual cycle (28).

Acknowledgements

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