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Changes In Tumor Infiltrating Lymphocytes Of Peripheral Blood And Tissue During Chemotherapy In Patients With Gastric Cancer

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ABSTRACT

To study the state of systemic immunity and local immunity before and during chemotherapy in patients with gastric adenocarcinoma.

From 2017 to 2018 at the Tashkent city branch of the Republican specialized scientific and practical medical center of oncology and radiology 20 primary metastatic patients with gastric adenocarcinoma received chemotherapy. The sampling of biological material (peripheral blood, tumor tissue) was carried out twice (before treatment and during the first control examination, after 3 courses). The percentage of the degree of infiltration of tumor tissue by lymphocytes (CD45+CD14-TILs) was estimated by flow cytometry; T cells (CD3+CD19-TILs); B cells (CD3-CD19+TILs); NK cells (CD3-CD16+CD56+TILs); CD16 and CD8 effector cells and their cytotoxic potential (CTP) (CD16+Perforin+TILs; CD16CTPTILs), (CD8+Perforin+TILs; CD8CTPTILs); regulatory T cells - NKT cells (CD3+CD16+CD56+TILs), CD4 (CD4+CD25+CD127-TILs) and CD8 (CD8+CD11b-CD28-TILs) regulatory cells and these parameters of systemic results. The factor of a favorable prognosis for PFS in patients with metastatic gastric cancer in the peripheral blood was an increase in the number of CD8 + T-regulatory

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cells (5.1% - 12.1%, p = 0.019), and in tumor tissue - an increase in the perforin potential of effector CD16 cells (0.5% - 4.9%, p = 0.030) and their cytotoxic potential (13.2% - 55.7%, p = 0.011). When assessing the changes in the indices of local immunity during chemotherapy, it was noted a negative effect of an increase of T cells (22.0% - -9.7%, p = 0.012), NKT - cells (207.9% - -13.8%, p = 0.002) and CD4 + T-regulatory cells (190.7% - -25.2%, p = 0.002). In contrast, an increase in the level of effector CD16 cells during chemotherapy increases the likelihood of surviving PFS - 9 months (-69.5% - 9.1%, p = 0.013).

Indicators of local and systemic immunity serve as additional prognostic factors for gastric cancer.

KEYWORDS

Metastatic gastric cancer; cellular immunity; local immunity, chemotherapy.

INTRODUCTION

Gastric cancer (GC) ranks 5th place among oncology diseases (1,313,000 cases) and is the 3rd cause of death from cancer (819,000 deaths) in the world [1]. Currently, there is no doubt that a malignant tumor is a dynamic system, considered in combination with all morphological components that form its microenvironment: stromal cells, cells of the immune system, blood and lymphatic vessels and extracellular matrix [1,2]. Tumor-infiltrating lymphocytes (TILs) are the subject of active research [3-5].They play a key role in the concept of "antitumor immunity". In addition, the cells of systemic immunity are responsible for both suppressing tumor growth [6] and, on the contrary, correlate with a poor prognosis [7,8]. Thus, over the past 20-30 years, great success has been achieved in the treatment of gastric cancer. An important step in the development of new therapeutic agents was the understanding of the role of prognostic and predictive factors, including the significance of the subpopulation composition of immunocompetent cells and tumor biology.

The aim of this work was the comprehensively study the structure of tumor-infiltrating lymphocytes (TILs), systemic immunity before and during treatment in metastatic patients with gastric adenocarcinoma.

MATERIALS AND METHODS

The prospective study included patients with metastatic gastric cancer (GC) who were treated at the Tashkent city branch of the Republican specialized scientific and practical medical center of oncology and radiology in the period from 2017 to 2018. Patients underwent 9 two-week cycles of chemotherapy (CTx) of the 1st line or 6 three-week courses with subsequent observation until the disease progressed. Analysis of the immune status by morphological material and peripheral blood was carried out twice (before the start of treatment and after 3-4 courses of chemotherapy). The main criteria for the inclusion of patients in the study: age over 18

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years, morphological verification of the tumor - gastric adenocarcinoma, regardless of the stage of the disease. The main exclusion criteria were: a history of inflammatory diseases in the last 3 months, supportive antibacterial and immunomodulatory therapy at the time of enrollment in the study.

LABORATORY METHODS

The analysis of indices of subpopulations of peripheral blood lymphocytes and tumor tissue was carried out by flow cytometry in order to determine the structure of immune cells: the degree of infiltration of tumor tissue by lymphocytes (CD45+CD14-TILs); T cells (CD3+CD19-TILs); B cells (CD3-CD19+TILs); NK cells (CD3-CD16 +CD56+TILs); effector cells CD16 (CD16+Perforin+TILs), CD8 (CD8+Perforin+TILs) and their cytotoxic potential - CD16CTPTILs and CD8CTPTILs; subpopulations of regulatory T cells - NKT cells (CD3+CD16+CD56+TILs), regulatory CD4 (CD4+CD25+CD127-TILs) and CD8 (CD8+CD11b-CD28-TILs) cells and these parameters of cellular immunity.

FLOW CYTOMETRIC ANALYSIS

The structure of subpopulations of immunocompetent cells was assessed by binding to monoclonal antibodies of various specificities by multivariable quantitative analysis on a FACSCalibur flow cytometer (BD Biosciences). For each sample, at least 500–5000 cells were analyzed in a CD45+gate. DotPlot analysis of cytograms was used with the commercial BD CellQuest PRO software (BD Biosciences). Further processing of the FSC files of primary cytometric data was performed using the WinMDI software package, version 2.8.

STATISTICAL PROCESSING OF RESULTS

Statistical processing of the material and calculations of indicators were carried out using the statistical software package Statistica for Windows v.10 and SPSS v21. The significance of differences between quantitative indicators was calculated by the Student's t test for normally distributed values or by the nonparametric Mann – Whitney and Wilcoxon tests. To compare the qualitative 2 parameters, Fisher's exact test and ⍰2 were used. Differences were considered significant at p <0.05 (95%accuracy). The degree of relationship between the parameters was assessed using the Spearman correlation analysis.

Statistical processing of the material and calculations of indicators were carried out using the statistical package of licensed programs Statistica for Windows v. 10 (univariate analysis, Spearman correlation analysis, descriptive statistics comparing quantitative indicators according to Mann–Whitney, Kaplan–Meier analysis) and SPSS v21 (ROC curves, multivariate analysis). Quantitative variables deviated from the normal distribution (Kolmogorov – Smirnov test) and are presented by the median indicating the 25th and 75th quartiles. Categorical variables were expressed as percentages and absolute values.

The statistical significance of differences between quantitative indicators was calculated using the Student's t-test using the nonparametric Mann – Whitney and Wilcoxon tests. To compare the qualitative parameters, Fisher's exact test and $\chi 2$ were used.

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Differences were considered significant at p <0.05 (acceptable level of α-error 5%). The degree of relationship between the parameters was assessed using the Spearman correlation analysis. Determination of the boundaries with the optimal ratio of sensitivity and specificity was performed by constructing the ROC curve.

RESEARCH RESULTS

Patient characteristics

The study included 20 patients with mGC - 9 (45%) men and 11 (55%) women. The age of the patients ranged from 44 to 64 years (mean age 58). Depending on the division of patients into age groups - up to 45 years old; 46-60 years old; over 60 years old, patients mainly belonged to the 3rd group - 9 (45%), respectively. In patients with mGC, the tumor was poorly differentiated - 12 (60%) and represented by the intestinal type Lauren 12 (60%). HER2neu status was positive in 6 (30%) patients. In 20 patients, no disturbances in the repair system were noted (MSS). The average follow-up time for patients was 16.4±6.2 months. (from 0.7 to 23.6 months, median 18.5 months). In the surgical group, 2 patients (4%) had a high level of MSI.

Study of cellular immunity in peripheral blood. The results are shown in Table 1.

Table 1. Dynamics of indices of the immunophenotype of peripheral blood lymphocytes during treatment in patients with metastatic gastric cancer.

Indicators of cellular immunity in the blood	Before chemotherapy		During chemotherapy		p
	median	quartiles	median	quartiles	
CD3+CD19-	75,4	66,9-81,8	77,9	65,6-85,2	0,090
CD3-CD19+	0,7	0,6-1,5	1,3	0,6-2,6	0,053
CD3-CD16+CD56+	20,2	9,4-24,6	13,8	9,3-26,2	0,864
CD3+CD4+	37,2	29,8-51,6	39,7	34,8-47,3	0,233
CD3+CD8+	24,7	20,4-36,2	31,4	28,3-35,7	0,140
CD16+Perforin+	17,7	14,6-27,2	14,1	5,9-20,2	**0,031**
CD16CTP	81,9	60,9-93,2	64,3	49,2-83,4	**0,009**
CD8+Perforin+	22,9	10,5-27,3	17,1	11,5-26,8	0,691
CD8CTP	64,3	45,7-66,0	49,3	41,8-70,9	0,112

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CD3+CD16+CD56+	11,4	8,8-20,0	16,2	11,7-18,7	0,069
CD4+CD25+CD127-	6,2	5,2-8,4	47,0	32,3-62,5	**0,007**
CD8+CD11b-CD28-	12,1	10,2-17,6	18,4	11,7-45,6	**0,031**

Thus, the percentage of effector CD16+cells and their cytotoxic potential before chemotherapy was higher than during treatment (p= 0.031; p=0.009). However, after 3-4 courses of chemotherapy, there is a statistically significant increase in the percentage of T-regulatory cells with the phenotypes CD4+CD25+ CD127- (p=0.007) and CD8+CD11b-CD28 (p=0.031).

To assess the possibility of predicting early progression in gastric cancer patients according to blood parameters already before the start of treatment, we compared the results of the study of systemic cellular immunity in subgroups where the progression of the disease was recorded up to 6 months and later (Table 2).

Table 2. Differences in blood counts before treatment depending on the risk of early progression in patients with metastatic gastric cancer (up to 6 months, n = 5/after 6 months, n=15)

Indicators of cellular immunity in the blood before treatment	Progression up to 6 months (n=5)		Progression after 6 months (n=15)		**p**
	median	quartiles	median	quartiles	
CD3+CD19-	64,1	63,6-64,2	75,4	66,9-81,8	0,168
CD3-CD19+	1,3	1,1-1,6	0,7	0,6-1,5	0,081
CD3-CD16+CD56+	30,6	30,2-31,1	20,2	9,4-24,6	0,168
CD3+CD4+	37,4	35,0-40,4	37,2	29,8-51,6	1,000
CD3+CD8+	24,2	20,5-24,9	24,7	20,4-36,2	0,553
CD16+Perforin+	26,9	24,3-30,0	17,7	14,6-27,2	0,306
CD16CTP	84,1	81,3-88,1	81,9	60,9-93,2	0,933
CD8+Perforin+	25,2	24,1-29,0	22,9	10,5-27,3	0,230

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CD8CTP	76,4	68,9-78,4	64,3	45,7-66,0	0,142
CD3+CD16+CD56+	8,5	7,6-10,8	11,4	8,8-20,0	0,306
CD4+CD25+CD127-	8,1	6,5-8,4	6,2	5,2-8,4	0,800
CD8+CD11b-CD28-	5,1	2,3-8,6	12,1	10,2-17,6	**0,019**

It follows from the table that a high level of CD8 T-regulatory cells with the CD8+CD11b-CD28- phenotype before treatment is a predictor of a favorable prognosis (p = 0.019).

The nature of changes in indicators during chemotherapy can also be informative for predicting early progression, so we compared the magnitude of this change depending on the timing of progression.

However, in patients examined in dynamics, early progression was not noted (up to 6 months). Therefore, to analyze the dynamics of indicators, we have chosen the border of the progression time frame in 9 months. Among the "linear" structure of lymphocytes, the median of T and B cells increases in patients with progression before and after 9 months, 8.3% versus 2.4%; 283.3% versus 22.9%, respectively. The level of NK cells decreases in patients with breast cancer with progression up to 9 months, and increases back with progression after 9 months (-35.7% versus 10.2%). Medians of CD4+ and CD8+ cytotoxic lymphocytes before and during therapy increased in patients with gastric cancer with progression before and after 9 months, 27.2% versus 4.2%; 11.7% versus 26.9%, respectively.

The medians of effector CD16 and CD8 cells decreased in patients with disease progression up to 9 months and grew back with progression after 9 months - 69.5% versus 9.1%, - 1.8% versus 11.4%, respectively. The values of their cytotoxic potential decreased in both cases, –21.5% versus –16.2%, –9.6% versus –1.2%, respectively. At the same time, the percentage of effector CD16 cells with the CD16+ Perforin+ phenotype changed significantly depending on the time of progression (before and after 9 months) - 69.5% versus 9.1%, respectively (p=0.013). The deltas of the values of T-regulatory cells with the CD4+ and CD8+ phenotype, as well as NKT cells, grew and amounted to 750.0% versus 494.1%, 0.6% versus 141.0% and 44.3% versus 34.5%, respectively.

Thus, before treatment, patients with mGC are characterized by an increase in the level of effector CD16+ T-cells (p=0.031) and their cytotoxic potential (p=0.009), while 3-4 courses of chemotherapy increase the level of CD4+ and CD8 + T-regulatory cells (0.007), (0,031) respectively. At the same time, the level of CD8+ T-regulatory cells is a predictor of a favorable prognosis for progression-free survival (PFS). Patients with a high level of CD8+ T-regulatory cells before the start of treatment have a statistically significantly higher probability of surviving PFS-6 months (p=0.019). However, an increase in the level of effector CD16 cells during chemotherapy increases the likelihood of surviving PFS-9 months (p=0.013).

Study of the cellular composition of tumor tissue by flow cytometry.

The results are shown in Table 3.

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Table 3. Dynamics of indicators of the immunophenotype of lymphocytes, infiltrating the tumor, during treatment in metastatic gastric cancer patients.

Indicators of cellular immunity in tissue	Before chemotherapy		During chemotherapy		p
	median	quartiles	median	quartiles	
CD45+CD14-TILs	161,0	59,0-178,0	83,0	45,0-220,0	0,778
CD3+ CD19-TILs	86,6	72,2-92,0	84,0	78,9-91,2	0,683
CD3-CD19+TILs	4,2	0,7-17,7	0,0	0,0-3,0	0,096
CD3-CD16+CD56+TILs	6,9	2,7-10,1	6,1	2,4-13,6	0,826
CD3+CD4+TILs	42,0	26,6-64,6	24,4	14,4-45,0	0,096
CD3+CD8+TILs	3,1	1,5-3,9	5,4	1,7-9,5	**0,009**
CD16+Perforin+TILs	5,0	3,9-7,1	6,2	3,6-12,5	0,074
CD16CTPTILs	55,7	25,1-56,8	64,7	52,1-78,3	**0,012**
CD8+Perforin+TILs	9,5	5,3-49,6	38,5	7,2-46,5	0,059
CD8CTPTILs	19,4	11,5-86,7	67,5	26,6-81,2	0,099
CD3+CD16+CD56+TILs	10,3	5,9-13,7	11,1	9,5-19,4	0,221
CD4+CD25+CD127-TILs	8,6	3,3-16,2	11,5	5,2-15,6	0,638
CD8+CD11b-CD28-TILs	47,9	33,3-69,6	43,1	38,1-50,0	0,638

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It follows from the table that the cytotoxic potential of CD16+TILs (p=0.012) and CD3+CD8+TILs (p=0.009) cells increases statistically significantly during chemotherapy in patients with breast cancer.

To assess the ability to predict early progression in breast cancer patients already before starting treatment, we similarly compared the results of a study of local immunity in subgroups where disease progression was recorded up to 6 months and later (Table 4).

Table 4. - Differences in tumor tissue before treatment, depending on the risk of early progression in patients with metastatic gastric cancer (up to 6 months, n=5/after 6 months, n=15).

Indicators of cellular immunity in tissue before treatment	Progression up to 6 months (n=5)		Progression after 6 months (n=15)		p
	median	quartiles	median	quartiles	
CD45+CD14-TILs	3,5	3,3-7,3	2,3	1,7-10,5	0,662
CD3+CD19+TILs	85,9	73,6-87,0	86,6	72,2-92,0	1,000
CD3-CD19+TILs	3,1	2,6-5,3	4,2	0,7-17,7	0,540
CD3-CD16+CD56+TILs	6,0	3,6-7,3	6,9	2,7-10,1	0,725
CD3+CD4+TILs	30,0	22,6-53,7	42,0	26,6-64,6	0,662
CD3+CD8+TILs	46,6	46,3-67,5	30,7	20,0-54,7	0,096
CD16+Perforin+TILs	0,5	0,0-1,9	4,6	3,9-7,1	**0,030**
CD16CTPTILs	13,2	0,0-17,4	55,7	25,1-56,8	**0,011**
CD8+Perforin+TILs	4,1	0,0-22,4	9,5	5,3-49,6	0,161
CD8CTPTILs	5,4	0,0-59,9	19,4	11,5-86,7	0,334
CD3+CD16+CD56+TILs	20,4	16,9-22,0	10,3	5,9-13,7	0,054
CD4+CD25+CD127-TILs	10,7	6,1-15,0	8,6	3,3-16,2	0,793
CD8+CD11b-CD28-TILs	41,2	31,1-52,9	42,3	33,3-69,6	0,553

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Thus, a low level of perforin potential of effector CD16 cells (p=0.030) and their cytotoxic potential (p=0.011) before chemotherapy is an unfavorable factor and may indicate a higher risk and probability of progression up to 6 months.

When assessing the nature of changes in indicators during chemotherapy to predict early progression, we also chose the 9-month progression time limit. It was noted that patients with progression up to 9 months are characterized by a decrease in the degree of infiltration of tumor tissue with lymphocytes, NK cells, perforin potential of effector CD8 cells and cytotoxic potential of CD16 (-7.5% - 18.1%, p=0.031) and CD8-cells, as well as CD8+T regulatory cells, and, on the contrary, an increase in T cells (22.0% - -9.7%, p=0.012), CD8+CTP, perforin potential of effector CD16 cells, NKT cells (207, 9% - -13.8%, p=0.002) and CD4+ T-regulatory cells (190.7% - -25.2%, p=0.002).

However, in patients with progression after 9 months, there is an increase in the degree of infiltration of tumor tissue with lymphocytes, NK cells, perforin potential of effector CD8 cells and cytotoxic potential of CD16 and CD8 cells and CD8+ T regulatory cells, and the level of T cells, CTLs of CD8+ cells , the perforin potential of effector CD16 cells, NKT cells and CD4+ T regulatory cells decreases.

Thus, patients with gastric cancer with progression up to 9 months are characterized by a statistically significant increase in T cells (p=0.012), NKT cells (p=0.002), and CD4+ T regulatory cells (p=0.002). On the contrary, an increase in the perforin potential of effector CD16 cells (p=0.030) and their cytotoxic potential (p=0.011) is a favorable prognostic factor and increases the likelihood of achieving long-term PFS.

These changes in the indices of local and systemic immunity in dynamics indicate an increase in cytotoxic CD8+ lymphocytes, NKT cells and CD4+ T regulatory cells in peripheral blood and tumor tissue during treatment.

However, a high level of CD8+ T regulatory cells in peripheral blood and a low level of perforin potential of effector CD16 cells and their cytotoxic potential in tumor tissue prior to treatment indicate a low and high risk and probability of progression up to 6 months, respectively. At the same time, an increase in the level of effector CD16 cells in peripheral blood and tumor tissue before and during treatment is a favorable prognostic factor and increases the likelihood of progression after 9 months.

On the contrary, an increase in T cells, NKT cells, and CD4+ T regulatory cells in tumor tissue is characteristic of patients with gastric cancer with a probability of progression up to 9 months.

DISCUSSION

Cancer development is a complex process that depends on the interaction of individual cells in the tumor, the microenvironment, and the immune system, which can both stimulate and suppress tumor growth and invasion [9].

Our study provides a new understanding of the mechanisms underlying T-cell dysfunction during chemotherapy in gastric cancer patients. Thus, in 20 patients with metastatic gastric cancer after 3-4 courses of chemotherapy, there was a decrease in the perforin potential of CD16 + cells (CD16+Perforin+), 17.7% versus 14.1%, (p=0.031) and the cytotoxic potential of CD16+ cells (CD16CTP), 81.9% - 64.3%, (p=0.009).

On the contrary, the content of regulatory minor populations of peripheral blood lymphocytes with phenotypes

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CD4+CD25+CD127- and CD8+ CD11b-CD28 increased statistically significantly after 3-4 courses of chemotherapy and amounted to 6.2% - 47.0%, (p=0.00007) ; 12.1% - 18.4%, (p=0.031), respectively. However, the results of the study by He Q. et al. including 105 patients with locally advanced gastric cancer are multidirectional. Blood samples were collected before and 1 week after the last administration of chemotherapy. The percentage of CD3+CD8+ lymphocytes increased after chemotherapy, and the content of CD4+CD25+CD127 regulatory T cells decreased (p=0.003 and p<0.001, respectively).

The increase of T-lymphocytes is due to the activation of T-cell immunity, but in our study there was only a slight tendency to their growth after 3-4 courses of chemotherapy, from 24.7% to 31.4%. However, when assessing the magnitude of changes in systemic immunity indicators before and during chemotherapy from the "linear" structure of peripheral blood, only T-cells (CD3+CD19-) have a tendency to their increase. In addition, in patients with a high level of CD3+CD8+CTLs and a low level of CD4+CD25+CD127-Tregs, an increase in OS was noted (p=0.012% and p=0.048%, respectively).

According to a number of studies in patients with various types of neoplasms, a correlation was found between CD3+CD19- T cells and CD3+ CD8+CTLs and a favorable prognosis [143;155]. However, according to He Q. et al., The content of CD3+CD19- T cells and CD3+CD4+CTLs after chemotherapy did not change and did not correlate with the clinical course of the disease, which is consistent with the results of our study [10].

Accordingly, the correlation between CD4+CD25+CD127-Tregs and clinical outcome points to their key role in the prognosis of gastric cancer patients and may serve as a biomarker for identifying patients with the best response to neoadjuvant chemotherapy.In comparing the results of the study of systemic cellular immunity in subgroups where the progression of the disease was recorded in terms of up to 6 months and later, it was revealed that a high level of CD8 T-regulatory cells before treatment is a predictor of a favorable prognosis, 12.1% versus 5.1% , (p=0.019).

Our results are consistent with a recent study of the subpopulation composition of peripheral blood lymphocytes in patients with head and neck squamous cell carcinoma (HNSCC). When analyzing 29 patients with early (I-II stages, n = 16) and late (III-IV stages, n = 13) stages of HNSCC, it was revealed that patients with early stages of HNSCC (group A) had a statistically significant increase in the percentage of CD8+CD11b-CD28 lymphocytes, 40.0% - 28.6% [11]. According to the results of the analysis Zabotina T.N. and others in the analysis of 29 patients with early and late stages of HNSCC in patients with I-II stages there was an increase in the content of CD16 cells compared with III, IV stages, 22.1% versus 18.0% [11].

Accordingly, an increase in the level of effector CD16 cells with the CD16+Perforin+ phenotype during chemotherapy increases progression-free survival, which is also consistent with our results.

Taking into account our results and the data of foreign researchers, it can be assumed that in the peripheral blood of patients with gastric cancer, the implementation of effector functions during chemotherapy occurs at the expense of CD16 cells, thereby increasing the likelihood of surviving PFS - 9 months.

A number of studies on the distribution of lymphocyte subpopulations in tumor tissue have shown that TILs are associated with the

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response to chemotherapy and the prognosis of the disease [12].

Thus, in our study, when assessing changes in the subpopulation composition in tumor tissue before and during chemotherapy, a statistically significant increase in the percentage of the cytotoxic potential of effector CD16+TILs and CD3+CD8+TILs was revealed, amounting to 3.1% versus 5.4%, (p=0.009), 55.7% versus 64.7%, (p=0.012), respectively. An increase in the value of CD3+CD8+ TILs together with CD3+CD16+CD56+ TILs, CD3+CD19-TILs after chemotherapy is also observed in patients with locally advanced breast cancer [13].

Further, a comparative analysis of changes in local and systemic immunity indices in dynamics revealed an increase in cytotoxic CD8 lymphocytes, NKT cells, and CD4-T regulatory cells in peripheral blood and tumor tissue in patients with gastric cancer. According to some authors, an increase in the level of Tregs both in the tumor microenvironment and in the peripheral blood is recorded in patients with gastrointestinal tract cancer, breast cancer, pancreatic cancer, hepatocellular cancer, etc. [14; 15; 16].

Despite conflicting opinions about changes in peripheral blood lymphocytes and in tumor tissue samples in gastric cancer, in a recent Japanese study of 44 patients with cervical cancer, a comparative analysis of immune cells in peripheral blood and tumor tissue revealed that the amount of CD3 + CD19- T- lymphocytes are significantly higher in tumor tissue than in peripheral blood 68.58% - 55.59%, (p <0.001). However, there was a significant decrease in the population of CD3+CD4+T cells in tumor tissue compared with peripheral blood 54.04% versus 45.85%, (p<0.013), while the level of CD3+CD8+ T cells increased significantly in tumor tissue compared with peripheral blood 32.37% versus 44.92%, (p<0.001).

Moreover, the proportion of NK cells was significantly higher in tumor tissue than in peripheral blood, 0.33% versus 4.38% (p <0.079). When comparing the distribution of various subsets of lymphocytes between relapsing patients and patients without signs of progression in tumor tissue of 35 patients, a statistically significant increase in CD3+CD4+TILs in patients without progression was found 48.07% versus 33.12%, (p=0.018) and an increase in CD3+CD8+TILs in recurrent patients with cervical cancer 57.92% versus 42.87%, (p=0.015). However, the data of 44 patients on peripheral blood were significantly insignificant [17].

CONCLUSION

Accordingly, the results showed that the distribution of lymphocyte subpopulations in the peripheral blood is not an optimal method for predicting the disease, while the determination of lymphocyte subpopulations in tumor tissue has prognostic significance. The results obtained in this study confirm that the most complete information on the state of the antitumor immune response in cancer patients can be obtained only with the simultaneous study of immune cells of peripheral blood and cells infiltrating tumor tissue.

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