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INFLUENCE OF THE IMMUNE MICROENVIRONMENT ON THE COURSE AND PROGRESSION OF PRIMARY FALLOPIAN TUBE CANCER

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Among all malignancies, primary fallopian tube carcinoma is the most insidious and challenging to diagnose and treat appropriately. The tumor microenvironment plays a crucial role in supporting the functioning of cancer cells and actively influences them. Understanding the prognostic potential of the presence of tumor-infiltrating immune cells is important not only for its intensity but also for the subpopulation composition of the infiltrating cells.

Objective - to evaluate the qualitative composition of the immune microenvironment of tissue in primary serous carcinoma of the fallopian tubes.

The study was conducted on 66 samples of tumor tissue from fallopian tubes. Molecular and biological markers used in the study included Ki-67, p53, VEGF, ER, PR, CD3, CD79α, CD68, and myeloperoxidase (MPO). The criteria for assessing molecular and biological markers were conducted according to commonly accepted methods and according to the manufacturer's protocol.

The presence of tumor-infiltrating inflammatory infiltrate was established in 40.9% of primary serous carcinoma of the fallopian tubes. The presence and intensity of infiltration depended on the degree of atypia of cancer cells ($p<0.05$). The cellular component of the microenvironment of primary serous carcinoma of the fallopian tubes is predominantly represented by B-lymphocytes. A decrease in the level of T-cell infiltration in tumor tissue was somewhat associated with lymph node metastasis ($p<0.001$). The presence of macrophages in tumor tissue was significantly higher in the group of patients with primary serous carcinoma of the fallopian tubes with metastasis to regional lymph nodes ($p<0.001$). A moderate negative correlation was found between the presence of T-lymphocytes in the tumor microenvironment and ER ($r=-0.48$, $p<0.001$) and PR ($r=-0.43$, $p<0.001$). A negative correlation of moderate strength was established between the presence of B-lymphocytes, ER ($r=-0.56$, $p<0.001$), and PR ($r=-0.50$, $p<0.001$). A correlation was found between the presence of T- and B-lymphocytes in the tumor microenvironment and Ki-67 expression ($r=0.28$, $p<0.05$ and $r=0.27$, $p<0.05$ respectively). A moderate correlation was established between the presence of T-lymphocytes ($r=0.47$, $p<0.001$), B-lymphocytes ($r=0.58$, $p<0.001$), and p53 expression. No statistically significant correlation was found between the presence of T-lymphocytes, B-lymphocytes, macrophages in the tumor microenvironment, and VEGF expression ($p>0.05$).

The presence of inflammatory tumor microenvironment predominantly localizes in low-grade neoplasms (66.7%), and its qualitative and quantitative composition is associated with decreased expression of steroid hormone receptors in tumor tissue ($p<0.001$) and correlates with Ki-67 ($p<0.05$) and p53 ($p<0.001$) expression. Decreased levels of CD3+ T-lymphocytes in primary fallopian tube carcinoma tissue and significant infiltration of the tumor by CD68+ macrophages are associated with regional metastasis and are indicators of an unfavorable prognosis.

Key words: *immune microenvironment, tumor immune infiltrate, fallopian tube cancer, inflammatory tumor microenvironment, serous fallopian tube carcinoma.*

Connection of the publication with planned research works.

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Introduction.

Primary fallopian tube cancer (PFTC) ranks last among oncological diseases of the female reproductive system. However, the mortality from it is quite high [1]. The incidence of PFTC varies from 0.14% to 1.8% among all malignant tumors of the female reproductive system [2, 3]. The causes of occurrence, frequency, diagnostic possibilities, prevention and treatment of PFTC remain not only problematic and relevant, but also poorly covered in modern medical literature. It is currently known that this type of neoplasm in practical oncogynecology occurs much more often and can be the source of serous tumors of the ovaries and peritoneum [4-6]. Increased oncological alertness, improvement of diagnostic methods, and the introduction of immunohistochemical (IHC) research methods into practice have contributed to a relative increase in the incidence of this type of neoplasm over the past fifty years [7].

The tumor microenvironment not only plays an important role in supporting the functioning of cancer cells, but also actively influences it. There are data on the antagonistic effect of immunocytes on tumor cells. Moreover, a significant number of publications demonstrate that the presence of immune system cells in the immediate tumor environment is a negative factor that correlates with accelerated growth and metastasis of cancer [8, 9]. The tumor immune microenvironment can not only directly or indirectly affect its development, but also have prognostic significance. Penetrating into the tumor, immune system cells can play the role of producers of various pro-inflammatory factors, thereby maintaining the state of chronic inflammation and stimulat-

ing the growth and progression of neoplasms [10, 11]. At the same time, the cells of the predominantly lymphoid line of the immune system (T – and B-lymphocytes, NK-cells) implement various scenarios of antitumor immune defense (production of antibodies, contact cell and complement-dependent cytolysis, etc.), which leads to the limitation of tumor growth [12]. It should be noted that there are currently no data on the influence of the qualitative and quantitative composition of the tumor microenvironment on the phenotype and progression of PFTC.

The aim of the study.

To evaluate the qualitative composition of the immune microenvironment of tissue in primary serous carcinoma of the fallopian tubes.

Object and research methods.

The study was conducted on surgical material obtained from 66 patients with serous adenocarcinoma of fallopian tubes (SAFT). Written informed consent was obtained from the patients for the publication of this study and accompanying images. There are no potentially identifiable human images or data is presented. Ethical approval based on the Declaration of Helsinki with the registration of this study was provided by the Bioethics Commission of the Academic and Research Medical Institute of Sumy State University, Sumy, Ukraine (protocol № 14/02 from 18/02/2021).

The immunophenotype of neoplastic cells was studied in the immunohistochemical study using molecular biological markers reflecting characteristics of proliferation (Ki-67), apoptosis (p53), neoangiogenesis (VEGF) and tumor hormonal status (ER, PR). The qualitative composition of the tumor immune microenvironment was studied using antibodies to CD3 (T-lymphocyte population marker), CD79α (B-lymphocyte marker), CD 68 (macrophage marker), and to myeloperoxidase (MPO) protein (a marker of neutrophil and eosinophil granulocytes).

The obtained preparations were examined using a Carl Zeiss Primo Star microscope with simultaneous digital output of a ZEN 2 (blue edition) image system and taking pictures with a Zeiss AxioCam ERc 5s digital camera (Germany).

Data processing was carried out using Microsoft Excel 2010 software with the Attestat 12.0.5 application. The probability estimation of discrepancies of the compared indicators was carried out using the Student's t-test. Identification and assessment of the nature of the relationship between the indicators was carried out using rank correlation with the calculation of the non-parametric Spearman's rank correlation coefficient (r). The results were considered statistically significant at a probability level of more than 95% ($p<0.05$).

Research results and their discussion.

During the morphological study in the stroma and parenchyma of the tumor tissue of SAFT, the presence of lymphocyte-macrophage infiltration of varying degrees of severity was revealed – from insignificant, where isolated lymphocytes and small lymphoid infiltrates are determined, to more pronounced, in the form of focal clusters based on the type of formation of lymphoid follicles, mainly located on the border of the tumor – the perifocal zone. At the periphery of tumor tissue growth zones, an inflammatory rim is formed in places, represented by a cluster of immunocompetent cells (lymphocytes, macrophages, granulocytes). Cells of hematogenous origin infiltrating the tumor represent a heterogeneous population. Since it is impossible to determine their qualitative composition during histological examination, we differentiated them according to the expression of the corresponding markers during immunohistochemical examination.

The presence of a tumor-infiltrating inflammatory infiltrate in histological examination was observed in 27 cases (40.9%) of SAFT. The dependence of the presence of an inflammatory infiltrate on the degree of neoplasia differentiation is shown in **table.**

Table – Presence of inflammatory infiltrate in SAFT depending on the degree of neoplasia differentiation

The grade of differentiation of SAFT	The number of cases of SAFT with the presence of an inflammatory tumor microenvironment
G1	2
G2	7
G3	18

That is, the presence of an inflammatory infiltrate in the inter – and intratumoral zones of the tumor microenvironment prevails in poorly differentiated neoplasms ($p<0.05$). Therefore, the assumption that the accumulation of lymphocytes in tumor tissue is necessarily a favorable condition may not be true.

The cellular component of the microenvironment of SAFT is mainly represented by B-lymphocytes, which were located diffusely and have different density of distribution in the tumor stroma and tumor complexes and is from 30 to 80% of all cells **(fig. 1).**

When studying the number of T-lymphocytes, it was found that their number is from 10 to 50% of the total number of cells in the microenvironment. The expression of $CD3^+$ manifested

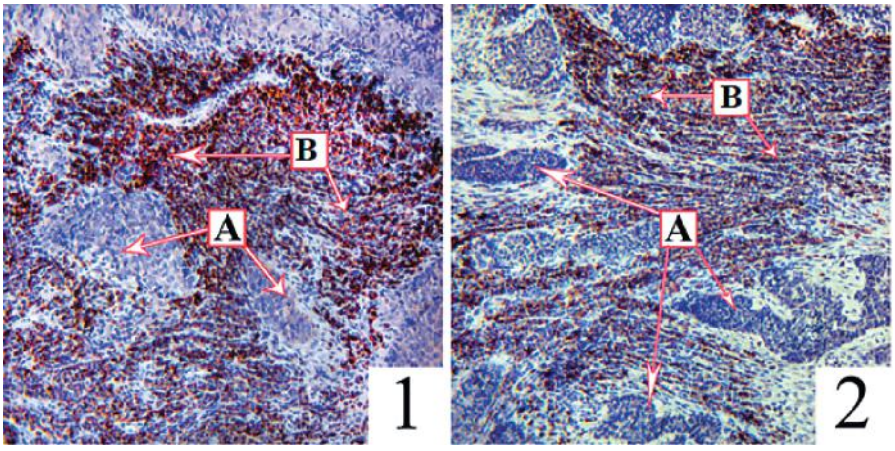

Figure 1 – SAFT of low differentiation: 1, 2 – IHC study of 79α receptors in tumor tissue of FT. Magnification: x100. Designation: A – cancer cells; B – diffusely located $79α^+$ lymphocytes.

in the form of brown staining of T-lymphocyte membranes of varying intensity in the inter – and intratumoral zones of the microenvironment with the predominant localization in the zones of SAFT invasion and the location of these cells mainly near the glandular component of the tumor **(fig. 2).**

Statistical processing of the results revealed a negative correlation between the level of B – and T-lymphocytes (r= -0.85, p<0.001). It was found that a decrease in the level of T-cell infiltration in the tumor tissue has a certain relationship with regional metastasis in the lymph nodes (t=9.68, p<0.001).

Macrophages ($CD68^+$-positive cells) were located in the form of infiltrates of varying severity with a predominant distribution at the periphery of SAFT **(fig. 3).** Additionally, the number of $CD68^+$ cells slightly decreased as the distance from the tumor increased. At the same time, the number of macrophages ranged from 1 to 20% of the total number of microenvironment cells. The increased density of $CD68^+$ cells in tumor tissue was significantly higher in the group of patients with PFTC with metastases in regional lymph nodes (t=8.85, p<0.001). According to our results, a low level of $CD3^+$-T-lymphocytes is associated with an increase in the level of $CD79\alpha^+$-B-lymphocytes and $CD68^+$-macrophages and indicates an unfavorable course of the disease.

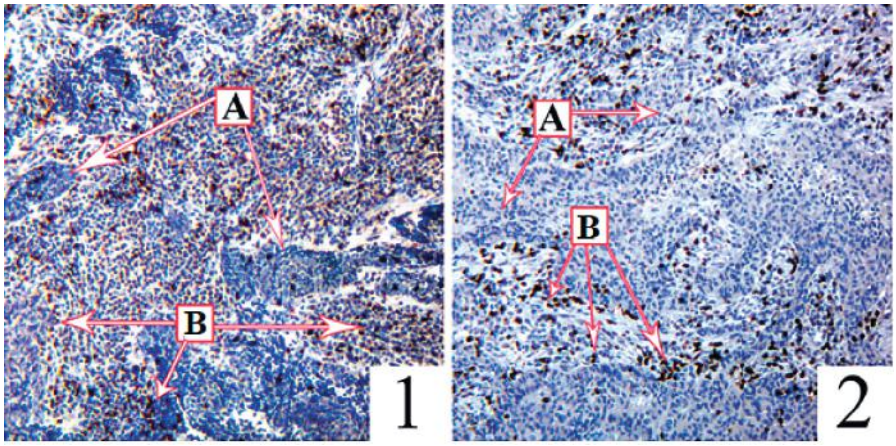

Figure 2 – SAFT of low differentiation: 1, 2 – IHC study of CD3 receptors in tumor tissue of FT. Magnification: x100. Designation: A – cancer cells; B – diffusely located $CD3^+$ T-lymphocytes.

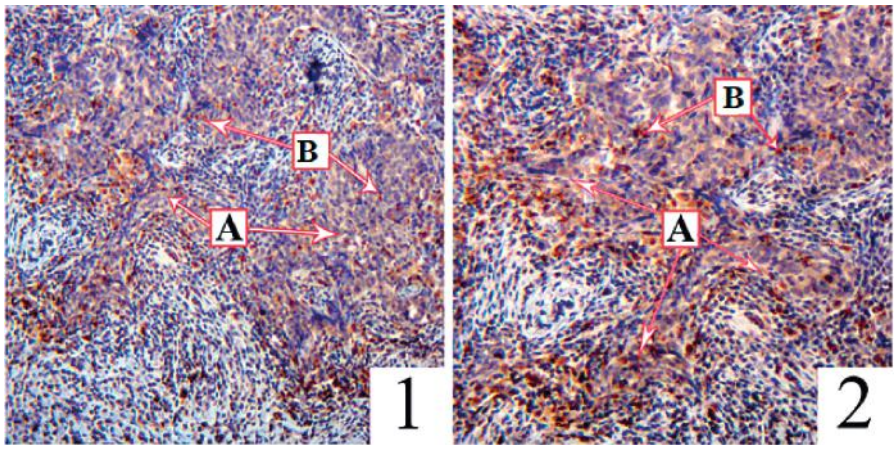

Figure 3 – SAFT of low differentiation: 1, 2 – IHC study of CD68 receptors in tumor tissue. Magnification: x100. Designation: A – cancer cells; B – diffusely located CD68+-cells (macrophages).

Detection of neutrophil and eosinophil granulocytes in the tumor microenvironment was carried out by studying the expression of myeloperoxidase (MPO). The expression of MPO manifested in the form of brown cytoplasmic staining of 1-8% of microenvironment cells, which were located individually or in small groups between the cells of the immune inflammatory infiltrate. A positive correlation was found between the presence of T-lymphocytes (r=0.4, p<0.05) and granulocytes, as well as a negative correlation between the presence of B-lymphocytes (r= -0.55, p<0.01), macrophages (r=0.5, p<0.001) and the presence of granulocytes in the tumor microenvironment.

When studying the relationship between ER and PR expression and the presence of an inflammatory immune infiltrate in the tumor tissue, a moderate negative correlation was found between the presence of T-lymphocytes in the tumor microenvironment and ER (r= -0.48, p<0.001) and PR (r= -0.43, p<0.001). A negative relationship of moderate strength was also found between the presence of B-lymphocytes, ER (r= -0.56, p<0.001) and PR (r= -0.50, p<0.001). Considering the fact that SAFT in the vast majority of cases (83.3%) is a hormone-dependent tumor, it becomes clear that the presence of an inflammatory immune microenvironment in cancer tissue reduces the level of receptor expression for steroid hormones.

When evaluating the expression of other prognostically important receptors (Ki-67, p53, VEGF) and the presence of an inflammatory infiltrate in SAFT, a correlation was found between the presence of T – and B-lymphocytes in the tumor microenvironment and Ki-67 expression (r=0.28, p<0.05 and r=0.27, p<0.05 respectively). A correlation of moderate strength was also found between the presence of T-lymphocytes (r=0.47, p<0.001), B-lymphocytes (r=0.58, p<0.001) and p53 expression in the tumor infiltrate. No statistically significant correlation was found between the presence of T-lymphocytes, B-lymphocytes, and macrophages in the tumor microenvironment and VEGF expression (p>0.05).

Conclusions.

The presence of an immune infiltrate in the tumor microenvironment is predominanatly localized in poorly differentiated neoplasms (66.7%). Its qualitative and quantitative composition is associated with a decrease in receptor expression for steroid hormones in the tumor tissue (p<0.001) and positively correlates with the intensification of Ki-67 (p<0.05) and p53 (p<0.001) expression. A decrease in the number of T-lymphocytes in the tissue of primary fallopian tube cancer and a significant infiltration of tumors by macrophages is associated with the promotion of regional metastasis in the lymph nodes and may be an indicator of an unfavorable

prognosis of neoplasms. That is why the study of the tumor microenvironment of serous adenocarcinoma of fallopian tubes can be used as a prognostic marker of the course of malignant tumors.

Prospects for further research.

To investigate the relationship between the immune microenvironment of uterine tube cancer and the level of expression of sex hormones in the epithelium.

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ВПЛИВ ІМУННОГО МІКРООТОЧЕННЯ НА ПЕРЕБІГ ТА ПРОГРЕСУВАННЯ ПЕРВИННОГО РАКУ МАТКОВИХ ТРУБ

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Резюме. Серед різних випадків злоякісних утворень рак маткових труб є найскладнішим для діагностики та лікування. Клітинне середовище має значний вплив на життєдіяльність ракових клітин і відіграє ключову роль у їх функціонуванні. Для оцінки прогностичного значення імунного інфільтрату у пухлині важливо враховувати як інтенсивність його присутності, так і склад клітин, що його утворюють.

Мета дослідження: провести оцінку якісного складу імунного середовища в тканині первинного серозного раку маткових труб.

Об'єкт і методи. Дослідження було здійснено на 66 зразках пухлинної тканини маткових труб. Для аналізу використовувалися молекулярно-біологічні маркери: Ki-67, p53, VEGF, ER, PR, CD3, CD79α, CD68 та мієлопероксидаза (MPO). Оцінку молекулярно-біологічних маркерів проводили відповідно до загальноприйнятих методик та протоколу виробника.

Результати. Була виявлена присутність пухлинного запального інфільтрату у 40,9% пацієнтів з первинним серозним раком маткових труб. Присутність та інтенсивність інфільтрації залежали від ступеня атипії ракових клітин ($p<0,05$). Клітинний склад мікрооточення пухлини складався переважно з В-лімфоцитів. Зменшення рівня Т-клітинної інфільтрації в пухлинній тканині мало певний зв'язок з метастазуванням у лімфатичні вузли ($p<0,001$). Присутність макрофагів у пухлинній тканині була значно вищою в групі пацієнтів з метастазами в регіонарних лімфатичних вузлах ($p<0,001$). Був встановлений помірний негативний кореляційний зв'язок між присутністю Т-лімфоцитів та ER ($r=-0,48$, $p<0,001$) і PR ($r=-0,43$, $p<0,001$). Також був виявлений негативний зв'язок помірної сили між присутністю В-лімфоцитів, ER ($r=-0,56$, $p<0,001$) та PR ($r=-0,50$, $p<0,001$). Встановлено кореляційний зв'язок між присутністю Т – і В-лімфоцитів в пухлинному середовищі та експресією Ki-67 ($r=0,28$, $p<0,05$ та $r=0,27$, $p<0,05$ відповідно). Також був встановлений кореляційний зв'язок помірної сили між присутністю Т – і В-лімфоцитів в пухлинному інфільтраті ($r=0,47$, $p<0,001$), а також експресією p53. Статистично значущого зв'язку між присутністю Т – і В-лімфоцитів, макрофагів у пухлинному середовищі та експресією VEGF не виявлено ($p>0,05$).

Висновки. Присутність запального пухлинного середовища переважно спостерігається у низькодиференційованих неоплазіях (66,7%) і її якісний та кількісний склад пов'язаний із зниженням експресії рецепторів стероїдних гормонів у пухлинній тканині ($p<0,001$) та корелює з експресією Ki-67 ($p<0,05$) та p53 ($p<0,001$). Зниження рівня CD3+-Т-лімфоцитів у тканині первинного раку маткових труб та значна інфільтрація пухлини CD68+ макрофагами пов'язані з регіональним метастазуванням і є індикатором несприятливого прогнозу.

Ключові слова: імунне мікрооточення, імунний інфільтрат пухлини, рак маткових труб, запальне мікрооточення пухлини, серозна карцинома маткових труб.

INFLUENCE OF THE IMMUNE MICROENVIRONMENT ON THE COURSE AND PROGRESSION OF PRIMARY FALLOPIAN TUBE CANCER

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Abstract. Among various cases of malignant formations, primary fallopian tube cancer stands out as the most challenging in terms of diagnosis and treatment. The cellular environment significantly influences the viability of cancer cells and plays a pivotal role in their functioning. To assess the prognostic significance of tumor immune infiltrate, it is crucial to consider not only its intensity but also the composition of the cells forming it.

Objective: to conduct an evaluation of the qualitative composition of the immune microenvironment in the tissue of primary serous fallopian tube cancer.

Object and methods. The study was conducted on 66 samples of tumor tissue from the fallopian tubes. Molecular-biological markers used for analysis included Ki-67, p53, VEGF, ER, PR, CD3, CD79α, CD68, and myeloperoxidase (MPO). The evaluation of molecular-biological markers was carried out according to generally accepted methodologies and the manufacturer's protocol.

Results. The presence of tumor-associated inflammatory infiltrate was detected in 40.9% of patients with primary serous fallopian tube cancer. The presence and intensity of infiltration depended on the degree of atypia of cancer cells ($p<0.05$). The cellular composition of the tumor microenvironment consisted predominantly of B-lymphocytes. Decreased levels of T-cell infiltration in tumor tissue were somewhat correlated with lymph node metastasis ($p<0.001$). The presence of macrophages in tumor tissue was significantly higher in the group of patients with metastases in regional lymph nodes ($p<0.001$). A moderate negative correlation was established between the presence of T-lymphocytes and ER ($r=-0.48$, $p<0.001$) and PR ($r=-0.43$, $p<0.001$). A negative correlation of moderate strength was also found between the presence of B-lymphocytes, ER ($r=-0.56$, $p<0.001$), and PR ($r=-0.50$, $p<0.001$). A correlation was established between the presence of T – and B-lymphocytes in the tumor microenvironment and the expression of Ki-67 ($r=0.28$, $p<0.05$ and $r=0.27$, $p<0.05$, respectively). A moderate correlation was also found between the presence of T – and B-lymphocytes in the tumor infiltrate ($r=0.47$, $p<0.001$) and the expression of p53. No statistically significant association was found between the presence of T – and B-lymphocytes, macrophages in the tumor microenvironment, and VEGF expression ($p>0.05$).

Conclusions. The presence of an inflammatory tumor microenvironment is predominantly observed in poorly differentiated neoplasms (66.7%), and its qualitative and quantitative composition is associated with a decrease in the expression of steroid hormone receptors in tumor tissue ($p<0.001$) and correlates with Ki-67 expression ($p<0.05$) and p53 ($p<0.001$) expression. A decrease in the level of CD3+ T-lymphocytes in the tissue of primary fallopian tube cancer and significant infiltration of the tumor by CD68+ macrophages are associated with regional metastasis and are indicators of an unfavorable prognosis.

Key words. immune microenvironment, tumor immune infiltrate, fallopian tube cancer, inflammatory tumor microenvironment, serous fallopian tube carcinoma.

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Conflict of interest:
The Authors declare no conflict of interest.

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