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ORIGINAL ARTICLE

Lymphocyte subsets in peripheral blood as prognostic factors in colorectal cancer

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Summary

Purpose: *To evaluate changes in different parameters of the immune status of colorectal cancer patients before their surgical treatment and to look for a possible impact these parameters could exert on overall survival.*

Patients and methods: *Forty patients with histologically confirmed colorectal cancer in stage II (n=22), III and IV (n=18) were eligible for inclusion in the present study. Heparinized venous blood (5 ml) of patients was examined 5 days before surgery. Their preoperative white blood cell (WBC) count was ≥3.0*10^9/l, hemoglobin ≥10 g/l, and platelets ≥180*10^9/l. The indices of cellular immunity determined for all of the patients were the following: total leukocyte number, absolute number and percents of total lymphocytes, monocytes and neutrophils. The percentage and absolute number of lymphocytes subsets CD3$^+$, T-helpers (CD4$^+$), T-cytotoxic cells (CD8$^+$), immunoregulation index (CD4$^+$/CD8$^+$), B-lymphocytes (CD20$^+$), natural killer (NK) cells (CD16$^+$) were measured by immunofluorescence methods.*

Results: *Cox regression analysis showed no dependence of the survival on estimated cellular immunity parameters of colorectal cancer patients in stage II. However, the number of circulating lymphocytes and in particular of T cells and NK cells was an independent prognostic variable for overall survival of stage III and IV patients. Analyzing the dependence of survival on immunological indices of colorectal cancer patients, a significant dependence of survival was determined on the absolute preoperative number of total lymphocytes and lymphocyte subsets (total lymphocytes levels ≥1.2*10^9/l, ≥CD3$^+$ ≥0.8*10^9/l, CD4$^+$ ≥0.3* 10^9/l, CD8$^+$ ≥0.3*10^9/l, and CD16$^+$ ≥0.25*10^9/l). Cox regression analysis showed that higher absolute number of lymphocyte subpopulations may be associated with longer survival of colorectal cancer patients in stage III and IV.*

Conclusion: *This study suggests total number of lymphocytes ≥1.2*10^9/l, CD3$^+$ ≥0.8*10^9/l, CD4$^+$ ≥0.3* 10^9/l, CD8$^+$ ≥0.3*10^9/l, and CD16$^+$ ≥0.25*10^9/l before surgery have a beneficial effect on overall survival of colorectal cancer patients in advanced stages (III and IV) of the disease.*

Key words: colorectal cancer, lymphocytes subsets, prognostic factors, surgery, survival

Introduction

The exact causes of cancer of the colon and rectum are not known, but risks for carcinogenesis appear to be associated with genetic, dietary, lifestyle factors and immunodeficiency disorders [1-4]. Cancer arises frequently in a background of immunodeficiency. On the other hand, tumor deepens an already existing deficiency of the immune system, and immunosuppression is caused by an intricate array of local and systemic physiological responses. Neoplastic diseases alter the immune response in cancer patients, and patients with cancer have a variety of immunological abnormalities [5]. Tumor-induced immune suppression is a fundamental problem in cancer biology [6].

Patients with cancer exhibit a poorly functioning immune system [2,4,7,8] manifested by decreased T-cell proliferation, reduced CD4$^+$/CD8$^+$ ratio [9] and a deficient production of T-helper cytokines [7].

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McMillan et al. have reported that reduction of $CD4^+$ T-lymphocytes occurs before detectable recurrence of colorectal cancer and this fact is important in tumor recurrence in colorectal cancer [10]. In another study it was underlined that cell-mediated immune responses are an essential aspect of tumour-host interactions in colorectal cancer. The progression from colorectal adenoma to colorectal cancer depends on a complex pathway involving the activities of activated T-lymphocytes. The immune response is initiated when either cytotoxic T-lymphocytes (CTL, $CD8^+$ cells) or $CD4^+$ T-helper cells recognize antigens from a human cancer cell. The cell-mediated response is largely initiated and controlled by the actions of various cytokines, which exert profound effects on T-cell proliferation, cell-cell adhesion, apoptosis, and host immunity [11].

Surgery is the most frequent primary treatment for colorectal cancer. This treatment deepens an already existing injury in the immune system and these infringements correlate with the degree of tissue trauma [12]. Patients with advanced cancer exhibit multifaceted defects in their immune capacity, which are likely to contribute to an accelerated disease progression. A chronic inflammatory condition is associated with increased oxidative stress and this has been suggested as one of the responsible mechanisms behind the tumor-induced immune suppression [5].

The aim of this study was to estimate preoperatively the values of different parameters of the immune status in colorectal cancer patients and to evaluate their impact on overall survival after colorectal cancer surgery.

Patients and methods

Forty patients with histologically confirmed stage II, III, and IV colorectal carcinoma were eligible for inclusion in the present study (stage II – 22 patients, stage III-IV-18 patients).

Thirty patients were treated by low anterior resection and 10 by abdominopelvic resection. The patients' median age was 64 years (range 50-75). There were 22 males (55%) and 18 females (45%). Five ml of the patients' venous blood were examined 5 days before surgery.

Their preoperative WBC count was $\geq 3.0*10^9$/l, hemoglobin ≥ 10 g/l, and platelets $\geq 180*10^9$/l .

The investigated parameters were determined only preoperatively. After surgery all patients were administered anticancer chemotherapy (5-fluorouracil + leucovorin).

The indices of cellular immunity determined for all 40 patients were the following: total leukocyte number, absolute number and percents of lymphocytes, monocytes and neutrophils. The percentage and absolute number of lymphocytes subsets $CD3^+$, T-helpers ($CD4^+$), T-cytotoxic cells ($CD8^+$), immunoregulation index ($CD4^+/CD8^+$), B-lymphocytes ($CD20^+$), NK cells ($CD16^+$) were measured by immunofluorescence methods. Reagents used were purchased from Sorbent (Russia).

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. The impact of the parameters of lymphocyte subsets on survival was evaluated by distributing the patients into subgroups according to gradual cut-off levels in each lymphocyte subset parameters. The survival of patients was analyzed using the Kaplan-Meier method. The difference between survival curves was determined using the log-rank test. For a more precise survival analysis Cox regression method was also used (in order to reject an influence of patients' age, sex, and stage). The level of statistical significance was accepted at $p \geq 0.05$.

Results

Cox regression analysis showed no impact on overall survival of the estimated cellular immunity parameters of colorectal cancer patients in stage II (absolute lymphocytes number: p=0.34; $CD3^+$: p=0.16; $CD4^+$: p=0.63; $CD8^+$: p=0.15; $CD16^+$: p=0.58).

However, Cox analysis showed that stage III and IV patients with higher absolute lymphocytes number had longer survival ($p \leq 0.05$, $\beta = -1.84$). Overall survival was also analyzed by testing different cut-off numbers of total lymphocytes. The analysis showed that the overall survival of stage III-IV patients was significantly higher with preoperative lymphocytes levels $>1.2*10^9$/l ($p < 0.016$, log - rank test) compared to patients with preoperative levels $\leq 1.2*10^9$/l (Figure 1), resulting in a 3-year survival of 50% and 0%, respectively. Also, the overall survival of stage III-IV patients with preoperative $CD3^+$ lymphocyte levels > $0.8*0^9$/l was significantly higher ($p < 0.017$, log - rank test) compared to patients with $CD3^+$ lymphocyte levels $\leq 0.8*10^9$/l (Figure 2), resulting in a 3-year survival of 60% and 0%, respectively. Cox analysis confirmed this tendency for $CD3^+$ lymphocytes ($p < 0.007$, $\beta = -6.4$). The overall survival of patients with preoperative $CD4^+$

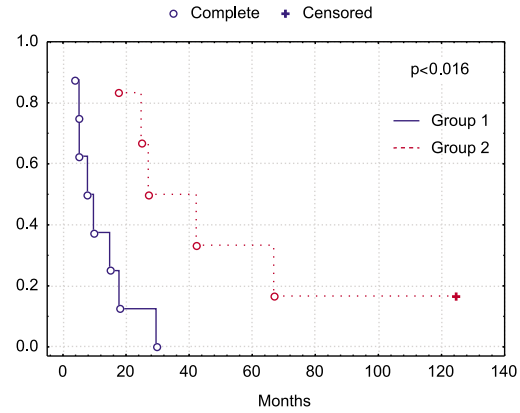

Figure 1. Impact on overall survival of stage III-IV colorectal cancer patients in relation to the total number of lymphocytes before surgery.

Group 1: lymphocytes ≤ 1.2*10^9/l; group 2: lymphocytes > 1.2*10^9/l

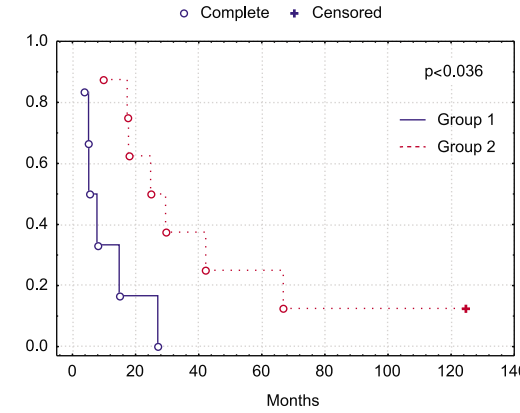

Figure 4. Impact on overall survival of stage III-IV colorectal cancer patients in relation to the number of CD8$^+$ lymphocytes before surgery.

Group 1: CD8$^+$ lymphocytes ≤ 0.35*10^9/l; group 2: CD8$^+$ lymphocytes > 0.35*10^9/l

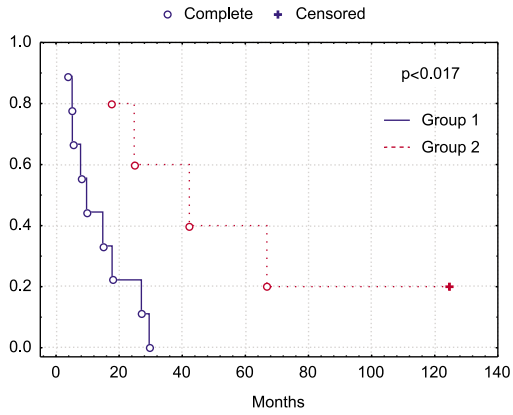

Figure 2. Impact on overall survival of stage III-IV colorectal cancer patients in relation to the number of CD3$^+$ lymphocytes before surgery.

Group 1: CD3$^+$ lymphocytes ≤ 0.8*10^9/l; group 2: CD3$^+$ lymphocytes > 0.8*10^9/l

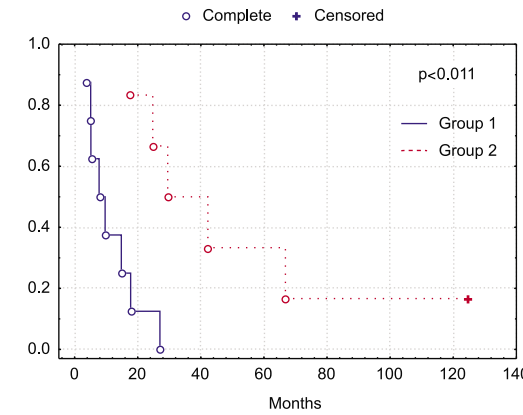

Figure 5. Impact on overall survival of stage III-IV colorectal cancer patients in relation to the number of CD16$^+$ lymphocytes before surgery.

Group 1: CD16$^+$ lymphocytes ≤ 0.25*10^9/l; group 2: CD16$^+$ lymphocytes > 0.25*10^9/l

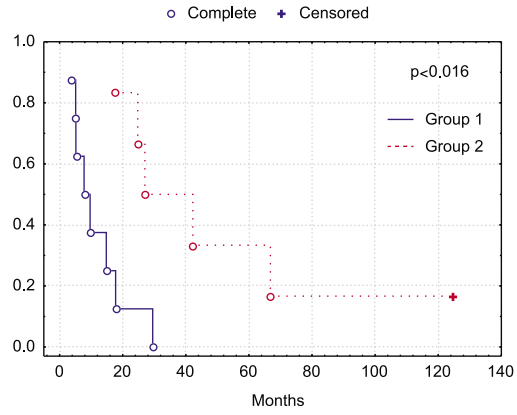

Figure 3. Impact on overall survival of stage III-IV colorectal cancer patients in relation to the number of CD4$^+$ lymphocytes before surgery.

Group 1: CD4$^+$ lymphocytes ≤ 0.3*10^9/l; group 2: CD4$^+$ lymphocytes > 0.3*10^9/l

lymphocyte levels > 0.3*10^9/l was significantly higher (p <0.016, log - rank test) compared to patients with preoperative CD4$^+$ lymphocytes levels ≤ 0.35*10^9/l (Figure 3), resulting in a 3-year survival of stage III-IV patients of 50% and 0%, respectively. The overall survival of patients with preoperative CD8$^+$ lymphocyte levels > 0.3*10^9/l was significantly higher (p <0.036, log - rank test) compared to patients with preoperative CD8$^+$ lymphocytes levels ≤ 0.3*10^9/l (Figure 4), resulting in a 3-year survival of 38% and 0%, respectively. Cox regression analysis confirmed these tendencies: CD4$^+$ (p <0.021, β= –7.8), and CD8$^+$ lymphocytes (p <0.047, β= –5.7). The overall survival of patients with preoperative CD16$^+$ lymphocytes levels > 0.25*10^9/l was significantly higher (p < 0.011, log - rank test)

compared to patients with preoperative $CD16^+$ lymphocytes levels < $0.25*10^9$/l (Figure 5), resulting in a 3-year survival of 50% and 0%, respectively. Cox regression analysis confirmed these tendencies: $CD16^+$ $p < 0.009$, $\beta = -11.8$.

Discussion

It is known that the prognosis of cancer depends on either tumor aggressiveness and on the host immune response. Although we do not completely understand the mechanisms that underlie the specific immunologic alterations, it is clear that both functional and quantitative defects in immunity develop with cancer, especially in advanced stages [13].

Cox regression analysis showed no impact on survival of the estimated immunity parameters of colorectal cancer patients in stage II. However, our data analysis has shown that the number of circulating lymphocytes, and in particular of T-cells and NK cells, is an independent prognostic variable for the overall survival of stage III and IV colorectal cancer patients.

Analyzing the relationship between overall survival and immunological indices of colorectal cancer patients, a significant impact on survival was detected, depending on the absolute preoperative number of total lymphocytes and lymphocyte subsets (total lymphocytes levels $\geq 1.2*10^9$/l, $CD3^+ \geq 0.8*10^9$/l, $CD4^+ \geq 0.3*10^9$/l, $CD8^+ \geq 0.3*10^9$/l, and $CD16^+ \geq 0.25*10^9$/l). Cox regression analysis also showed that stage III-IV patients with higher absolute number of lymphocyte subpopulations may be associated with longer survival. Some authors underline that immunosuppression is associated with $CD3^+$ $CD4^+$ $CD8^+$ T-cells level depression, and this is related with worse prognosis for patients with stage III gastric cancer. The 5-year disease-free survival rates of patients with stage III gastric cancer were poor with lower values of $CD3^+$ and $CD4^+$ T-cells [14]. The data of Paholyuk et al. point to the involvement of the NK cells in the control of tumor growth in stage II colorectal cancer patients [15]. Our results showed no relationship of survival with the preoperative NK cells number in the peripheral blood of stage II colorectal cancer patients. Such a relationship in our study was seen only in stage III-IV colorectal cancer patients.

We conclude that this study suggests that higher levels of the total number of lymphocytes ($\geq 1.2*10^9$/l), $CD3^+ \geq 0.8*10^9$/l, $CD4^+ \geq 0.3*10^9$/l, $CD8^+ \geq 0.3*10^9$/l, and $CD16^+ \geq 0.25*10^9$/l before surgery have a beneficial effect on overall survival of colorectal cancer patients in advanced stages (III and IV) of the disease.

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