

The importance of T-lymphocyte subsets on overall survival of colorectal and gastric cancer patients

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Key words: gastric cancer; colorectal cancer; lymphocyte subsets; immune system.

Summary. The aim of this study was to evaluate the influence of cellular immunity parameters on overall survival of colorectal and gastric cancer patients after surgery. The parameters of cellular immunity (CD3⁺, CD4⁺, CD8⁺, CD20⁺, and CD16⁺) were determined by immunofluorescence method.

Cox regression analysis showed no impact of the estimated preoperative and postoperative parameters of cellular immunity on overall survival of colorectal cancer patients and similarly of gastric cancer patients in stage II. However, the analysis showed that the survival of colorectal and gastric cancer patients in stage III depended on immunological parameters determined before surgery: CD3⁺ ($P=0.007$ and $P=0.007$, respectively), CD4⁺ ($P=0.021$ and $P=0.011$, respectively), and CD8⁺ ($P=0.047$ and $P=0.007$) counts. Only the survival of colorectal cancer patients depended on natural killer cell number ($P=0.009$). Kaplan-Meier analysis showed that patients with stage III colorectal and gastric cancer had better survival rates when absolute number of CD3⁺ lymphocytes, determined before surgery, was greater than $0.8 \times 10^9/L$, CD4⁺ – greater than $0.25 \times 10^9/L$, CD8⁺ – greater than $0.3 \times 10^9/L$. Colorectal cancer patient survived longer when the number of natural killer cells CD16⁺ was more than $0.25 \times 10^9/L$.

This study suggests that higher levels of the absolute number of lymphocyte subsets before surgery have a beneficial effect on overall survival of gastric and colorectal cancer patients in stage III.

Introduction

The exact causes of digestive system cancer are not known, but risk of carcinogenesis appears to be associated with genetic, dietary, lifestyle factors and immunodeficiency disorders (1–7). Cancer arises frequently in a background of immunodeficiency induced by the various harmful habits as tobacco smoking and alcohol consumption. On the other hand, tumor deepens an already existing disbalance of the immune system, and it is caused by an intricate array of local and systemic physiological responses (8); therefore, patients with cancer have a variety of immunological abnormalities (9). Tumor-induced immune suppression is a fundamental problem in cancer biology (3, 10, 11).

Patients with digestive system cancer exhibit a poorly functioning immune system (3, 12), with decreased T-cell proliferation, reduced CD4⁺/CD8⁺ ratio (13), and a deficient production of T-helper cytokines (12, 14); equally it was underlined that cell-mediated immune responses are an essential aspect of tumor-

host interactions (15).

Surgery is the most frequent primary treatment for gastric and colorectal cancer. This treatment can deepen already existing injury in the immune system (9, 16).

The aim of this study was to evaluate the perioperative values of the cellular immunity parameters in gastric and colorectal cancer patients and to estimate their importance on overall survival after surgery for gastric and colorectal cancer.

Patients and methods

Seventy-three patients diagnosed with primary colorectal and gastric cancer in the Department of Abdominal Surgery, Institute of Oncology, Vilnius University, Lithuania, were enrolled into the study. We studied patients with histologically documented gastric and colorectal cancer in stage II and III with ECOG performance status 0–2 and reasonable hematology, liver and renal functions.

Twenty-two colorectal cancer patients in stage II

($T_{3-4}N_0M_0$) and 15 in stage III ($T_{1-4}N_+M_0$) were treated by colectomy, low anterior resection, or abdominoperineal resection (R0). The median age of patients was 64 years (range 50–75).

Fourteen gastric cancer patients in stage II ($T_1N_2M_0$; $T_2N_1M_0$; $T_3N_0M_0$) and 22 in stage III ($T_2N_2M_0$; $T_3N_1M_0$; $T_4N_0M_0$) were treated by total or partial gastrectomy (R0). The median age of patients was 63 years (range 49–73).

The patients with gastric and colorectal cancer included in this study had a preoperative erythrocyte

count greater than or equal to $3.0 \times 10^{12}/L$, hemoglobin level greater than or equal to 10.0 g/L, leukocyte count greater than or equal to $3.0 \times 10^9/L$, and platelet count greater than or equal to $180 \times 10^9/L$.

Heparinized venous blood (5 mL) of each patient was examined 5 days before surgery and 14 days after surgery.

Leukocyte number, absolute number and percentage of total lymphocytes for all the investigated persons were measured using an automated blood cell counter. The parameters of cellular immunity ($CD3^+$,

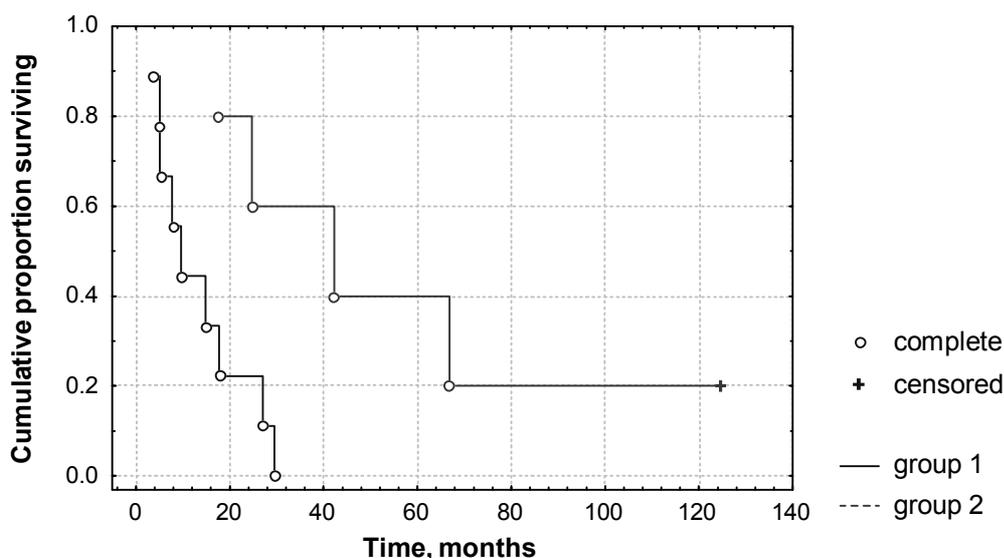


Fig. 1. Overall survival of stage III colorectal cancer patients (n=15) in relation to $CD3^+$ lymphocyte count before surgery

Group 1 – $CD3^+$ lymphocyte count $\leq 0.8 \times 10^9/L$; Group 2 – $CD3^+$ lymphocyte count $> 0.8 \times 10^9/L$; $P=0.017$, log-rank test.

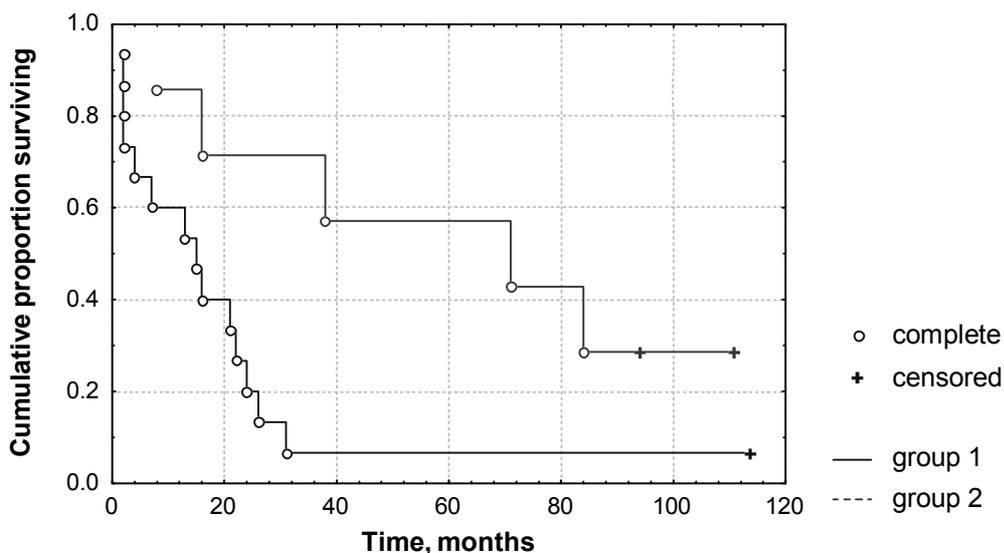


Fig. 2. Overall survival of stage III gastric cancer patients (n=22) in relation to $CD3^+$ lymphocyte count before surgery

Group 1 – $CD3^+$ lymphocyte count $\leq 0.8 \times 10^9/L$; Group 2 – $CD3^+$ lymphocyte count $> 0.8 \times 10^9/L$; $P=0.0201$, log-rank test.

CD4⁺, CD8⁺, CD20⁺, and CD16⁺) were determined by immunofluorescence method (Becton Dickinson GmbH, Heidelberg, Germany).

After surgery, colorectal cancer patients in stage II (T₄N₀M₀) and in stage III and gastric cancer patients in stage III received anticancer chemotherapy with 5-fluorouracil plus leucovorin.

Statistical analysis

The survival was calculated from the date of operation to the date of death or the last date the patient

was known to be alive. All patients were followed-up via the Lithuanian Cancer Registry.

Cox regression analysis was used in order to reject an influence of patient's age and sex. The level of statistical significance was accepted at $P \leq 0.05$; β value shows in what direction (positively or negatively) the analyzed parameter influences overall survival. Negative β value shows that analyzed parameter positively influences overall survival of colorectal patients. Kaplan-Meier method was used for more precise survival analysis. The impact of lymphocyte subsets on

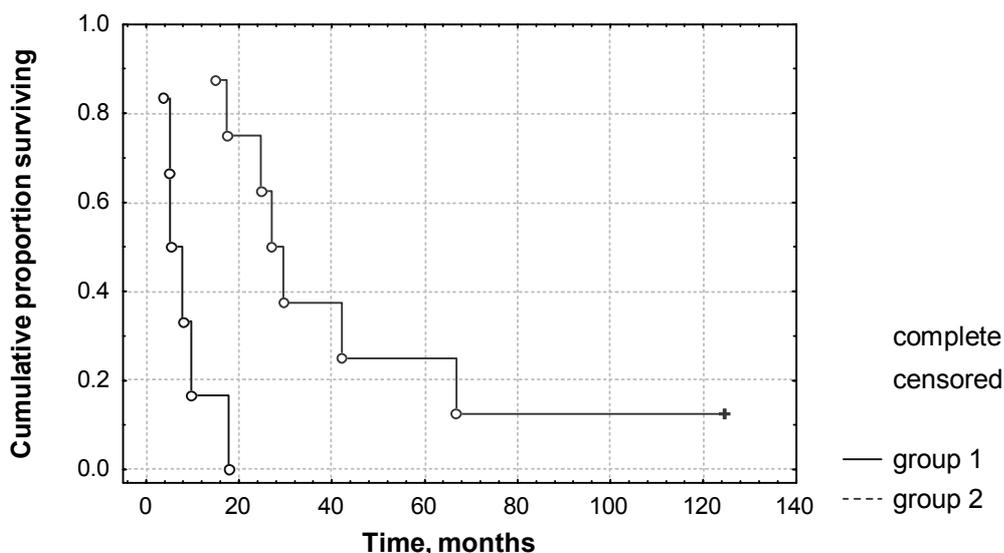


Fig. 3. Overall survival of stage III colorectal cancer patients (n=15) in relation to CD4⁺ lymphocyte count before surgery

Group 1 – CD4⁺ lymphocyte count $\leq 0.3 \times 10^9/L$; Group 2 – CD4⁺ lymphocyte count $> 0.3 \times 10^9/L$;
P=0.017, log-rank test.

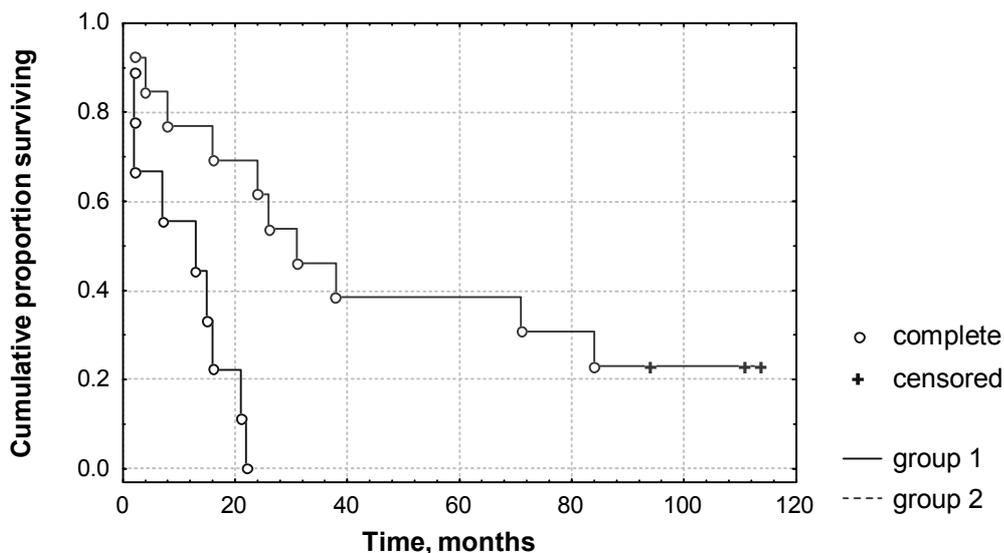


Fig. 4. Overall survival of stage III gastric cancer patients (n=22) in relation CD4⁺ lymphocyte count before surgery

Group 1 – CD4⁺ lymphocyte count $\leq 0.3 \times 10^9/L$; Group 2 – CD4⁺ lymphocyte count $> 0.3 \times 10^9/L$;
P=0.014, log-rank test.

survival was evaluated by distributing the patients into subgroups according to gradual cut-off levels of each lymphocyte subset. The difference between survival curves was determined using log-rank test.

Results

Cox regression analysis revealed no impact of estimated preoperative and postoperative parameters of cellular immunity on overall survival of colorectal and gastric cancer patients in stage II.

Cox regression analysis showed that only preope-

orative parameters of cellular immunity had an impact on overall survival of colorectal and gastric cancer patients in stage III, but postoperative parameters (14 days after surgery) had no impact.

Cox regression analysis revealed longer survival for colorectal cancer patients in stage III with a higher preoperative absolute number of CD3⁺ lymphocytes (P=0.007; β=-6.4), CD4⁺ (P=0.021; β=-7.8) and CD8⁺ lymphocytes (P=0.047; β=-5.7) and for gastric cancer patients in stage III with a higher absolute number of CD3⁺ lymphocytes (P=0.006; β=-5.9),

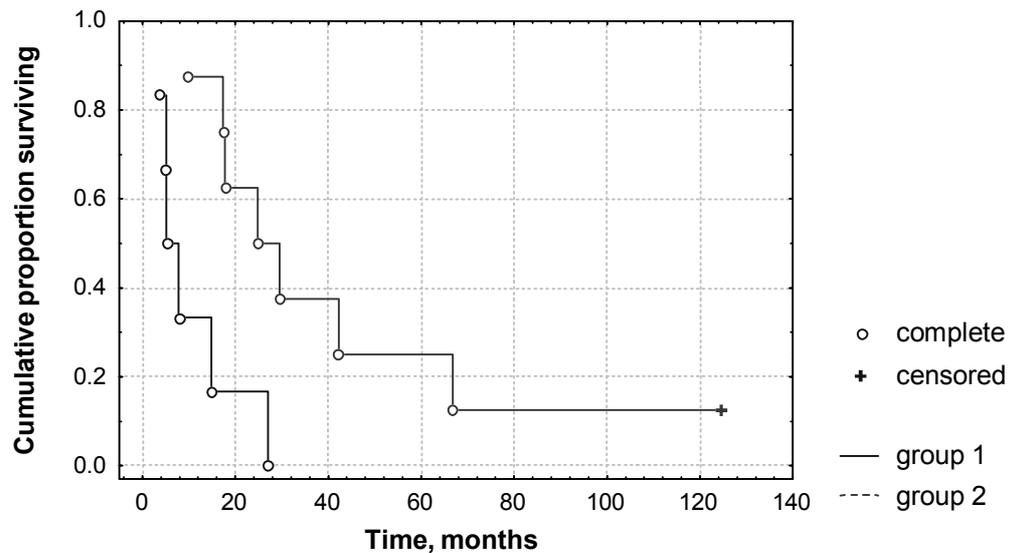


Fig. 5. Overall survival of stage III colorectal cancer patients (n=15) in relation to CD8⁺ lymphocyte count before surgery
 Group 1 – CD8⁺ lymphocyte count ≤0.3×10⁹/L; Group 2 – CD8⁺ lymphocyte count >0.3×10⁹/L; P=0.036, log-rank test.

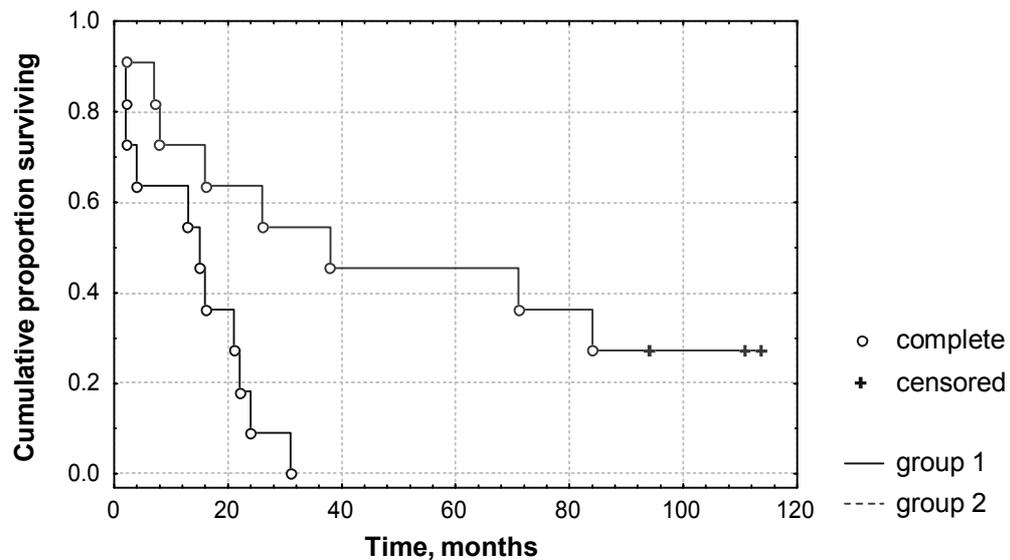


Fig. 6. Overall survival of stage III gastric cancer patients (n=22) in relation to CD8⁺ lymphocyte count before surgery
 Group 1 – CD8⁺ lymphocyte count ≤0.3×10⁹/L; Group 2 – CD8⁺ lymphocyte count >0.3×10⁹/L; P=0.018, log-rank test.

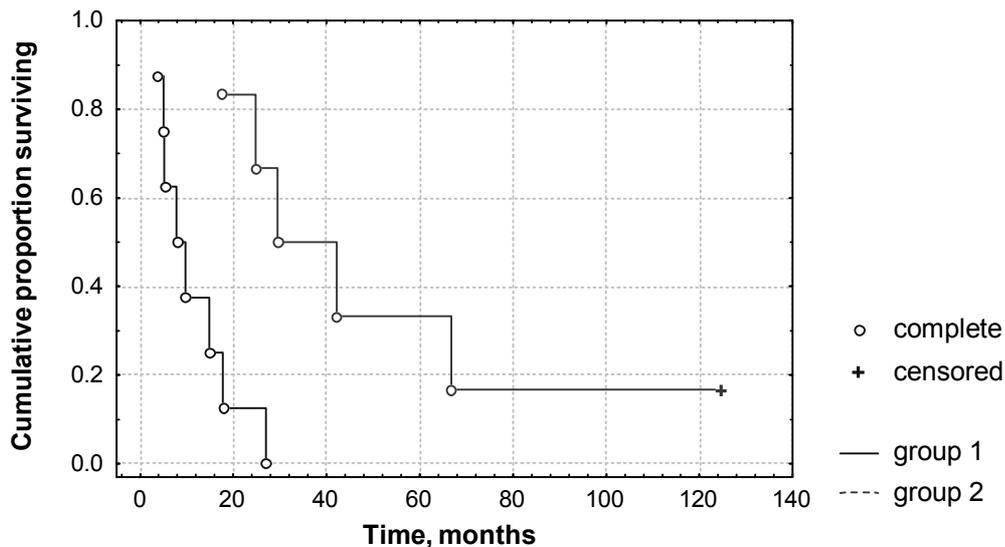


Fig. 7. Overall survival of stage III colorectal cancer patients (n=15) in relation to CD16⁺ lymphocyte count before surgery

Group 1 – CD16⁺ lymphocyte count $\leq 0.25 \times 10^9/L$; Group 2 – CD16⁺ lymphocyte count $> 0.25 \times 10^9/L$; P=0.011, log-rank test.

CD4⁺ (P \leq 0.011; β =-6.9) and CD8⁺ lymphocytes (P=0.0075; β =-5.1). Cox regression analysis showed that colorectal cancer patients with a higher absolute number of CD16⁺ survived longer (P=0.009; β =-11.8). Overall survival was also analyzed by testing different cut-off levels of lymphocyte subsets. The analysis showed that the overall survival of colorectal and gastric cancer patients in stage III was significantly better with a preoperative CD3⁺ lymphocyte count greater than $0.8 \times 10^9/L$ (P=0.017 and P=0.02, respectively; log-rank test) compared to patients with CD3⁺ lymphocyte count lower than or equal to $0.8 \times 10^9/L$ (Figs. 1 and 2). The overall survival of colorectal cancer patients with preoperative CD4⁺ lymphocyte count greater than $0.3 \times 10^9/L$ was significantly better (P=0.016 and P=0.014, respectively; log-rank test) compared to patients with preoperative CD4⁺ lymphocyte count lower than or equal to $0.3 \times 10^9/L$ (Figs. 3 and 4). The overall survival of patients with preoperative CD8⁺ lymphocyte count greater than $0.3 \times 10^9/L$ was significantly better (P=0.036 and P=0.018, respectively; log-rank test) compared to patients with preoperative CD8⁺ lymphocyte count lower than or equal to $0.3 \times 10^9/L$ (Figs. 5 and 6). The overall survival of colorectal cancer patients with preoperative CD16⁺ lymphocyte count greater than $0.25 \times 10^9/L$ was significantly better (P=0.011, log-rank test) compared to patients with preoperative CD16⁺ lymphocyte count lower than or equal to $0.25 \times 10^9/L$ (Fig. 7).

Discussion

It is known that the prognosis of cancer depends on tumor aggressiveness and host immune response. Although the mechanisms that underlie the specific immunologic alterations are not completely understood, it is clear that both functional and quantitative defects in immunity develop with cancer, especially in advanced stages (10, 17).

Other factors such as harmful habits (tobacco smoking and alcohol consumption) can also influence the changes of parameters of cellular immunity (18, 19). In the case of advanced cancer, these changes in parameters are basically affected by disease.

Data in the literature show that functional activity of immunocompetent cells has an important role in the function of immune system, but quantitative indicators of lymphocyte subsets are also important.

The main present finding appeals on analysis of overall survival of colorectal and gastric cancer patients, emphasizing the role of absolute counts of T-lymphocyte subsets.

Cox regression analysis showed no impact on overall survival of estimated preoperative and postoperative parameters of cellular immunity in colorectal and gastric cancer patients in stage II. However, our study showed that T-cell subsets are an independent prognostic variable for overall survival in gastric and colorectal cancer patients in stage III. NK (CD16⁺) cell number in peripheral blood is an independent prognostic

variable for survival only in colorectal cancer patients.

Analyzing the relationship between overall survival and immunological indices of colorectal and gastric cancer patients, a significant impact on survival was detected, depending on preoperative absolute number of lymphocyte subsets (CD3⁺ greater than $0.8 \times 10^9/L$, CD4⁺ greater than $0.25 \times 10^9/L$, CD8⁺ greater than $0.3 \times 10^9/L$, and CD16⁺ greater than $0.25 \times 10^9/L$) only in colorectal cancer patients. Cox regression analysis also showed that the higher absolute number of lymphocyte subpopulation might be associated with a longer survival of colorectal and gastric cancer patients in stage III. Our study has shown that similar tendencies of overall survival depend on quantitative parameters of cellular immunity of colorectal and gastric cancer patients in stage III. Thus, status of the immune system of patients with advanced cancer before treatment is important for survival of patients.

Some authors emphasize that immunosuppression is associated with CD3⁺, CD4⁺, CD8⁺ T-cell level depression, and this is related with worse prognosis in patients with stage III gastric cancer. The 5-year disease-free survival rates of patients with stage III gastric cancer were poorer at lower values of CD3⁺ and CD4⁺ T cell counts (20).

Wang *et al.* reported (8) that preoperative immune status of patients with gastric cancer was negatively related to cancer stage and CD3⁺, CD4⁺, CD4⁺/CD8⁺, CD16⁺ lymphocyte subsets decreased gradually with

progress of cancer.

The data of Paholyuk *et al.* (21) point to the involvement of NK cells in the control of tumor growth in stage II colorectal cancer patient. Our results showed the dependence of overall survival on preoperative NK cell number in peripheral blood of colorectal cancer patients in stage III.

Conclusions

Cox regression analysis showed no impact of preoperative and postoperative parameters of cellular immunity on overall survival in colorectal and gastric cancer patients in stage II. However, analysis showed the dependence of survival on preoperative immunological indices of colorectal and gastric cancer patients in stage III. Kaplan-Meier analysis showed that absolute number of lymphocyte subsets (CD3⁺ greater than $0.8 \times 10^9/L$, CD4⁺ greater than $0.25 \times 10^9/L$, and CD8⁺ greater than $0.3 \times 10^9/L$) before surgery were related to overall survival of patients with stage III colorectal and gastric cancer. CD16⁺ lymphocyte count greater than $0.25 \times 10^9/L$ was associated with overall survival only in colorectal cancer patients.

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T limfocitų subpopuliacijų reikšmė ligonių, sergančių storosios žarnos ir skrandžio vėžiu, gyvenimo trukmei

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Raktažodžiai: storosios žarnos vėžys, skrandžio vėžys, limfocitų subpopuliacijos, imuninė sistema.

Santrauka. *Tyrimo tikslas.* Įvertinti ląstelinio imuniteto rodiklių įtaką ligonių, sergančių II–III stadijos storosios žarnos ir skrandžio vėžiu, gyvenimo trukmei. Ląstelinio imuniteto rodikliai (CD3⁺, CD4⁺, CD8⁺, CD20⁺, CD16⁺) nustatyti imunofluorescenciniu metodu. Cox regresinė analizė parodė, kad ligonių, sergančių tiek III stadijos storosios žarnos, tiek skrandžio vėžiu, gyvenimo trukmė priklauso nuo ląstelinio imuniteto rodiklių, nustatytų prieš operaciją: CD3⁺ (atitinkamai – $p=0,007$ ir $p=0,007$), CD4⁺ (atitinkamai – $p=0,021$ ir $p=0,011$), CD8⁺ (atitinkamai – $p=0,047$ ir $p=0,007$). Nuo NK ląstelių (CD16⁺) absoliutaus kiekio priklausė tik III stadijos storosios žarnos vėžiu sergančių ligonių gyvenimo trukmė ($p=0,009$).

Kaplan-Meier gyvenimo trukmės analizė parodė, kad ligoniai, sergantys III stadijos storosios žarnos ir skrandžio vėžiu, kuriems prieš operaciją buvo nustatytas CD3⁺ limfocitų absoliutus kiekis $>0,8 \times 10^9/L$, CD4⁺ $>0,25 \times 10^9/L$, CD8⁺ $>0,3 \times 10^9/L$, gyvena ilgiau. Storosios žarnos vėžiu sergančių ligonių gyvenimo trukmė ilgesnė, kai CD16⁺ $>0,25 \times 10^9/L$.

Tyrimas parodė, kad didesnis absoliutus limfocitų kiekis turi įtakos geresniam ligonių, sergančių III stadijos storosios žarnos ir skrandžio vėžiu, išgyvenimui.

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