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ORIGINAL ARTICLE

LOCAL IMMUNITY FEATURES IN PATIENTS WITH NON-INVASIVE MUSCULAR BLADDER CANCER OF VARIOUS DEGREES OF MALIGNANCE

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ABSTRACT

Purpose of the study. To study the features of the local distribution of populations of immune system cells in patients with non-invasive muscular bladder cancer of various degrees of malignancy.

Materials and methods. The study included 51 patients with newly diagnosed non-muscle-invasive bladder cancer (papillary urothelial carcinoma) who received complex treatment and follow-up after 9 months at the oncourological department of the National Medical Research Center of Oncology. Patients were divided into two groups: group 1 – with a tumor of low malignant potential (Low grade – LG), *n* = 31; group 2 – with a tumor of high malignant potential (High grade – HG), *n* = 20. After 6–9 months, 24 patients were diagnosed with a relapse of the disease – in 48,4 % in patients of group 1 (*n* = 15) and in 45 % – in group 2 (*n* = 9). In cell suspensions obtained from the primary and recurrent tumors, as well as the perifocal zone, the relative number of populations of immunocompetent cells was estimated using flow cytometry. A comparison was made of the content of individual populations of lymphocytes in the tumor tissue, the perifocal zone of primary and recurrent lesions of various degrees of malignancy. Statistical processing was performed using Statistica 13.0.

Results. The development of a recurrent tumor of low malignant potential is accompanied by the involvement of cells of innate immunity (NK- and NKT-lymphocytes) into its microenvironment, which is associated with an imbalance in the number of main cells of adaptive immunity – a fairly pronounced decrease in the tumor of T-lymphocytes of the helper-inductor type was noted with a constant content cytotoxic T-lymphocytes, as well as the multidirectional nature of changes in DP- (decrease) and DN-lymphocytes (increase). A feature of the development of a recurrent tumor of high malignant potential is that it is accompanied by the involvement of innate immunity cells (NK- and NKT-lymphocytes) into its microenvironment, as well as multidirectional changes in DP- (decrease) and DN-lymphocytes (increase).

Conclusion. Studies of the population composition of tumors and their perifocal tissues of NMIBC revealed a number of features that are reflected in the redistribution of cytolytic cells, the formation of immunosuppressive conditions, which are reflected both in the manifestation of the biological properties of tumor cells and in changes in the cellular composition of bladder tissues involved in the process. development and progression of cancer.

Keywords:
bladder cancer, non-muscle-invasive bladder cancer, local cellular immunity, lymphocytes, relapse

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ОСОБЕННОСТИ ЛОКАЛЬНОГО ИММУНИТЕТА У ПАЦИЕНТОВ С НЕИНВАЗИВНО-МЫШЕЧНЫМ РАКОМ МОЧЕВОГО ПУЗЫРЯ РАЗЛИЧНОЙ СТЕПЕНИ ЗЛОКАЧЕСТВЕННОСТИ

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РЕЗЮМЕ

Цель исследования. Изучить особенности локального распределения популяций клеток иммунной системы у пациентов с немышечно-инвазивным раком мочевого пузыря различной степени злокачественности.
Материалы и методы. В исследование были включены 51 пациент с впервые выявленным немышечно-инвазивным раком мочевого пузыря (гистологическая верификация папиллярной уротелиальной карциномы), после комплексного лечения и динамического наблюдения в течение 9 мес. в онкоурологическом отделении ФГБУ «НМИЦ онкологии» Минздрава России. Пациенты были распределены на две группы: 1 группа – с опухолью низкого злокачественного потенциала (Low grade – LG), *n* = 31; 2 группа – с опухолью высокого злокачественного потенциала (High grade – HG), *n* = 20. Через 6–9 мес. у 24 пациентов был диагностирован рецидив заболевания – в 48,4 % у пациентов 1 группы (*n* = 15) и в 45 % – 2 группы (*n* = 9). В клеточных суспензиях, полученных из первичной и рецидивной опухоли, а также перифокальной зоны с использованием проточной цитометрии, оценивали относительное содержание иммунокомпетентных клеток. Проводили сравнение содержания отдельных популяций лимфоцитов в ткани опухоли, перифокальной зоны первичных и рецидивных образований различной степени злокачественности. Статистическая обработка выполнялась с использованием Statistica 13.0.
Результаты. Развитие рецидивной опухоли низкого злокачественного потенциала сопровождается привлечением в её микроокружение клеток врожденного иммунитета (NK- и NKT-лимфоцитов), что сопряжено с дисбалансом в количестве основных клеток адаптивного иммунитета – отмечено достаточно выраженное снижение в опухоли Т-лимфоцитов хелперно-индукторного типа при неизменном содержании цитотоксических Т-лимфоцитов, а также разнонаправленном характере изменения ДП- (снижение) и ДН-лимфоцитов (увеличение). Особенностью развития рецидивной опухоли высокого злокачественного потенциала является то, что оно сопровождается привлечением в её микроокружение клеток врожденного иммунитета (NK- и NKT-лимфоцитов), а также разнонаправленным изменением ДП- (снижение) и ДН-лимфоцитов (увеличение).
Заключение. Проведенные исследования популяционного состава опухолей, так и их перифокальных тканей немышечно-инвазивного рака мочевого пузыря позволили выявить особенности, отражающиеся в перераспределении цитолитических клеток, формированием иммуносупрессивных условий, отражающихся как на проявлении биологических свойств опухолевых клеток, так и на изменении клеточного состава тканей мочевого пузыря, вовлеченного в процесс развития и прогрессирования онкологического заболевания.

Ключевые слова:
рак мочевого пузыря, немышечно-инвазивный рак мочевого пузыря, локальный клеточный иммунитет, лимфоциты, рецидив

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RELEVANCE

According to the latest GLOBOCAN data, malignant neoplasms (MN) of the bladder account for 3 % of all cancer diagnoses in the world, a high prevalence is observed in developed countries [1]. According to world statistics, it continues to occupy the 10th line in the structure of general oncological morbidity [2]. Almost 75 % of urothelial carcinomas are non–invasive carcinomas with a high frequency of recurrence of the disease after surgical treatment without infiltration of the bladder wall or distant metastases, the remaining 25 % are a muscle-invasive form of bladder cancer, which is highly invasive and with the presence of distant metastases [3]. Among patients with superficial papillary lesions, multiple relapses are usually observed, and only 10–30 % of them develop invasive tumors of a high degree of malignancy [4].

Diagnostic methods for assessing non-musculoskeletal invasive bladder cancer (NMIBC), as well as relapse and progression, have a number of disadvantages: low sensitivity and specificity, therefore, there is a need to study this direction for more thorough diagnosis and detection of malignant neoplasms (MN) at the early stages of the development of relapse of the disease for adequate and timely treatment and selection management tactics of patients [5].

Currently, it is generally accepted that the development of tumors of various localization accompanies a violation of the antigenic homeostasis of the human body, which, at certain stages of the development of the pathological process, naturally causes the activation of various effector mechanisms of innate and adaptive immunity. Further development of neoplasm is accompanied by its complex interaction with other anatomical and physiological structures of the body, through the implementation of a number of stages, the study of which in order to identify new diagnostic and prognostic markers is an urgent task of modern oncoimmunology [6].

It has been shown that the distribution pattern and density of immune cells in the tumor reflect the activity of the immune system against tumor cells. In this context, various populations of immune cells have been studied by Russian and foreign authors. The close interaction of microenvironment cells with each other and with tumor cells leads to a change in their phenotype, gene expression and changes in functional activity. Previous studies have revealed that the density of T-lymphocytic infiltration by CD3- , CD8- or CD45R0-positive lymphocytes has a high prognostic value for various tumors. So, Sharma P. and co-authors demonstrated the best relapse-free survival and overall survival in 69 patients with muscle-invasive bladder cancer (IMBC) with a high content of CD8+ lymphocytes [7]. In addition, it has been shown that high levels of intracellular infiltration by CD3- and CD8-positive lymphocytes suggest better overall survival results among patients with BC [8].

However, despite the available information on the study of the role of individual cells of the immune system in the development of tumors and, in particular, BC, much remains unclear, including the features of the distribution of immune cells (IC) between the tumor and its perifocal zone, which may contribute to the nature of the development of the pathological process and the effectiveness of the treatment methods used. In connection with the above, the study of the role of the local distribution of cells of the immune system is an urgent task to identify new potential markers of the development of BC and the likelihood of its recurrence.

The aim of the study was to study the features of the local distribution of immune system cell populations in patients with noninvasive muscle bladder cancer of various degrees of malignancy.

MATERIALS AND METHODS

The study examined tumor tissue samples from 51 patients with newly diagnosed NMIBC. After the complex treatment in the volume: transurethral resection of the bladder + adjuvant intravesical chemotherapy No. 6 (TUR + IUCT), all patients were dynamically monitored for 9 months after the complex treatment. According to the results of histological analysis, patients with papillary urothelial carcinoma were divided into two groups: group 1 – with a tumor of low malignant potential (Low grade – LG), n = 31; Group 2 – with a tumor of high malignant potential (High grade – HG), n = 20. Every 3 months, patients underwent a control examination, including a cystoscopic examination of the bladder, as a result of which, after 6–9 months, 24 patients were diagnosed with a relapse of the disease – in 48.4 % of patients of group 1 (n = 15) and in 45 % of group 2 (n = 9).

Written informed consent was received from all patients to participate in the study.

Complex treatment was recommended to all patients with newly diagnosed NMIBC as treatment: TUR + IUCT. Intraoperatively, the material was collected – fragments of the tumor (TF) and the perifocal zone (PZ), as well as a similar sampling was carried out in patients with a relapse of the disease, which was detected during 6–9 months of dynamic observation. The preparations were delivered to the laboratory, where they were subjected to mechanical crushing followed by tissue homogenization using a BD Medimachine homogenizer, USA (2 ml of Cell Wash buffer was added to the TF fragments, homogenized for 30 seconds, the cell suspension was filtered using 50 μm Medicons, USA). The cells were deposited in a refrigerated centrifuge Eppendorf Centrifuge 5702R (Eppendorf AG, Germany) at 250g for 5 minutes. After removal of the supraplastic fluid, the cells were resuspended in 100 μl of "Cell Wash" buffer.

The cell suspension was treated with a panel of monoclonal antibodies: CD3 FITC/CD15+56 PE/CD45 PerCP-Cy5.5/CD4 PE-Cy7/CD19 APC/CD8 APC–Cy7 in accordance with the manufacturer's instructions (BD, USA). The results were evaluated using a FacsCantoII flow cytometer (BD, USA). At least 100,000 cells were accumulated in each sample for data analysis. The relative (percentage) content of cells of the desired phenotype to the total number of living cells was estimated. The content of individual populations of lymphocytes was compared (total number of lymphocytes (CD45+ cells, Lymph); CD45+CD3+ cells (total CD3+ lymphocytes); CD45+CD3+CD4+ cells (helper T-lymphocytes (Th)); CD45+CD3+CD8+ cells (cytotoxic T-lymphocytes (CTL)); CD45+CD3+CD4+CD8+ cells (double positive lymphocytes, DP); CD45+CD3+CD4-CD8 cells (double negative lymphocytes, DN); CD45+CD16+CD56+ cells (NK-lymphocytes); CD45+CD3+CD16+CD56+ cells (NKT-lymphocytes); CD45+CD19+ cells (B lymphocytes)) in the tumor tissue, the perifocal zone of primary and recurrent formations of varying degrees of malignancy.

Statistical processing was carried out using the STATISTICA 13 package (StatSoft Inc., USA). The nature of the distribution of the obtained data was evaluated using the Shapiro-Wilk criterion. Since the obtained results of the evaluation of the determined parameters did not obey the law of normal distribution, they are presented in the form of median (Me) and interquartile range – 25 and 75 percentiles (Me [LQ; UQ]). The reliability of the differences was assessed using the nonparametric Mann-Whitney criterion. The results were considered statistically significant at $p < 0.05$.

RESEARCH RESULTS AND DISCUSSION

When analyzing the nature of the development of the tumor process, the likelihood of disease progression and the peculiarities of the local immunological status, a special role is assigned to identifying the distribution of individual populations of ICC between the tumor tissue itself and its perifocal zone.

The results of such a comparison in low-grade tumors are presented in Figures 1, 2, 3.

It can be seen from the presented results that, in comparison with the tumor in the perifocal zone, there is an increase in the content of a number of lymphocytes: CD4+, CD45+CD3+CD4+CD8+, CD45+CD3+CD4-CD8- and CD45+CD19+ cells, respectively, by 30 % (45.8 (41.9; 46.9) vs. 35.1 (33,7; 41,2), p = 0,048), 133 % (0,7 (0,49; 1,4) against 0.3 (0.2; 0.5), p = 0,041), 93 % (6,2 (5,9; 8,1) against 3.2 (2.4; 3.9), p = 0.042) and 85 % (10.9 (7.5; 14.3) vs. 5.9 (2.8; 7.3), p = 0.035). The tendency to decrease in the relative number of CD8+ T-lymphocytes and CD45+CD16+CD56+ in the CD is noteworthy, which indicates their accumulation in the tumor tissue (Fig. 1).

In the case of considering the features of the distribution of IC between primary TFand its PP in patients with subsequent relapse (a group of primary relapsing), in this case, an increase in lymphoid infiltration of PP compared with TFwas revealed, which is probably realized due to an increase in cells with potential cytolytic activity – CD8+, CD45+CD16+CD56+ lymphocytes (Fig. 2). These indicators were higher in the PP compared to the TF by 130 % (38.2 (24.2; 47.9) vs. 16.6 (8.8; 21.1), p = 0,037), 23 % (50.4 (53.9; 67.6) against 41.0 (37.2; 44.9), p = 0.048) and 316 % (20.8 (12.4; 25.8) vs. 5.0 (3.9; 7.3), p = 0.028). Against this background, a significant decrease in CD45+CD16+CD56+ and CD45+CD19+ cells in the PP with their probable accumulation in the tumor was revealed (Fig. 2). These indicators in the PP were lower than the values in the tumor by 54 % (3.5 (2.3; 4.5) vs. 7.6 (5.8; 10.2), p = 0.035) and 53 % (4.7 (3.9; 8.3) vs. 10.0 (7.9; 17.2), p = 0.042).

Probably, in conditions of increasing the likelihood of further relapse, there is a redistribution of IC between the tumor tissue and the perifocal zone with

an increase in the latter lymphocytic infiltration, CD8+ and CD45+CD3+CD16+CD56+, as well as a decrease in CD4+, CD45+CD19+ and CD45+CD16+CD56+ cells. At the same time, the multidirectional nature of the distribution of the effector cells of innate immunity is revealed – NK lymphocytes accumulate in the tumor, while NKT lymphocytes concentrate in the perifocal zone.

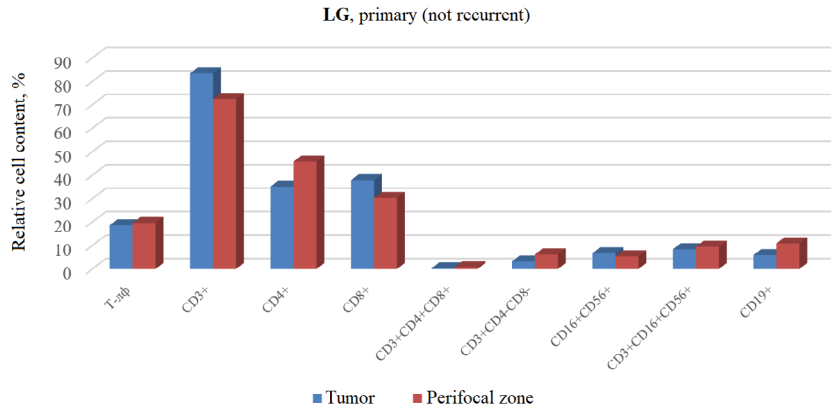

Fig. 1. Percentage of IC in the tumor and perifocal zone of patients with low-grade NMIBC, group 1 (LG) primary (non-recurrent).
Note: * – $p < 0.05$.

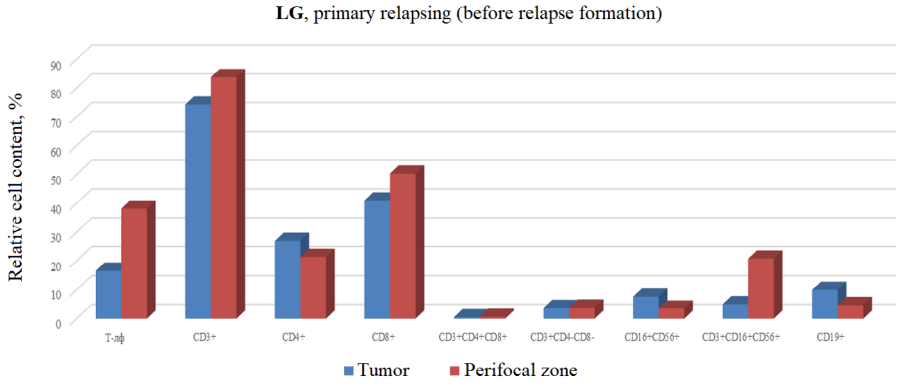

Fig. 2. Percentage of IC in the tumor and perifocal zone of patients with low-grade NMIBC, group 1 (LG) Primary relapsing (before relapse).
Note: * – $p < 0.05$.

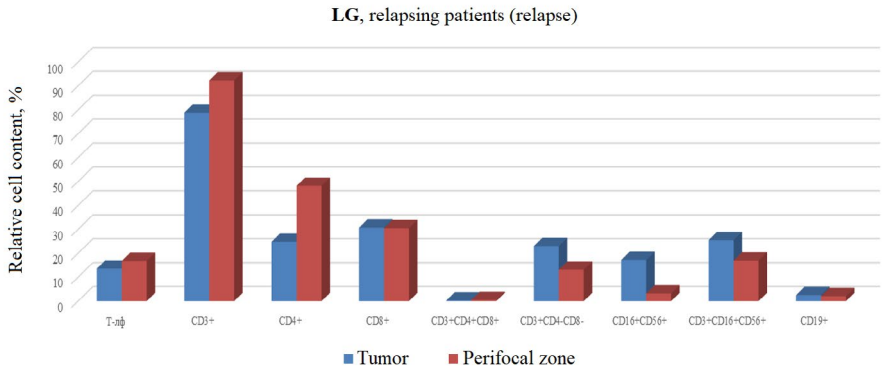

Fig. 3. Percentage of IC in the tumor and perifocal zone of patients with low-grade NMIBC, group 1 (LG) – relapsing patients (relapse).
Note: * – $p < 0.05$.

The analysis of the obtained results of determining the features of the distribution of IC in a recurrent tumor and its perifocal zone revealed the following (Fig. 3).

With the development of relapse, the accumulation of CD4+ and CD45+CD3+CD4+CD8+ cells in the tumor was observed, the content of which exceeded the values in the TF by 94 % 48.2 (40.3; 56) vs. 24.8 (16.1; 33.4), p = 0.021) and 67 % (0.5 (0.41; 0.75) vs. 0.3 (0.25; 0.35), p = 0.045). Against the background of the noted changes in the PD, a decrease in the number of CD45+CD3+CD4-CD8- (DN-lymphocytes) and effector cells of innate immunity – NK- and NKT-lymphocytes, which accumulate in the tumor, was found. The specified parameters in the PP were lower than in the TF by 43 % (13.1 (8; 18.2) versus 23.0 (18.5; 30), p = 0.046), 81 % (3.2 (2.6; 3.7) against 17.2 (10; 24.3), p = 0.033) and 33 % (17.0 (12; 21.9) vs. 25.5 (20.4; 30.5), p = 0.037).

Thus, the development of a recurrent tumor of low malignant potential is accompanied by the involvement of innate immunity cells (NK- and NKT-lymphocytes) into its microenvironment, which is associated with an imbalance in the number of main cells of adaptive immunity – a sufficiently pronounced decrease in helper-inductor type T-lymphocytes in the tumor was noted with a constant content of cytotoxic T-lymphocytes, as well as the multidirectional nature of the changes in DP- (decrease) and DN-lymphocytes (increase).

The results of comparing the distribution of IC populations between PP and TF in high-grade bladder tumors are presented in Figures 4, 5, 6.

From the presented results, it can be seen that compared with the tumor in the perifocal zone, there is an increase in the content of a number of lymphocytes: CD8+, CD45+CD3+CD4+CD8+, CD45+CD16+CD56+ and CD45+CD3+CD16+CD56+ cells, respectively, by 50 % (33.9 (27.1; 49.3) against 22.6 (16.4; 25.8), p = 0.026), 350 % (1.8 (1.4; 3.6) against 0.4 (0.25; 1.45), p = 0.008), 92 % (7.1 (4.9; 14.3) against 3.7 (2.6; 5.5), p = 0.031) and 134 % (13.8 (10.6; 20.3) vs. 5.9 (3.4; 6.6), p = 0.017). There was a decrease in the relative number of CD4+ and CD45+CD19+ cells in the PP, respectively, by 26 % (29.6 (16.8; 33.3) versus 39.8 (35.4; 47.9), p = 0.045) and 50 % (5.9 (2.5; 7.3) versus 11.7 (10.5; 16.4), p = 0.042), which indicates their accumulation in the tumor tissue (Fig. 4).

In the case of considering the features of the distribution of IC between the primary TF and its PP in patients with subsequent relapse (a group of primary relapsing), in this case, an increase in the infiltration of PP compared with the main populations of adaptive and innate immunity was revealed (Fig. 5). CD4+ content in the PP was statistically significant compared with the OP, CD8+ and CD45+CD3+CD4+CD8+ cells, as well as CD45+CD16+CD56+ and CD45+CD3+CD16+CD56+ cells, respectively, by 26 % (50.0 (49; 51.6) vs. 39.8 (30.6; 48.2), p = 0.037), 51 % (28.3 (27.9; 28.7) vs. 18.7 (12.7; 26.6), p = 0.046), 57 % (0.55 (0.52; 0.57) against 0.35 (0.23; 0.43), p = 0.047), as well as by 117 % (6.3 (4.8; 9.3) vs. 2.9 (2.1; 4.7), p = 0.041) and 52 % (6.4 (6.2; 8.6) vs. 4.2 (2.9; 5.1), p = 0.043). Against this background, a significant decrease in the content of B-lymphocytes in the PS with their probable accumulation in the tumor was revealed. These indicators in the PP were 46 % lower than the values in the tumor (11.4 (11.1; 19.3) vs. 21.0 (18.9; 30), p = 0.044).

Probably, in conditions of an increase in the likelihood of further relapse, there is a redistribution of IC between the tumor tissue and the perifocal zone with an increase in adaptive and innate immunity cells in the latter, potentially possessing cytolytic activity, as well as a decrease in the number of B lymphocytes.

The analysis of the obtained results of determining the features of the distribution of IC in a recurrent tumor and its perifocal zone revealed the following (Fig. 6).

With the development of relapse, CD45+CD3+CD4+CD8+ cells and CD45+CD19+ cells accumulated in the tumor, the content of which exceeded the values in the TF by 50 % (0.3 (0.25; 0.39) vs. 0.2 (0.1; 0.24), p = 0.047) and 396 % (27.3 (15.1; 32.4) vs. 5.5 (3.7; 6.05), p = 0.005). Against the background of the noted changes in the PD, a decrease in the number of total lymphoid infiltration, as well as CD45+CD3+CD4-CD8- (DN-lymphocytes) and effector cells of innate immunity – CD45+CD3+CD16+CD56+ (NKT-lymphocytes), which accumulate in the tumor, was found. The specified parameters in the PP were lower than in the TF by 54 % (20.8 (12.4; 29.2) vs. 45.2 (32; 48.7), p = 0.044), 88 % (1.5 (1.1; 3.5) against 12.2 (7.0; 15.3), p = 0.039) and 42 % (3.6 (2.1; 3.7) vs. 6.2 (3.6; 6.1), p = 0.042).

The data obtained earlier in our laboratory, which are also consistent with the results of similar studies, indicate that the immune microenvironment of tu-

mors largely contributes to their progression, among the variants of which are metastasis and recurrence [9]. Despite the fact that the latter is often regarded as a surgical problem arising due to the non-radicality of the operation, more and more data are accumulating indicating that a number of cellular factors, such as USC, as well as cells of the immune microenvironment, are involved in relapse. In particular, the review by Yan Chen et al. in 2022, which is a meta-analysis of recent studies on the role of the microenvironment in

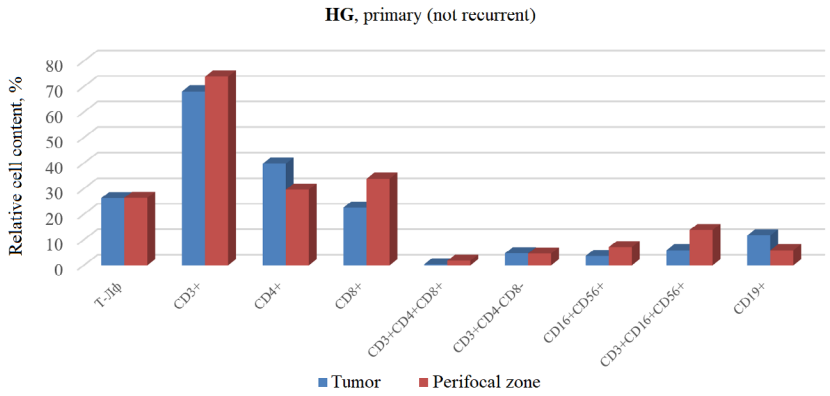

Fig. 4. The percentage of IC in the tumor and perifocal zone of patients with low-grade NMIRMP, group 2 (HG) primary (not recurrent). Note: * – $p < 0.05$.

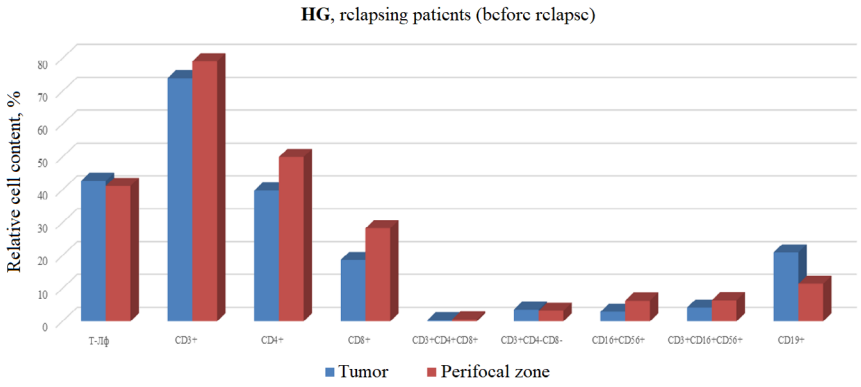

Fig. 5. Percentage of IC in the tumor and perifocal zone of patients with low-grade NMIBC, group 2 (HG) Primary relapsing (before relapse formation). Note: * – $p < 0.05$.

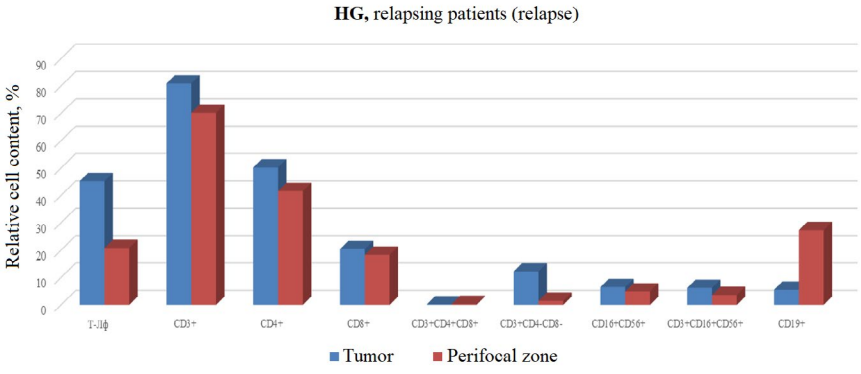

Fig. 6. Percentage of IC in the tumor and perifocal zone of patients with low-grade NMIBC, group 2 (HG) – relapsing patients (relapse). Note: * – $p < 0.05$.

the recurrence of gastric cancer, emphasizes the role of a high number of CD8+, CD4+Tm, NK lymphocytes, M1 macrophages, and a low content of M2 macrophages, Tregs lymphocytes, mast cells for long-term relapse-free survival of patients [10].

It is assumed that some minor subpopulations of T-lymphocytes, in particular, DP and DN, may play an important role in the process of relapse. According to the results of the study of the number of DP T-lymphocytes in the blood of patients with tumors of urological localization, including RMP, an increase in the level of these cells was found, and their heterogeneity was revealed, represented by CD4highCD8low and CD4+CD8 high DP subpopulations with that phenotype and related to Th2 [11].

DN T cells also attract a lot of attention. The review by Zhiheng Wu et al., 2022, summarizes reports on the multidirectional effects of these cells on tumor growth – from stimulation to antigen-independent cytolysis and the possibility of using them for adoptive immunotherapy [12]. At the same time, their phenotypic and functional heterogeneity is noted, as well as a change in their activity in the tumor microenvironment [13].

CONCLUSION

The conducted studies of the population composition of tumors and their perifocal tissues of NMIRMP revealed some features that are characterized by the development of a recurrent tumor of high malignant potential (HG) by the involvement of innate immunity cells (NK- and NKT-lymphocytes) in its microenvironment, as well as the multidirectional nature of changes in DP- (decrease) and DN-lymphocytes (increase). The emerging immunosuppressive conditions affect both the manifestation of the biological properties of tumor cells and the change in the cellular composition of bladder tissues involved in the development and progression of cancer.

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Shevchenko A. N. – development of research design, systematization and analysis of the data obtained, consultation, final conclusions;
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Hvan V. K. – collection of clinical material;
Khomutenko I. A. – collection of clinical material;
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