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THE Ki-67 MARKER FOR ASSESSING THE EFFECTIVENESS OF SYSTEMIC OR REGIONAL NEOADJUVANT POLYCHEMOTHERAPY IN PATIENTS WITH LOCALLY ADVANCED BREAST CANCER

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Over the past decades, breast cancer (BC) is the most common cancer and one of the key causes of mortality and disability among women in developed countries. *Aim*: Determination of the role of Ki-67 index in assessing the quality of neoadjuvant polychemotherapy treatment using regional or systemic delivery routes of pharmacological agents in patients with locally advanced breast cancer (LABC). *Materials and Methods*: The retrospective analysis of 30 clinical trials of LABC treatment based on selective intra-arterial therapy in patients with BC (T4A-DN0-3M0) was used. *Results:* The decrease in Ki-67 level in LABC after selective intra-arterial polychemotherapy was more pronounced than after systemic polychemotherapy. No correlation of the tumor metastatic potential with a Ki-67 level was detected. *Conclusion*: Assessment of Ki-67 expression allows to evaluate effectively the biological properties of the tumor, predict the course of the disease and choose the optimal tactics of neoadjuvant polychemotherapy (regional or systemic variant) as part of integrated antitumor treatment.

***Key Words*: Ki-67, immunohistochemistry, locally advanced breast cancer, neoadjuvant polychemotherapy.**

Over the past decades, breast cancer (BC) is the most common cancer type and one of the key causes of mortality and morbidity among women in developed countries. The epidemiological status of the disease and the wide range of available diagnostic and therapeutic information both explain the increased interest to the issues of diagnosis and evaluation of the quality of treatment of this pathology in scientists and public activists in the field of health care [1].

The etiological model of BC is based on the classic idea of each disease-specific exogenous and endogenous factors ratio with a strong shift of accent onto the genotypic component, leaving the trigger role to external factors. The epidemiological distribution of contributing factors declaims the increased risk area in Western countries, which is highly associated with the cultural and social characteristics of the life and family planning organization among the developed countries populations.

Medical and economic expediency create the preconditions for the development and implementation of the best curative tactics. Considering the physiological and morphological features of the target area, the urgent issues are the development of targeted anti-tumor methods, in particular, the physical concentration of chemotherapeutic effects on the hearth of locally advanced breast cancer (LABC). The ultimate goals of polychemotherapy (PCT) in LABC cases, the pathogenic points of therapeutic application, the composition of paneling substances are now discussed [2, 3].

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Abbreviations used: BC – breast cancer; LABC – locally advanced breast cancer; PCT – polychemotherapy; SIPCT – selective intra-arterial polychemotherapy; SPCT – systemic polychemotherapy.

The method of selective intra-arterial polychemotherapy (SIPCT) in general is one of the modern ways of influencing the course of the oncological process.

Schematically, the essence of the method is an artificial invasive controlled short-term increase in peak concentration of active substances in the regional microcirculation by saturation of afferent inflow with active molecules [4]. This strategy is implemented by catheterization of the internal thoracic artery on the side of the tumor localization and followed by isolated potentiated perfusion of the affected area by chemotherapy preparations [5, 6]. The goal is to achieve common results for all targeting methods: increasing local exposure and reducing the systemic toxic response of used antitumor drugs.

In the context of initially inoperable forms of LABC at the planning stage of treatment, it is important to address the key issue of potential effectiveness of proposed method and to predict the sensitivity of the tumor to the neoadjuvant treatment course, so to choose the method and route of the chemotherapeutic drug administration. A convenient and reliable way of the future outcome calculating is the proliferative activity index of the tumor cells as an indirect characteristic of the degree of sensitivity to artificial damage. A promising solution to this question is the use of immunohistochemical indicers that correlate with cytomorphological characteristics of cell proliferation. One of these indices is the Ki-67 [7].

In the long run, the study of a diverse panel of specific BC biomarkers and their compilation will allow a comprehensive calculation of the tumor proliferative activity index, which will enable more accurately assess the tumor current status and to predict the effectiveness of the planned treatment.

The aim of the research was to assess the role of Ki-67 index in assessing the quality of neoadjuvant PCT using regional or systemic delivery routes of pharmacological agents in patients with LABC, determine the Ki-67 reference range to predict the effectiveness of treatment, and develop the adequate tactic for complex preoperative antitumor treatment.

MATERIALS AND METHODS

The current study was based on the selective analysis of the data on 30 patients with LABC of grade T4A-DN0-3M0 who received the comprehensive neoadjuvant treatment with the SIPCT at the Donetsk Regional Cancer Center and the University Clinic of Odessa National Medical University in 2000–2017. Before including the patient in the study protocol, a personal informed written voluntary consent to participate in the study was obtained in accordance with the WMA Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects, 2013 form (No. 117A protocol of the Odessa National Medical University Bioethics Commission meeting dated 12.05.2017). At the neoadjuvant stage, all patients received 2–3 courses of regional or 3–4 courses of systemic polychemotherapy (SPCT) for the purpose of cytoreduction and the possibility of further radical surgical intervention.

The sample was standardized by age and clinical parameters, range of 42–75 years. The average age of the patients was 49 ± 7.2 years. In all patients immunohistochemical investigation of the Ki-67 expression in BC biopsy samples was performed in parallel with routine laboratory and instrumental examination and morphological study of the biopsy material.

Proliferative activity was determined by standard immunohistochemical assessment using Ki-67 antibody (Thermo Fisher Scientific, USA). The fraction of 500 tumor cells was chosen as the minimum number of proliferating cells for evaluation. Ki-67 values were expressed as the specific percentage of cells with positive staining. Most patients in both groups (60%) had a Ki-67 value in the range of 10–20%, with a median value of 15%. Therefore, Ki-67 values were divided into 2 groups: < 15% and > 15%. In accordance with the recommendations of the St. Gallen consensus, in which it is recommended to establish a threshold value from 15 to 30%, the results of our study showed a borderline level of 15%.

After analyzing the data obtained, all patients were divided into 2 groups. The first study group consisted of 15 patients (50%) with initially inoperable forms of LABC, which were treated by SIPCT as a neoadjuvant treatment course. The second group included 15 patients (50%) with initially inoperable forms of LABC, treated with SPCT at the neoadjuvant treatment stage.

For statistical analysis of the data, the statistics of dynamic series were used. To evaluate the significance of these changes the nonparametric Pearson χ^2 test was applied.

RESULTS

In the first group, prior to performing neoadjuvant PCT, the referent values of Ki-67 were: < 15% in 6 patients (40%) with M ± m = 12.9 ± 0.9; > 15% in 9 patients (60%) with M ± m = 24.4 ± 2.9. After conducting PCT, the Ki-67 range had the following dynamics: the proportion of patients with a low Ki-67 level (< 15%) increased by 50% (from 6 to 12) with the overall index mean decline to 5.2 ± 2.9 ($p = 0.049$). The average rate in 3 patients with high levels of Ki-67 (> 15%) was 19.7 ± 1.6 ($p = 0.398$).

In the second group, before the PCT has been performed, the range of Ki-67 was: < 15% in 7 patients (47%) with M ± m = 13.6 ± 0.9; > 15% at 8 (53%) with M ± m = 25.3 ± 3.0. After neoadjuvant PCT, the reference values of Ki-67 decreased to 12.1 ± 0.8 ($p = 0.507$). The average index in group with a high level of Ki-67 (> 15%) became 20.1 ± 2.8 ($p = 0.398$). No changes in the quantitative and qualitative composition of groups were revealed (Fig. 1).

By the Pearson χ^2 factor there is a statistically significant advantage in treatment outcomes in patients of the first group ($\chi^2 = 4.367$, $p = 0.07$ with the critical values of 3.841 and 0.05, respectively).

For patients of the first group: the average life expectancy was 3.4 ± 0.9 years, with the total 3-year survival rate — 66% (10 patients). 13 patients (86.7%) has achieved the operative treatment status. Distant metastases in the period of 36 months after operative treatment were detected in 5 cases (33%).

For patients the second group, the average life expectancy was 2.3 ± 1.0 years, and 6 patients (40%) survived 3-year postoperative term. In 7 patients (46%)

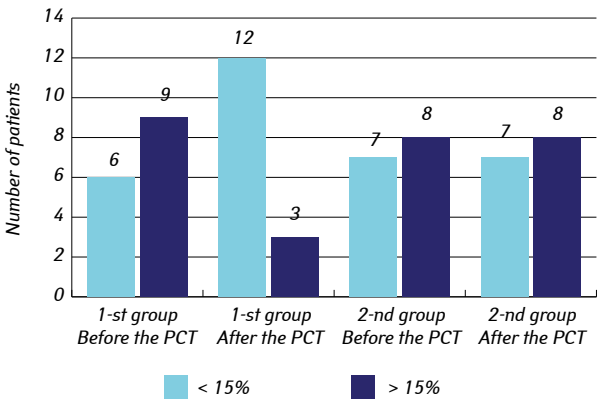

Fig. 1. The analysis of the qualitative comparison of the groups before and after the PCT by Ki-67 expression

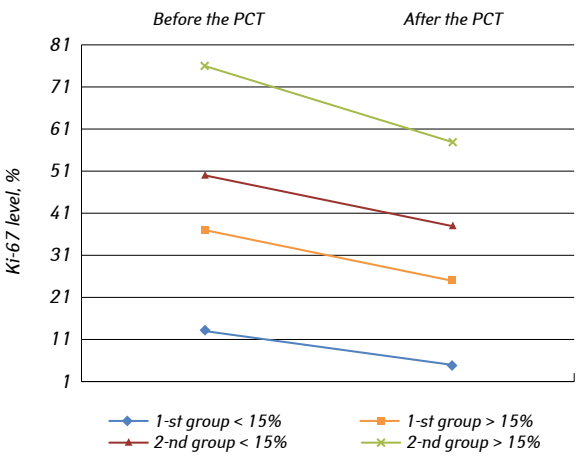

Fig. 2. Average Ki-67 values before and after the PCT

the aim of operative treatment possibility was gained. Distant metastases within 36 months period after the operative treatment were observed in 6 patients (40%).

No correlation between the tumor metastatic potential and a Ki-67 level was detected.

DISCUSSION

It is known that the majority of the oncological pathologies are initiated either via exogenous and endogenous damage to the DNA structure or the damage to the repair system in these lesions followed by the formation and accumulation of negative mutations in the key genes, resulting in acquisition of functional and morphological features of the autonomous growth [6]. The further biological behavior of each future tumor (growth rate, the risk of metastasis, as well as the clinical course and the disease outcomes) is determined by qualitative and quantitative changes in the genetic apparatus. An independent integral characteristic describing the formed tumor phenotype is its proliferative activity. An important feature of proliferative activity is its clear correlation with the grade of neoplasm malignancy. Thus, this indicator has two important clinical roles: the predictive (defining the clinical course of the disease and the metastatic risks) and the descriptive (an indirect characteristic of the current state of tumor and its changes in response to treatment) [7].

Different approaches are known for evaluating the proliferative activity of the tumor: optical microscopy (counting of mitotic figures), electron microscopy, flow cytometry, and immunohistochemical methods: direct (use of labeled nucleotides) and indirect (determination of specific antigens).

The task of our research was to improve the method for evaluating the treatment effectiveness in patients with LABC by determining the proliferative activity of tumor cells based on immunohistochemical assessment of Ki-67 antigen. It allows predicting the sensitivity of the neoplasm to the neoadjuvant PCT and choosing the mode of PCT. This, in our opinion, will allow defining an adequate tactics for the treatment of BC patients.

The nuclear antigen Ki-67, first described by Gerdes *et al*. [8], is a dimeric molecule consisting of two polypeptide chains with a total molecular weight of 358 694 Da and a length of 3256 amino acids encoded by the MKI-67 gene located in humans on a short arm of the 10th chromosome.

During the interphase, the protein is determined exclusively in the nuclear matrix associated with the chromosomes. In the active phases of the cell cycle (G_1, S, G_2, and M), the expression of the protein and its content in the cytoplasm increase, especially when the cell progresses through the synthetic stage; in the G_0 phase, they decrease. The Ki-67 function is not well known, but a clear relationship with cell proliferative activity is determined. Consequently, Ki-67 is a reliable marker for cellular proliferative activity [9].

The index of proliferative activity has different referent values for different scenarios of oncological pathology. On the basis of the immunohistochemical diagnostic test for the BC, the following types are distinguished: low-aggressive tumor — Ki-67 < 15%, aggressive — > 15%, highly aggressive — > 30% [10].

By the dynamics of Ki-67 index the method of SIPCT is more effective way of neoadjuvant management of patients with primary inoperable forms of BC and has better results in terms of inhibition and reversal of tumor progression compared with systemic approach. On the basis of the current research, the authors proposed evaluating the Ki-67 dynamics to determine the effectiveness of the performed treatment by the regression of tumor proliferative activity. The proposed method can be effectively used to estimate the biological properties of the tumor, to predict the course of the disease (favorable or unfavorable) and to choose the optimal tactic of neoadjuvant PCT (regional or systemic) as a part of an integrated antitumor treatment.

REFERENCES

1. Yamauchi H, Woodward WA, Valero V, *et al.* Inflammatory breast cancer: what we know and what we need to learn. Oncologist 2012; **17**: 891–9.

2. Wirtz HS, Buist DSM, Gralow JR, *et al.* Frequent antibiotic use and second breast cancer events. Cancer Epidemiol Biomarkers Prev 2013; **22**: 1588–99.

3. Ording AG, Garne JP, Nyström PMW, *et al.* Hospital recorded morbidity and breast cancer incidence: a nationwide population-based case-control study. PLoS One 2012; **7**: e47329.

4. Landercasper J, Bailey L, Buras R, *et al.* The American Society of Breast Surgeons and quality payment programs: ranking, defining, and benchmarking more than 1 million patient quality measure encounters. Ann Surg Oncol 2017; **24**: 3093–106.

5. Greenlee H, DuPont-Reyes MJ, Balneaves LG, *et al.* Clinical practice guidelines on the evidence-based use of integrative therapies during and following breast cancer treatment. CA Cancer J Clin 2017; **67**: 194–232.

6. Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, *et al.* Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. CA Cancer J Clin 2017; **67**: 378–97.

7. Sueishi M, Takagi M, Yoneda Y. The forkhead-associated domain of Ki-67 antigen interacts with the novel kinesin-like protein Hklp2. J Biol Chem 2000; **275**: 28888–92.

8. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983; **31**: 13–20.

9. Viale G, Regan MM, Mastropasqua MG, *et al.* Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Nat Cancer Inst 2009; **100**: 207–12.

10. Yerushalmi R, Woods R, Ravdin PM, *et al.* Ki-67 in breast cancer: prognostic and predictive potential. Lancet Oncol 2010; **11**: 174–83.