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Comprehensive Assessment of the Immunophenotype of Peripheral Blood Lymphocytes, Inflammatory Factors, the Immunophenotype of Lymphocytes Infiltrating the Tumor, and Clinical Indicators for Overall Survival and Progression-Free Survival in Patients with Gastric Cancer

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Abstract: To study the state of cellular immunity and local immunity in patients with gastric adenocarcinoma.

From 2017 to 2018 at the N.N. Blokhin NMRC of Oncology 45 primary patients with gastric adenocarcinoma (25 - with stages I – III, 20 - with stage IV) received surgical/combined treatment or chemotherapy, respectively. Peripheral blood and tumor tissue were collected before starting treatment. The percentage of the degree of infiltration of tumor tissue by lymphocytes (CD45+CD14-TILs) was assessed by flow cytometry; T cells (CD3+CD19-TILs); B cells (CD3-CD19+TILs); NK cells (CD3-CD16+CD56+TILs); effector cells CD16 and CD8 and their cytotoxic potential (CD16+Perforin+TILs; CD16CTPTILs), (CD8+Perforin+TILs; CD8CTPTILs); subpopulations of regulatory T cells- NKT cells (CD3+CD16+CD56+TILs), regulatory CD4 cells (CD4+CD25+CD127-TILs) and CD8 (CD8+CD11b-CD28-TILs) and parameters of systemic immunity. Intratumoral and stromal subpopulations of CD4+TILs, CD8+TILs, CD4+/CD8+TILs ratios were studied by immunohistochemistry. Also, the cellular composition of peripheral blood was investigated. The prognostic significance of immune cells, inflammation factors (neutrophil-lymphocyte index, platelet-lymphocyte index) and clinical characteristics (patient's age (both by years and by groups: up to 45 years, 46-60 years, over 60 years), disease stage, degree of differentiation (G), Lauren type and MSI status for overall survival (OS) and progression-free survival (PFS).

Results. The factor of a favorable prognosis for PFS in patients with local and locally advanced forms of gastric cancer was an increase in the number of CD3+CD19-TILs (HR0.865,95%CI0.782–0.957,p=0.005), and poor prognosis - an increase in NK cells; HR1.382,95%CI1.087–1.758,p=0.008. There was a negative effect of the relative content of NK cells, an increase in the level of neutrophils in the peripheral blood on the OS of patients with metastatic GC(HR1.42,95%CI1.06-1.89,p=0.017 and HR1.64,95%CI1.12–2.40,p=0.011).At the same time, an increase in the age of patients, the level of neutrophils and platelets (HR1.106, 95%CI1.002-1.199, p=0.015; HR1.714, 95%CI1.063-2.764,p=0.027 and HR1.017,95%CI1.006-1.029, p=0.003) reduce PFS in patients with metastatic gastric cancer.

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Indicators of local immunity, the cellular composition of peripheral blood, characterizing the systemic inflammatory response, as well as indicators of systemic immunity can serve as additional prognostic factors in gastric cancer.
Key words: gastric adenocarcinoma; cellular immunity; local immunity; subpopulation of lymphocytes; neutrophil-lymphocyte index; platelet-lymphocyte index.

Introduction

Gastric cancer (GC) ranks 5th 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 lymph 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 suppression of tumor growth [6] and, on the contrary, correlate with a poor prognosis [7,8]. The predictive value of systemic inflammatory indices based on the calculation of the ratios of neutrophils, lymphocytes and platelets in the peripheral blood of patients with colorectal cancer [9], gastric cancer [5,10] and hepatocellular cancer [11] makes it possible to use these indicators in clinical practice at the stage of planning complex treatment patients with malignant neoplasms, and as early as possible to identify signs of a relapse of the disease. 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 to comprehensively study the structure of tumor-infiltrating lymphocytes (TILs), systemic immunity and inflammation indices in patients with gastric adenocarcinoma and assess their prognostic significance.

Materials and methods

The prospective study included gastric cancer patients undergoing treatment at the N.N. Blokhin National Medical Research Center of Oncology in the period from 2017 to 2018. Analysis of the status of systemic immunity in the peripheral blood, local immunity in the tumor tissue was carried out once, before the start of treatment. The main criteria for the inclusion of patients in the study: age over 18 years, morphological tumor verification - 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 inclusion in the study. The treatment was carried out according to existing standards (stages I – III - surgical or combined treatment; stage IV - drug therapy).

Laboratory methods

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

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(CD3+CD16+ CD56+TILs), regulatory CD4 (CD4+CD25+CD127-TILs) and CD8 (CD8+CD11b-CD28-TILs) cells and these parameters of cellular immunity. In order to determine the spatial distribution of intratumoral (iTILs) and stromal (sTILs) subpopulations of lymphocytes, the indices of subpopulations of CD4 + TILs were studied by immunohistochemistry; CD8+TILs and CD4+/CD8+TILs ratios. To identify the prognostic value of clinical markers of inflammation, the following ratios were calculated (based on the results of a clinical blood test at the initial assessment stage): 1) neutrophil-lymphocyte index (NLI); 2) platelet-lymphocyte index (PLI).

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 FACSC 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.

Immunohistochemical analysis

The following primary monoclonal antibodies were used on formalin-fixed paraffin tissue samples: anti-CD4 clone 113 (dilution 1:200; Sino Biological, BDA, Beijing, PR China) and anti-CD8SP16 clone (dilution 1:150; Thermo Scientific, Fremont, CA). The number of immune cells was determined separately in the intratumoral and peritumoral tissues. By microscopic examination (×400; BX51; Olympus, Tokyo, Japan), each section was assessed for the presence of immune cells. The number of immune cells in 10 fields was accumulated and then averaged to calculate the average for 1 computerized 400 x microscopic field (0.1590 mm2/field).

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 using the Student's t test for normally distributed values or by the nonparametric Mann – Whitney and Wilcoxon tests. To compare qualitative 2 parameters, Fisher's exact test and кач е2 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 (one-way 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 represented by the median indicating the 25th and 75th quartiles. Categorical variables were expressed as percentages and absolute values.

The statistical significance of the differences between the 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. 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

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the boundaries with the optimal ratio of sensitivity and specificity was performed by constructing the ROC curve.

Main parameters to be assessed

OS and PFS were the main endpoints in this study. OS was defined as the time from the moment of diagnosis of gastric cancer to the moment of death of the patient from any cause or to the date of the last contact with the patient. PFS was defined as the time from the initiation of treatment of the disease to the time of registration of the growth of existing manifestations of the disease or the appearance of new metastatic foci. Comparison of survival curves - using the log-rank test. Survival rates were calculated from real data on the life expectancy of each patient at the end of the study using the Kaplan – Meier method.

Research results

Patient characteristics

The study included 45 patients with gastric cancer - 19 (42.2%) men and 26 (57.8%) women. The age of the patients varied from 37 to 80 years (mean age 60.9 ± 10.9 years, median 62 years). 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 group 3 (18 (72.0%) and 9 (45%) in groups I and II, respectively. 25 (55.6%) patients with localized gastric cancer received surgical treatment (of which 16 (64%) patients received adjuvant therapy) and 20 (44.4%) stage IV patients received chemotherapy (group II) at the N.N.Blokhin NMRC of Oncology in 2017–2018 yy.

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 groups I and II, the tumor is poorly differentiated (56% and 60%) and is represented by the intestinal type Lauren (64% and 60%). 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. Indicators of systemic cellular immunity in patients with gastric cancer.

Indicators of systemic immunity	**Surgical group (I) (n = 25)**		**Chemotherapy group (II) (n = 20)**		**p**
	median	**quartiles**	**median**	**quartiles**	
CD3+CD19-	74,3	71,4-84,7	72,5	64,2-81,8	0,560
CD3-CD19+	1,6	1,3-2,2	1,0	0,6-1,5	**0,017**
CD3-CD16+CD56+	11,8	7,3-25,0	21,3	9,4-30,4	0,140
CD3+CD4+	39,7	29,6-45,5	37,3	32,0-49,1	0,828
CD3+CD8+	32,8	23,0-41,2	24,7	20,5-33,6	0,074
CD16+Perforin+	12,1	8,5-18,7	19,1	14,6-27,2	**0,016**
CD16CTP	66,7	42,5-75,5	81,9	63,0-90,3	**0,010**
CD8+Perforin+	19,0	14,6-25,3	23,5	12,8-27,3	0,515
CD8CTP	55,6	42,9-66,3	64,3	52,0-72,7	0,084
CD3+CD16+CD56+	15,9	8,7-25,1	11,1	8,7-18,4	0,167
CD4+CD25+CD127-	7,7	6,2-9,1	6,9	5,4-8,4	0,134
CD8+CD11b-CD28-	9,5	6,2-13,7	11,3	7,7-13,2	0,457

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The study of the subpopulation structure of peripheral blood lymphocytes in 45 patients with gastric cancer showed a decrease in the number of B cells at stage IV compared to stage I-III (1.0 versus 1.6; p = 0.017). On the contrary, in comparison with group I in group II patients, the content of CD16 + Perforin + effector lymphocytes and their cytotoxic potential was higher than 19.1% versus 12.1% (p = 0.016); 81.9% and 66.7%, (p = 0.010), respectively.
Investigation of the cellular composition of tumor tissue by flow cytometry. The results are shown in Table 2.

Table 2. Indicators of local immunity in patients with gastric cancer.

Indicators of local immunity	Surgical group (I) (n = 25)		Chemotherapy group (II) (n = 20)		p
	median	quartiles	median	quartiles	
CD45+CD14-TILs	6,8	1,9-14,0	3,4	1,9-10,5	**0,001**
CD3+ CD19-TILs	76,6	65,3-85,9	86,3	72,2-89,9	0,112
CD3-CD19+TILs	6,0	1,6-25,6	4,2	1,7-17,7	0,343
CD3-CD16+CD56+TILs	3,2	1,4-5,2	6,9	3,2-9,8	**0,019**
CD3+CD4+TILs	37,4	27,8-49,6	37,0	26,6-55,6	0,954
CD3+CD8+TILs	30,6	22,4-43,2	39,0	27,2-54,7	0,204
CD16+Perforin+TILs	0,5	0,0-1,2	4,3	1,9-6,0	**0,002**
CD16CTPTILs	17,9	0,0-30,8	42,3	15,3-56,3	0,181
CD8+Perforin+TILs	1,8	0,7-3,1	7,7	3,0-28,6 0	**0,002**
CD8CTPTILs	4,6	2,6-9,2	16,7	5,7-65,1	**0,034**
CD3+CD16+CD56+TILs	5,1	2,3-7,5	11,2	6,1-13,8 0	**0,002**
CD4+CD25+CD127-TILs	15,4	6,6-20,9	9,7	4,7-16,2	0,228
CD8+CD11b-CD28-TILs	53,8	43,2-60,3	41,9	33,3-69,6	0,262

Subgroup analysis of tumor-infiltrating lymphocytes (TILs) in 45 patients with gastric cancer showed that the median percentage of TILs (CD45+CD14-TILs) was significantly higher in patients with stages I – III of the disease compared with mR: 6.8% versus 3.4 % (p = 0.001). On the contrary, the median of the percentage of NK cells (CD3-CD16+CD56+TILs) increases as the stage of the disease increases from 3.2% in group I to 6.9% in group II (p = 0.019). The median percentage of effector CD16 (CD16+Perforin+TILs) in patients with mGC is significantly higher than in patients with local and locally advanced forms of gastric cancer 4.3–0.5%, (p= 0.002).
A similar pattern was found for the effector cells CD8 (CD8+Perforin+TILs) and CD8CTP, amounting to 7.7-1.8% (p=0.002) and 16.7-4.6% (p=0.034), respectively. A high content of NKT cells (CD3+CD16+CD56+TILs) was observed in patients with mGC compared with stages I – III of the disease 11.2–5.1% (p=0.002).
Study of the cellular composition of tumor tissue by immunohistochemistry. The results are shown in Table 3.

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Table 3. Indicators of local immunity in patients with gastric cancer.

Indicators of local immunity		Surgical group (I) (n = 25)		Chemotherapy group (II) (n = 20)		p
		median	quartiles	median	quartiles	
CD4+ TILs (cells/f.v.)	CD4+TILs	22,2	10,5- 38,5	0,4	0- 1,1	**0,001**
	CD4+iTILs	1,1	0,4- 7,4	0,2	0- 1,1	0,021
	CD4+sTILs	21,9	7,7- 26,5	0	0- 0	**0,003**
CD8+ TILs (cells/f.v.)	CD8+TILs	53,5	39,1- 106,9	3,0	0- 9,0	**0,002**
	CD8+iTILs	24,1	4,9- 37,3	3,0	0- 9,0	**0,0027**
	CD8+sTILs	38,0	11,7- 54,9	0	0-0	**0,004**
CD4+/ CD8+ TILs	CD4+/CD8+TILs	0,35	0,19- 0,54	0,04	0- 0,32	**0,001**
	CD4+/CD8+iTILs	0,06	0,007- 0,59	0,04	0- 0,27	0,157
	CD4+/ CD8+sTILs	0,48	0,20- 0,61	0	0-0	**0,002**

cells/f.v. – cells in the field of view.

Subgroup analysis of subpopulations of lymphocytes CD4+TILs; CD8+TILs, the ratio of CD4+/CD8+TILs by the method of immunohistochemistry revealed that the number of stromal TILs was statistically significantly higher in group I compared with group II, in whose patients these subpopulations of sTILs were not found (medians, 21.9 versus 0, p=0.0003; 38.0 versus 0, p=0.000004; CD4+/CD8+, p=0.00001, respectively). A significant relationship of the smallest value of CD4+ iTILs and CD8+iTILs was less frequently observed in patients with mGC (p=0.02, rs=0.70; p=0.0027, rs=0.65) compared with localized stages of the disease.
Study of indicators of clinical markers of inflammation. The results are shown in Table 4.

Table 4. Indicators of clinical markers of inflammation in patients with gastric cancer.

Indicators of clinical markers of inflammation		Surgical group (I) (n = 25)		Chemotherapy group (II) (n = 20)		p
		median	quartiles	median	quartiles	
Peripheral blood parameters (10^9/ l)	neutrophils	4,90	3,17-8,84	3,63	3,04-5,17	0,147
	platelets	265,0	222,0-311,0	270,0	221,0-348,5	0,793
	lymphocytes	2,13	1,65-2,75	1,68	1,44-2,40	0,337

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Inflammation indices	NLI	2,29	1,36-4,99	2,19	1,55-2,85	0,954
	TLI	120,19	92,11-168,56	154,0	113,0 - 228,76	0,278

When comparing the medians of peripheral blood indices and relative indices of inflammation in patients with local, locally advanced and metastatic forms of gastric cancer, no significant differences were found. At the same time, an increase in the number of neutrophils and NLI occurs mainly in men and is more pronounced in group I (r = –0.58; r = –0.74). An increase in the number of platelets is more common in the intestinal type of gastric cancer and is mainly presented in group II (r = –0.66).

The prognostic value of the immunophenotype of peripheral blood lymphocytes, inflammation factors, the immunophenotype of lymphocytes infiltrating the tumor, and clinical parameters in patients with gastric cancer.

During the observation period 18.5 (15.2–20.4) months. 3 (12%) of 25 patients in group I and 10 (50%) of 20 patients in group II (p = 0.007) died from the progression of the underlying disease. Disease progression was registered in 29 (62.2%) patients: in 20 (100%) in group II and in 9 (36%) in group 1. In group I, 1-year OS was 92.0 ± 5.4%, in group 2-70.0 ± 10.2%; 1-year PFS-64.0±9.6 and 10.0±6.7%, respectively.

To determine independent prognostic signs that affect the PFS and OS indicators, a sequential Cox regression analysis was performed. It included indicators of systemic immunity, indicators of systemic inflammation and indicators of local immunity, determined by flow cytometry and immunohistochemistry.

To assess the predictive value of survival indices, we used:

- NLI <1.43 - low, NLI = 1.43-3.05 - normal and NLI> 3.05 - high level
- PLI<111.6 - low, PLI = 111.6-208.7 - normal and PLI> 208.7 - high level.

According to univariate analysis (UVA), for OS of group I, CD3 + TILs (HR0.894; 95%CI0.813-0.983; p = 0.021), CD3+CD8+TILs (HR-0.782;95%CI 0.631-0.970; p = 0.026) and CD8+CD11b-CD28-TILs (HR-0.935; 95% CI 0.885-0.989; p = 0.018) were significant, respectively. For PFS in group I patients, CD3+TILs (HR-0.915; 95% CI 0.862-0.971; p = 0.003), CD3-CD16+CD56+TILs (HR-1.195; 95%CI1.042-1.369;p=0.011), PLI by ranges (RR-0.21; 95% CI 0.06-0.72; p = 0.013), CD4+/CD8+iTILs (RR-3.26; 95% CI 1.09-9.78 ; p = 0.035), respectively. (Figure 1.2)

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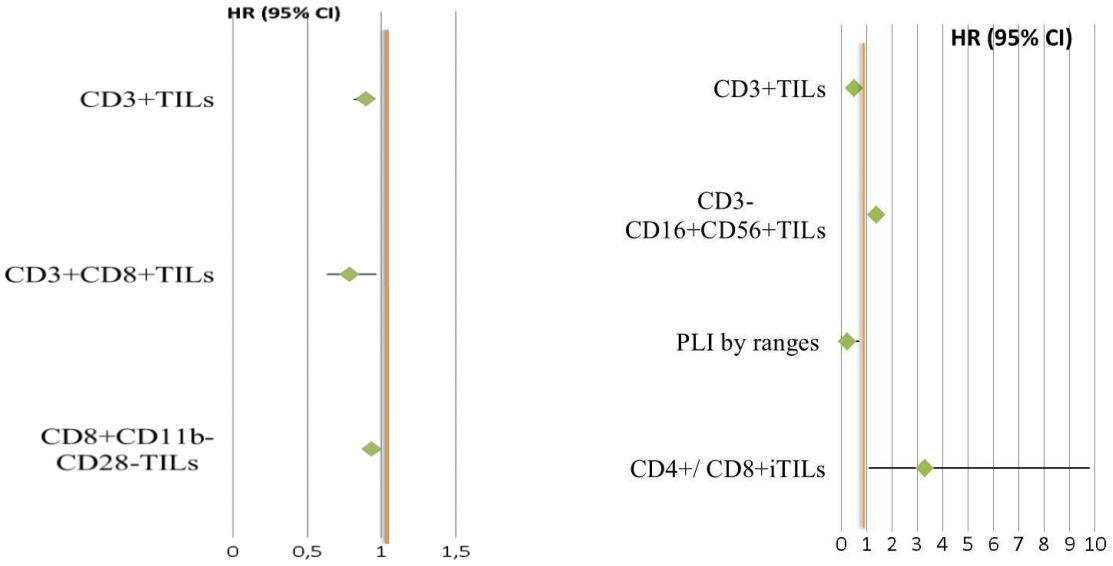

A. OverallsurvivalB. Progression-freesurvival

Figure 1 - The magnitude of the risks affecting OS and PFS in patients of group I.

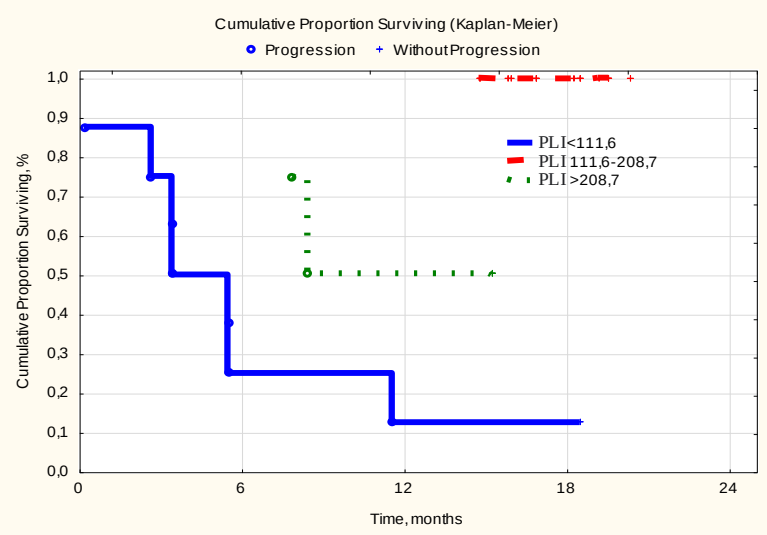

Figure 2 - The influence of the platelet-lymphocyte index on PFS in patients of groupI.

According to univariate analysis data for group II OS, the levels of neutrophils (HR-1.33; 95% CI 1.08-1.65; p = 0.008), platelets (HR-1.01; 95% CI 1.00-1,02; p = 0.006), CD3 + CD19- (RR-0.91; 95% CI 0.085-0.97; p = 0.007) and CD3-CD16 + CD56 + (RR-1.09; 95% CI1, 02-1.16; p = 0.007) of peripheral blood, as well as CD3-CD16 + CD56 + TILs (RR-1.202; 95% CI 1.011-1.429; p = 0.037) and CD3 + CD16 + CD56 + TILs (RR- 1.112; 95% CI 1.012-1.221; p = 0.027) of tumor tissue. For PFS of group II, the age of the patients (RR-1.06; 95% CI 1.01-1.12; p = 0.012), the level of neutrophils (RR-1.34; 95% CI 1.07-1.67; p = 0.010), platelets (RR-1.01; 95% CI 1.01-1.02; p = 0.0006), NLI (RR-2.80; 95% CI 1.36-5.75; p = 0.005), CD3-CD16 + CD56 + (RR-1.05; 95% CI 1.01-1.10; p = 0.023) and CTPCD16 (RR-1.06; 95% CI 1.01-1.11; p = 0.018) of peripheral blood, as well as CD45+CD14-TILs (RR-1.003; 95% CI 1.000-1.006; p = 0.041), CD3 + CD19-TILs (RR-0.952; 95% CI 0.908-0.998; p = 0.041), CD3-CD19 + TILs (RR-1.058; 95% CI 1.001-1.117; p = 0.045) of tumor tissue. (Figure 3,4,5,6)

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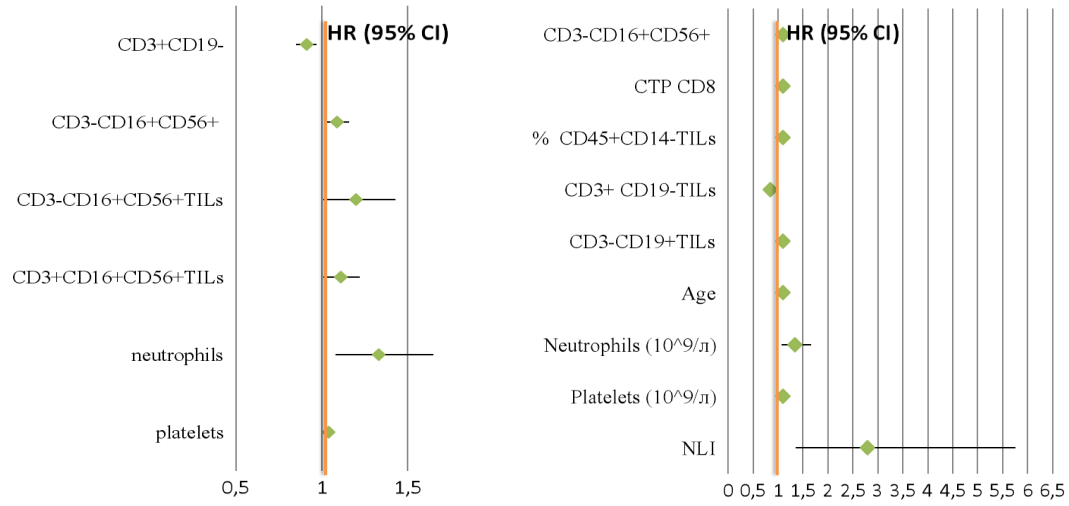
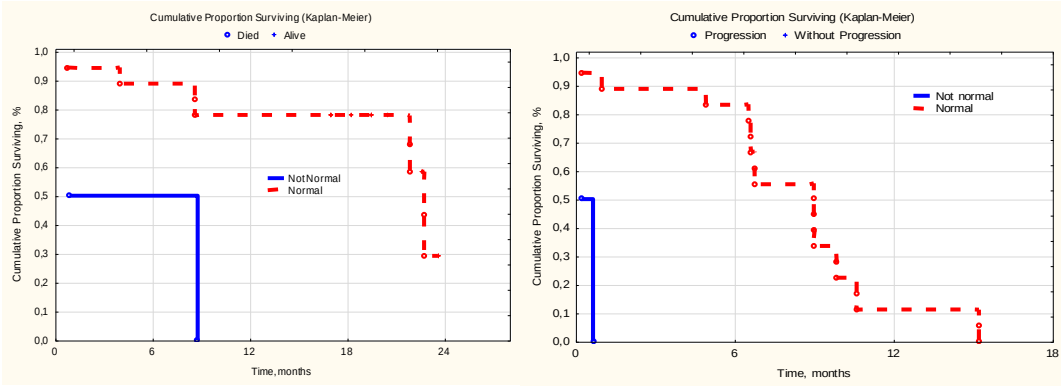


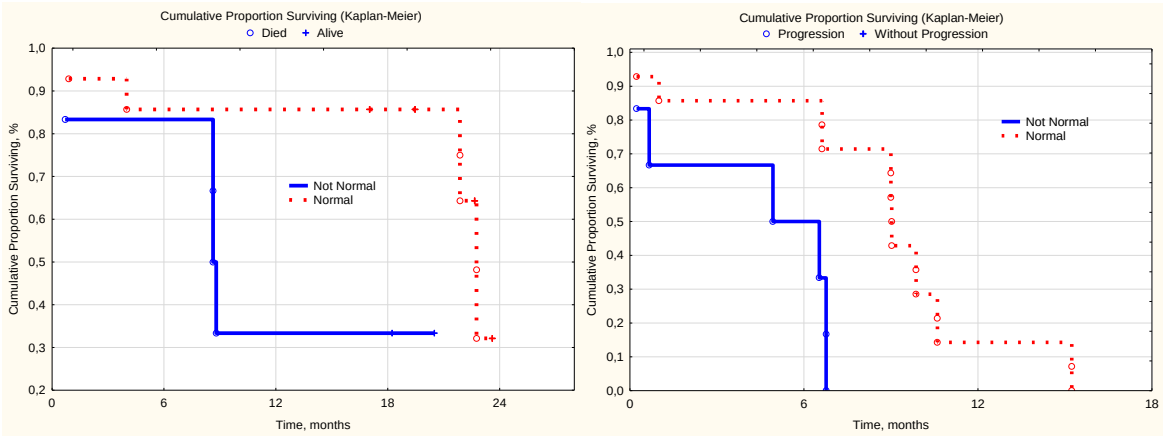
A. OverallsurvivalB. Progression-freesurvival

Figure 3 - The magnitude of the risks affecting OS and PFS in patients of group II.



A B

Figure 4 - Influence of the level of neutrophils (normal / not normal) on indicators of survival rate: A - OB; B - PFS in patients of group II



A B

Figure 5 - Influence of platelet level (normal / not normal) on indicators of survival rate: A - OB; B - PFS in patients of group II

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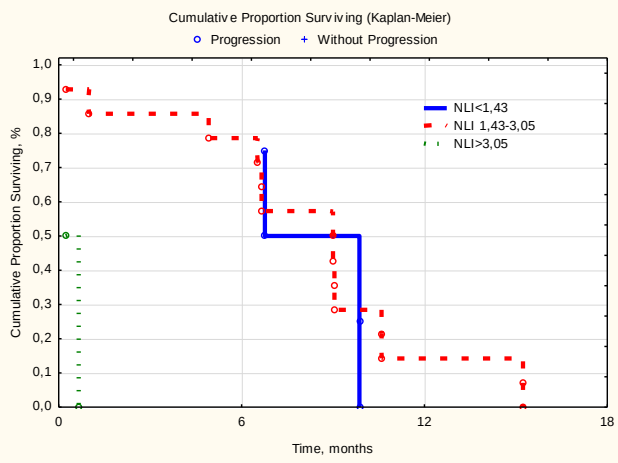

Figure 6 - The influence of the neutrophil-lymphocyte index on PFS in patients of group II.

Multivariate Cox regression analysis for group I OS did not allow constructing a significant mathematical model with independent factors. However, multivariate Cox regression analysis for PFS showed that an increase in the relative content of T cells (CD3 + CD19-TILs) is a favorable prognosis factor in patients of group I (RR 0.865, 95% CI 0.782–0.957, p = 0.005). In contrast, an increase in NK cells (CD3-CD16 + CD56 + TILs) worsens PFS (RR 1.382, 95% CI 1.087–1.758, p = 0.008).

Multivariate Cox regression analysis for group II OS showed that an increase in the level of neutrophils in the peripheral blood (RR 1.64, 95% CI 1.12–2.40, p = 0.011), as well as an increase in the infiltration of tumor tissue by NK cells (CD3-CD16 + CD56 + TILs) (RR1.42.95% CI1.06–1.89, p = 0.017) is a factor of poor prognosis for OS in patients with breast cancer.

Multivariate Cox regression analysis for PFS group II showed that an increase in age (RR 1.106, 95% CI 1.020-1.199, p = 0.015), neutrophil count (RR 1.714, 95% CI 1.063-2.764, p = 0.027) and platelets (RR 1.017, 95% CI 1.006–1.029, p = 0.003) worsen progression-free survival in patients with breast cancer.

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 [12]. The results of this study, where the content of T cells is a factor of a favorable prognosis for OS in patients with mGC, and an increase in NK cells worsens the indicators of OS and PFS in patients with mGC, are consistent with the study by Bass, A. et al. [13]. The study of the conjugation of 2 groups (T-cells and NK-cells) of 152 samples of peripheral blood of patients with gastric cancer showed that in patients of the T-cell group, in comparison with the NK-cell group, tumor invasion within the mucous membrane (T1) is significantly more often observed, 32 , 6% versus 14.3% (p = 0.03) and stage I of the disease is more often diagnosed, 36.8% versus 19% (p = 0.01). In patients of the NK-cell group, metastases to second-order lymph nodes are more often observed, 38% versus 17.6%, (p = 0.02) and distant metastases, 19.5% versus 4%, (p = 0.03) comparison with the opposite group [13]. On the one hand, the above data suggest that there are processes of mutual regulation between the indicated directions of differentiation of T and NK cell precursors. Possibly, this balance is one of the mechanisms of adaptation processes during the development of a stress reaction. On the other hand, such a conjugation may indicate the opposite: these cells are similar in origin, but

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their lymphopoiesis proceeds independently, therefore the factors causing lymphopenia of T cells do not affect NK cells, which are produced in a "normal" amount, and they become " relatively "more (and vice versa).

According to the latest data from foreign literature, NLI and ATP can be informative factors in predicting the course of gastric cancer in connection with the detected effect on the OS of patients. Thus, according to M. Mori et al., An increase in the NLI value in patients with gastric adenocarcinoma who received adjuvant chemotherapy is a factor of poor prognosis, affecting both OS and PFS [2]. These data are consistent with the results of univariate analysis (OFA), where NLI was a negative prognosis factor for PFS in patients with breast cancer. Using the ROC curve method, the boundaries with the optimal ratio of sensitivity and specificity were determined.

For neutrophils, the limit was 5×10^9 / L, and their number directly correlates with the stage of the disease. It turned out that the level of neutrophils reaches its maximum at stage III of the disease, and at stages I – II and IV, the indicators are in the range of 3.48–3.63 × 109 / l (p = 0.045). The method of constructing the ROC curve determined the boundaries with the optimal ratio of sensitivity and specificity for platelets, which was 270 × 109 / L. When analyzing PLI, its value increased as the disease progressed. So, the value of PLI at I-II stages of gastric cancer was 113, at IV - 154, (p = 0.045).

Similar results were obtained when analyzing data from 723 patients with adenocarcinoma of the stomach or gastroesophageal junction and showed that it is the increased rate of PLI that is a reliable factor in predicting the course of the disease, affecting both OS and relapse-free survival [3].

TILs act as the main determinants of the human immune response to tumor cells. The degree of infiltration of TILs is thought to be associated with the control of cancer growth and metastasis, as well as predicting the response to chemotherapy and radiation therapy.

Nevertheless, the results of studies on their study in malignant tumors of the breast, esophagus, and lungs are contradictory [14-16]. In particular, due to the small sample size and the study of various subgroups of T cells, the prognostic role of TILs in gastric cancer has not yet been fully determined. However, in the 2017 meta-analysis. with the inclusion of more than 30 studies, it was found that an increase in the relative content of T cells increases OS (RR 0.52, 95% CI 0.43–0.63, p = 0.001) [13]. Similar results were obtained in our study. The median high percentage of T cells correlated with OS and PFS in both groups.

At the same time, when performing multivariate analysis according to Cox, a correlation was found between the level of T-cells with an increase in PFS in patients with early and locally advanced forms of gastric cancer (RR 0.862, 95% CI 0.782-0.957, p = 0.005). In a univariate study, the effect of cytotoxic CD8 T-lymphocytes on OS in patients of group 1 was noted (RR 0.782, 95% CI 0.631–0.970, p = 0.026). The data are supported by the work of J. Lu et al., Who studied the role of CD3+ CD8+TILs in patients with gastric cancer after adjuvant chemotherapy, where their high level reduced the risk of progression (RR 0.37, 95% CI 0.23-0.57, p = 0.001) [17].

Regulatory T cells are currently playing a key role in evading immune surveillance. They are a type of T-lymphocyte with an immunoregulatory capacity that can inhibit the proliferation and secretion of cytokines by effector T-lymphocytes. A number of studies have confirmed that regulatory CD8 cells and NKT cells are factors of poor prognosis and correlate with the progression of the disease in gastric cancer [18–20].

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For example, the analysis of T-regulatory cells of previously untreated gastric cancer patients and the control group revealed that the percentage of NKT cells in tumors was significantly lower than in non-tumor tissues (18.10% versus 26.50%, p <0.01) In addition, a low level of T-regulatory cells is directly proportional to the clinical stage of the disease, including tumor size, tumor invasion, and distant metastases [21]. Thus, these data suggest that a decrease in the number of regulatory T cells is associated with tumor progression and a decrease in OS in patients with gastric cancer.

On the contrary, the obtained results of multivariate analysis in patients with breast cancer showed that an increase in the relative content of NKT cells is a factor of poor prognosis for OS (RR 1.127, 95% CI 1.025–1.239, p = 0.013). Another widely studied indicator of local immunity is NK cells. Natural cytotoxicity mediated by NK cells is believed to play an important role in inhibiting tumor metastasis, and a decrease in NK cell activity leads to a high incidence of tumors.

Despite compelling experimental data, the role of NK cells in immunological surveillance is still poorly defined. H. Takeuchi et al. a study of 156 patients with stage II – III gastric cancer revealed that the 5-year OS was higher in the group of patients where their percentage was higher (more than 25%), p = 0.05 [22]. According to multivariate analyzes, an increase in the level of NK cells worsened PFS in patients with early and locally advanced forms of gastric cancer (RR 1.382, 95% CI 1.087–1.758, p = 0.008) and OS in patients with mGC (RR 1.249, 95% CI 0.997– 1.564, p = 0.053). These data showed that NK cell activity may be related to tumor volume and spread.

For example, measuring the preoperative activity of NK cells can be important for the prognosis of patients with gastric cancer and for subsequent clinical treatment. Thus, in patients with gastric cancer, an increase in the level of T cells increases PFS, and, on the contrary, an increase in the percentage of NK cells is a factor of poor prognosis. For patients with mGC, while maintaining the linearity of the ratio of the main immune cells, the indices of local immunity affect both OS and PFS. An increase in the percentage of NK cells and NKT cells decreases OS. At the same time, with increasing age of patients and the degree of infiltration of tumor tissue with lymphocytes, the likelihood of disease progression increases. Accordingly, subpopulations of immunocompetent local immunity cells play a special role in gastric cancer and lead to different outcomes in patients.

Since the use of flow cytometry is possible only if the study is carried out in a cell suspension, further separate study of the intratumoral and stromal components becomes impossible.

Conclusion

Since both TILs are localized in tumor tissue, they are true TILs. Recent studies have shown that the composition and location of the immune infiltrate should be considered when evaluating TILs. It is unclear if there is a relationship between the location of TILs and disease prognosis. Accordingly, despite the subjective analysis component of the immunohistochemical staining method, this method assesses the spatial location of tumor-infiltrating lymphocytes. Our data are consistent with a meta-analysis of 31 studies examining the relationship between the density and distribution of TILs and prognosis in gastric cancer.

Analysis of 484 samples of tumor tissue from gastric cancer patients revealed a positive effect of CD8 + sTILs on OS, HR = 0.71; 95% CI, p = 0.029. Also, in a study with more than 1500 patients, CD8 + iTILs are a favorable prognosis factor for OS, HR = 0.73, 95% CI, p = 0.001 and PFS HR = 0.57; 95% CI, p = 0.029, respectively. At the same time, no statistically significant

effect of CD4 + TILs, regardless of distribution, on survival rates was found [13]. Accordingly, 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.

Despite the fact that the clinical prognostic factors for gastric cancer have been studied for a long time, the mechanisms of blocking antitumor immunosuppressive pathways and the ways of stimulating a local immune response remain not fully understood. It becomes obvious that the time when clinical characteristics of a tumor had a dominant role in patient treatment tactics is in the past.

Thus, our results indicate the possibility of using indicators of cellular immunity of tumor tissue, systemic immunity and inflammatory response in patients with gastric cancer as additional prognostic markers for assessing PFS and OS.

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