THE COMPARATIVE ANALYSIS OF EXPRESSION OF MOLECULAR-BIOLOGICAL MARKERS IN ONCOGINECOLOGICAL PATIENTS

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Abstract: *the aim of the study was to study the expression of the main molecular-biological markers in oncogynecological patients. The majority of patients with cervical cancer and ovarian cancer showed expression of molecular-biological markers - p53, VEGF and Ki-67. It was shown that the molecular prognostic markers p53, VEGF and Ki-67, as well as the level of proliferative activity of the tumor, have the greatest prognostic significance with respect to treatment effectiveness. In the OC, the HER-2 / neu marker was detected in 20.4% of patients. Due to the fact that the positive level of expression of p53, VEGF and Ki-67 markers have a negative effect on the 5-year survival of oncogynecologic patients, we have been tasked to study the expression of the above-mentioned proteins before the start of therapy. Therefore, the positive level of expression of p53, VEGF and Ki-67 in oncogynecologic patients, along with high proliferative activity of the tumor, may serve as a basis for conducting this category of women methods of accompanying immunotherapy with extracorporeal immunotherapy before the polychemotherapy. This study is devoted to a planned study of the results of immunotherapy, which will be performed by a patient with cervical cancer and ovarian cancer, depending on the expression of proliferative molecular biology markers to improve the results of polychemotherapy, increasing the 3- and 5-year survival. We hope that a preliminary assessment of the expression of molecular-biological markers prior to the appointment of treatment will affect the quality of the prognostic criterion for the course of the disease.*

Keywords: *ovarian cancer, cervical cancer, molecular-biological markers, apoptosis, proliferation.*

СРАВНИТЕЛЬНЫЙ АНАЛИЗ ЭКСПРЕССИИ МОЛЕКУЛЯРНО-БИОЛОГИЧЕСКИХ МАРКЕРОВ У ОНКОГИНЕКОЛОГИЧЕСКИХ БОЛЬНЫХ

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Аннотация: *целью исследования явилось изучение экспрессии основных молекулярно-биологических маркеров у онкогинекологических больных. У большей части больных раком шейки матки и раком яичников выявлена экспрессия молекулярно-биологических маркеров - p53, VEGF и Ki-67. Показано, что наибольшей прогностической значимостью в отношении эффективности лечения обладают молекулярно-биологические маркеры p53, VEGF и Ki-67, а также уровень пролиферативной активности опухоли. При РЯ маркер HER-2/neu обнаруживался у 20,4% больных. В связи с тем, что положительный уровень экспрессии маркеров p53, VEGF и Ki-67 оказывает негативное влияние на показатели 5-летней выживаемости онкогинекологических больных, нами поставлена задача изучить экспрессию вышеперечисленных белков до начала терапии. Следовательно, положительный уровень экспрессии p53, VEGF и Ki-67 у онкогинекологических больных, наряду с высокой пролиферативной активностью опухоли, может служить основанием для проведения данной категории женщин методы сопроводительной иммунотерапии с применением экстракорпоральной иммунотерапии до проведения полихимиотерапии. Данное исследование посвящено запланированному изучению результатов иммунотерапии, который будет проведен больным раком шейки матки и раком яичников, в зависимости от экспрессии пролиферативных молекулярно-биологических маркеров для улучшения результатов полихимиотерапии, увеличения 3- и 5-летней выживаемости. Надеемся, что предварительная оценка экспрессии молекулярно-биологических маркеров до назначения лечения повлияет на качество прогностического критерия течения заболевания.*

Ключевые слова: *рак яичников, рак шейки матки, молекулярно-биологические маркеры, апоптоз, пролиферация.*

Introduction. It has been established that modern methods of predicting and selecting further tactics for oncogynecologic patients are the study of the expression of important molecular-biological markers in tumor tissue that play a role in the mechanisms of apoptosis and proliferation of malignant cells, which directly affects the course of the oncological process [1, P.1293; 3,P.1419; 6,P. 1; 12,P. 433; 16,P. 3]. Moreover, it is known that

the use of modern methods of treatment, including preparations of monoclonal antibodies, is based on the detection of overexpression of molecular-biological markers in tumor tissues that are targets for targeted therapy [2, P. 395; 3, P. 1419; 7, P. 242; 10, P. 809]. Today, one of the most promising areas in the diagnosis of malignant tumors is the detection of tumor markers, which can provide additional information about the biological behavior of the tumor. Much attention is paid to the study of markers that characterize apoptosis and cell proliferation. These include the proteins p53, Bcl-2, Ki-67, etc. [11, P. 765; 16, P. 3].

The problem of oncogynecological diseases, including ovarian cancer and cervical cancer, is one of the most urgent and difficult sections of clinical oncology. The oncogynecological diseases account for more than 30% of all malignant diseases [2, P. 395; 5, P. 3999; 8, P. 242; 11, P. 765]. The reason for the high mortality of patients with malignant ovarian tumors and cervical cancer is likely to be the asymptomatic course of the disease in the early stages, which leads to late treatment to the doctor and, consequently, recognition of the disease in later stages [5, P. 3999; 7, P. 669; 8, P. 242; 12, P. 433]. Today, the successes of molecular genetic studies in oncology make it possible to treat malignant neoplasm as a disease characterized by the clonal evolution of transformed cells in organ tissues, and moreover, the study of the expression of molecular-biological markers makes it possible to understand the tumor's behavior, the possibility of targeting therapy [5, P. 3999; 9, P. 763; 10, P.809; 14, P. 485]. As is known, molecular-biological markers are divided into serum and tissue detectable directly in tumor cells. It should be noted that all tumor markers studied participate in the functioning of normal cells of the body and are direct participants in carcinogenesis. Of course, their determination during malignant growth provides additional information on the speed of its growth, the ability to metastasize and invade, and resistance to chemotherapy [5, P. 3999;10, P. 809; 14, P. 485; 15; P. 33]. In connection with the foregoing, the purpose of the study was to study the expression of major molecular-biological markers in oncogynecological patients.

Material and methods of research. Molecular biological tumor markers were examined in 68 patients with cervical cancer (CC) and 85 patients with ovarian cancer (OC) $T_{2-3}N_{0-1}M_0$ stages (II-III clinical stages) who were hospitalized in oncogynecology and chemotherapy departments in 2008, 2014 Immunohistochemical methods of investigation were carried out on histological preparations of the operational-biopsy material of the primary tumor of patients with CC and OC received before the initiation of therapy. Samples of tumor tissue were fixed in neutral buffered formalin with conventional standard wiring and paraffin waxing. Histological preparations were stained by conventional methods and immunohistochemical studies were performed. Paraffin sections were dewaxed and rehydrated using a standard procedure. To visualize the immunohistochemical reaction, a DAB + system was used [DakoCytomation, Denmark]. The staining results were evaluated using a light microscope "Leica" (Germany) under magnification x10; x20; x40. For the marker, the localization of staining in the cell (nucleus, cytoplasm, membrane) was evaluated. The number of positive cells was evaluated in the zones containing their maximum number. Patients in the immunohistochemical evaluation of p53 expression used monoclonal mouse antibodies to p53, Bc1-2, VEGF, and Ki-67 (DakoCytomation, Denmark). The following criteria for the evaluation of markers were used in the study: 1. The tumor was considered negative for p53 if there was no nuclear reactivity with antibodies in the tumor tissue or the number of stained cells was less than 25%; and positive for p53, if more than 25% of the nuclei of tumor cells were stained; 2. the tumor was considered negative for Bc1-2, VEGF, if there was no cytoplasmic reactivity with antibodies in the tumor tissue or the amount of stained cells was less than 25%; and positive if more than 25% of tumor cells were colored; 3. To estimate the proliferative activity (PA) of the tumor, the number of Ki-67 positive tumor cells per 300 tumor cells was counted. The Ki-67 index was determined by the formula: PA = number of Ki-67-positive cells x 100 / total number of cells. The proliferative activity of the tumor was estimated as the percentage of Ki-67 positive cells from the total number of tumor cells. High proliferative activity corresponded to the Ki-67 index> 40%, low proliferative activity of the tumor corresponded to the Ki-67 index <40%.

The results obtained and their discussion. A comparative analysis of the expression of molecular-biological markers showed that in the study of tumor marker expression in patients with CC, negative p53 level was observed in 28% of patients, positive in 72%). Analysis of the Bcl-2 study showed that an inverse pattern was found: a negative marker level was detected in 65.5% of patients with CC and positive in 44.5%. Corresponding indices in the study of VEGF showed that negative expression was detected in 21.5%, and positive in 78.8%. Analysis of the Ki-67 study showed that 24.8% of the individuals are negative, and 75.7% of the patients are positive. Therefore, in most patients with CC, the level of oncomarkers studied was positive, with the exception of Bcl-2. As is known, p53 gene suppressor encodes a nuclear protein that modulates gene expression, responsible for DNA repair, cell division and apoptosis. To date, there is no consensus in the literature about the dynamics of p53 expression in the progression of CC. According to several authors, it can both increase and decrease with this disease. The frequency of accumulation of mut-p53 increases with the growth of malignancy of tumors, while mutant p53 does not occur in benign tumors, and in malignant tumors the accumulation frequency increases to 46% [6, P. 1; 11, P. 765; 15, P. 33; 16, P. 3]. The Bcl-2 protein plays an important role in the regulation of apoptosis. It was shown that a high degree of expression of the tumor cell Bid (protein from the Bcl family, which plays an important role in the regulation of apoptosis and integrating signals for mitochondria)

correlates with the unfavorable cervical prognosis. Bcl-2 can completely delay apoptosis caused by p53 and other stimulants, including cytostatic drugs, but does not stop apoptosis caused by cytotoxic T-lymphocytes [14, P. 485; 16, P. 3]. In some studies, it is said that a sharp and significant increase in Bcl-2 expression in localized forms of CC compared with the initial stages and a subsequent decrease in expression in a locally advanced process [5, P. 3999; 9, P. 763; 14, P. 485]. It has been established that the main vascular activator is the vascular endothelial growth factor (VEGF), responsible for the proliferation and migration of endothelial cells, and is also directly related to tumor invasion and metastasis. The data supporting the participation of VEGF in the construction of the vascular bed, the growth and progression of malignant neoplasms have been accumulated. Moreover, the interaction of these ligands with transmembrane tyrosine kinase receptors is considered as the most important autocrine pathway of tumor promotion [16, P. 3]. As for the expression of the Ki-67 protein, it is known that this is a nuclear protein, the expression of which is noted in the active phase of the cell cycle, including mitosis. According to literature data, the expression of Ki-67 increases with cervical lesions. The proliferative index of Ki-67 is considered as an independent prognostic indicator of the occurrence of relapse, general and disease-free survival, a predictive factor for determining sensitivity to chemotherapy (CT) and radiation therapy. The Ki-67 index allows one to assess the degree of malignancy of the tumor and to predict the course of the disease in conjunction with other factors. It is shown that a high level of the Ki-67 index is associated with an unfavorable forecast. In particular, with a high level of Ki-67, there is a worsening of the disease-free and overall survival rates of patients with breast, ovarian, colon, bladder, soft tissue sarcomas, etc. [5, P. 3999; 12, P. 433; 14, P. 485].

Next, an analysis of the study of the main molecular-biological markers in patients with ovarian cancer was carried out. The analysis showed that the expression of molecular-biological tumor markers in patients with ovarian cancer is characterized by a negative level of p53 in 18.6% of patients, positive in 81.8%. Corresponding indices in the study of HER-2 / neu were found in 79.6% and 20.4% of patients, in the study of VEGF - in 14.4% and 86.5%, in the study of Ki-67 - in 18.9% and in 78.2% of women. Consequently, in most of the OI patients the level of oncomarkers studied was positive, with the exception of HER-2 / neu. It was found that the result of p53 activation is the stopping of the cell cycle and DNA replication; with a strong stress signal - the initiation of apoptosis. Violation of the mechanism of development of apoptosis can occur when the key gene of this process p53 loses its function. This can occur as a result of mutation of the p53 gene with the formation of a mutant oncoprotein - mut-p53, which is observed in conditions of pathology or as a result of blockade of p53 by other proteins, to which Bcl-2 primarily applies. Increasing the expression of mutated p53 in a tumor is accompanied by its greater aggressiveness, as the number of tumor cells undergoing apoptosis decreases. In OC, according to different researchers, mutant p53 is detected in more than half of patients already in the early stages of the disease [16, P. 3]. In some works devoted to ovarian cancer, it has been shown that the amplification of HER-2 / neu, occurring in 10-50%, indicates an unfavorable prognosis of the course of the disease [15, P. 33; 16, P. 3]. However, there are also conflicting data, so the practical significance of HER-2 / neu testing remains controversial today [5, P. 3999; 8, P. 242; 12, P. 433]. Recently, it was found that some mucinous ovarian carcinomas have amplification of the HER2 / neu gene and overexpression of its protein [2, P. 395; 16, P. 3]. In recently published results of a genomic analysis of 50 samples of clear-cell ovarian cancer, the presence of HER2 / neu amplification in 14% of cases was shown [10, P. 809; 12, P. 433]. It is obvious that at a rare frequency of HER2 / neu overexpression and amplification of its gene in the general ovarian carcinoma group in a subgroup of mucinous and clear cell carcinomas, this receptor is much more common. The main activator of angiogenesis is the vascular endothelial growth factor (VEGF), responsible for the proliferation and migration of endothelial cells, and is also directly related to tumor invasion and metastasis. In OC, various investigators have noted high expression of VEGF [5, P.3999; 15, P.33]. Studies of many scientists have shown that a high level of the Ki-67 index is associated with an unfavorable forecast. In particular, with a high level of Ki-67, there is a worsening of the disease-free and overall survival rates of patients with breast, ovarian, colon, bladder, soft tissue sarcomas, etc. In most cases, a high level of Ki-67 expression is detected in malignant ovarian tumors [8, P. 242].

Thus, the majority of patients with CC and the OC were present with molecular-biological markers p53, VEGF and Ki-67. The analysis showed that the molecular prognostic markers p53, VEGF and Ki-67, as well as the level of proliferative activity of the tumor, have the greatest prognostic significance with regard to the effectiveness of treatment. In the OC, the HER-2 / neu marker was detected in 20.4% of patients. As is known, a positive level of expression of p53, VEGF and Ki-67 markers has a negative effect on the 5-year survival of patients with OC [10, P. 809; 12, P. 433]. Therefore, the positive level of expression of p53, VEGF and Ki-67 in patients with cervical cancer and OC, along with high proliferative activity of the tumor, can serve as a basis for carrying out this category of patients, of course, taking into account the immunological characteristics of the patient's organism, carrying out accompanying immunotherapy with extracorporeal Immunotherapy in the accompanying regime before polychemotherapy. The present study is devoted to a further study of the results of immunotherapy, which will be carried out by patients with cervical cancer and ovarian cancer, depending on the expression of proliferative molecular biology markers for improving the results of polychemotherapy, increasing

the 3- and 5-year survival and for the prevention of metastasis. We hope that a preliminary assessment of the expression of molecular-biological markers prior to the appointment of treatment will affect the quality of the prognostic criterion of the course of the disease.

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