

T-lymphocyte subsets and lipid peroxidation in relation to survival among advanced gastric cancer patients

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Background. Surgery is the most frequent primary treatment for gastric cancer. This treatment deepens the already existing injury of the immune system, and these infringements correlate with the degree of tissue trauma. It is generally accepted that reactive oxygen species could be important causative agents of cancer and are associated with the different steps of carcinogenesis either through immunological mechanisms. Little is known about the changes that occur in the antioxidant and lipid peroxidation levels in response to surgical trauma.

The aim of this study was to evaluate changes of the parameters of immune and oxidant–antioxidant status during surgical treatment of gastric cancer patients and to evaluate the effect of these parameters on survival after gastric cancer surgery.

Materials and methods. Twenty two patients with histologically confirmed gastric carcinoma in stage III–IV were eligible for inclusion in the present study (14 patients in stage III and 8 in stage IV). The median age of patients was 62 years (range 44–75 years). There were 14 males and 8 females.

The indices of cellular immunity were measured by immunofluorescence methods. Indirectly the state of the antioxidant system is reflected by the intensity of lipid peroxidation, which was evaluated by the concentration of peroxidation products – diene conjugates and malondialdehyde.

Results. Analysis of the data showed that the overall survival of patients with gastric cancer stage III–IV, pre-surgical CD₃⁺ lymphocyte levels >0.8·10⁹/l was significantly higher (P= 0.0201, log-rank test) compared to patients with pre-surgical CD₃⁺ lymphocyte levels <0.8·10⁹/l. The overall survival of patients with pre-surgical CD₄⁺ lymphocyte levels >0.25·10⁹/l was significantly higher (P = 0.014, log-rank test) compared to patients with pre-surgical CD₄⁺ lymphocyte levels <0.25·10⁹/l. The overall survival of patients with pre-surgical CD₈⁺ lymphocyte levels >0.3·10⁹/l was significantly higher (P = 0.018, log-rank test) compared to patients with pre-surgical CD₈⁺ lymphocyte levels <0.3.

Analysis of the parameters of the lipid peroxidation process in relation to the survival indicates a significantly lower level of MDA concentration after surgery (9.2 and 13.2 nmol/ml respectively, p = 0.0257), while decrease of DK concentration after surgery was insignificant (6.2 and 7.3 nmol/ml respectively, p = 0.068).

Conclusions. This study suggests that higher levels of T lymphocyte subsets absolute number before surgery have a beneficial effect on the overall survival of advanced gastric cancer patients. Malondialdehyde concentration decreased after surgery of gastric cancer patients in stage III and IV. Lower levels of the parameters of lipid peroxidation after surgery may play a positive role in the survival of gastric cancer patients.

Key words: gastric cancer surgery, immune system, lipid peroxidation, survival

INTRODUCTION

According to the available cancer epidemiology data, the annual number of new cases increased significantly in the developing world. Gastric cancer is the leading cause of cancer-related mortality in the world as well as in Lithuania. Cancer arises frequently on the background of immunodeficiency and is associated with cellular immunodeficiency (1). Patients with gastric cancer have a variety of immunological abnormalities and exhibit a poorly functioning immune system (1, 2), manifested by a decreased T-cell proliferation, reduced CD_4/CD_8 ratio (3) and a deficient production of T-helper cytokines (2, 4). Malignant tumors have been suggested to cause an oxidative disorder in the antioxidative defense system (5–7). The level of lipid peroxidation products, especially malondialdehyde (MDA) level in blood, are considered to be the criterion for the oxidative status of the entire human organism (8).

Surgery is the most frequent primary treatment for gastric cancer. This treatment deepens the already existing injury in the immune and antioxidative systems and infringements correlated with the degree of tissue trauma (9, 10). The markers reflecting the immunological status and the level of oxidative stress of patients after surgical treatment might be of value in the prognosis of postoperative complications as well as of survival of patients.

The aim of this study was to evaluate the values of the parameters of protective systems of the gastric cancer patients and to evaluate the effect of the immune and oxidant-antioxidant status on the survival of patients after gastric cancer surgery.

MATERIALS AND METHODS

Twenty-two patients (14 males and 8 females) with histologically confirmed gastric carcinoma in stage III–IV were eligible for inclusion in the present study (14 patients in stage III and 8 in stage IV).

17 patients had been treated by resection and 5 by gastrectomy. The median age of patients was 62 years (range 44–75 years).

The patients included in this study were selected according to their odd number of surgical history. They had preoperative WBC count $> 3.0 \cdot 10^{12}/l$, hemoglobin > 100 g/l, leucocytes $> 3.0 \cdot 10^9/l$, platelets $> 180 \cdot 10^9/l$. Venous blood of cancer patients was tested before surgery (1st analysis) and 14 days after surgery (2nd analysis).

The indices of cellular immunity determined for all the investigated persons were the following: leucocyte number, total lymphocyte, monocyte and

neutrophile percentage and absolute number. By immunofluorescence methods the percentage and absolute number of total T lymphocyte population (CD_3^+), T helpers (CD_4^+), T cytotoxic cells (CD_8^+), immunoregulation index (CD_4^+ / CD_8^+), B lymphocyte (CD_{20}^+), NK cells (CD_{16}^+) (Sorbent, Russia) were measured.

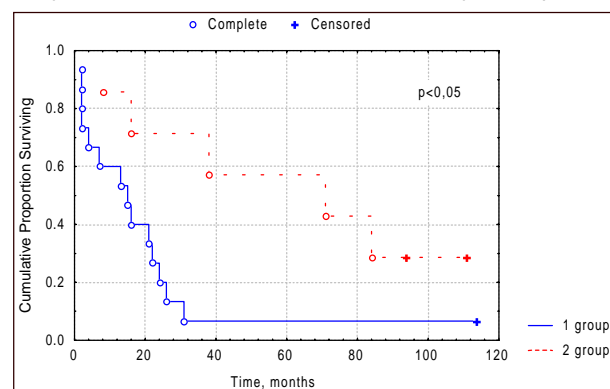
Indirectly the state of the antioxidant system is reflected by the intensity of lipid peroxidation, which was evaluated by the concentration of the peroxidation products, diene conjugates (DK) and malondialdehyde (MDA). Determination of DK is based on its extraction in heptane-isopropanol mixture and of MDA on the reaction with thiobarbituric acid.

The statistical analysis of cellular immunity parameters was performed and the intensity of lipid peroxidation determined by two-tailed, paired Student's t test.

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 influence of the parameters of lymphocyte subsets and lipid peroxidation on survival was evaluated by distributing the patients into subgroups according to the gradual cut-off levels in each lymphocyte subset and lipid peroxidation 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 the Cox regression method was also used (in order to reject the influence of patient's age, sex, gastric cancer disease stage). The significance was accepted at $P < 0.05$.

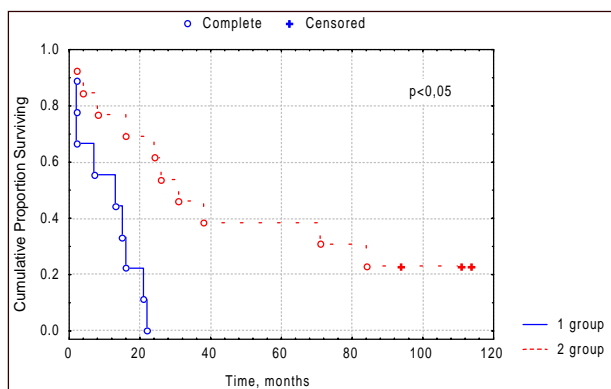
RESULTS

The cellular immunity reflecting parameters (total leucocyte, monocyte, total lymphocyte, and CD_3^+ , CD_4^+ , CD_8^+ , immunoregulation index (CD_4^+ / CD_8^+), CD_{20}^+ , CD_{16}^+



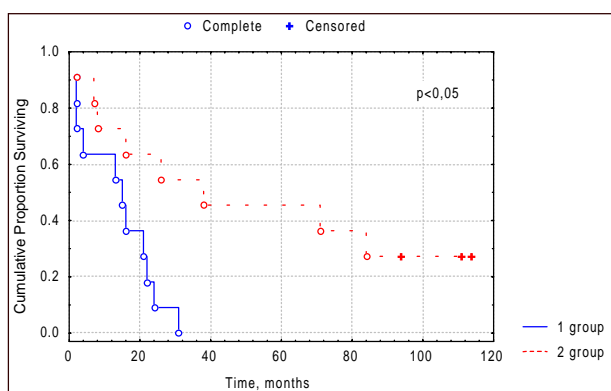
Graph 1. Dependence of overall survival of gastric cancer patients on the number of CD_3^+ lymphocytes before surgery

Group 1 – CD_3^+ lymphocytes $\leq 0.8 \cdot 10^9/l$, group 2 – CD_3^+ lymphocytes $> 0.8 \cdot 10^9/l$.



Graph 2. Dependence of overall survival of gastric cancer patients on the number of CD₄⁺ lymphocytes before surgery

Group 1 – CD₄⁺ lymphocytes ≤ 0.25·10⁹/l, group 2 – CD₄⁺ lymphocytes > 0.25·10⁹/l.

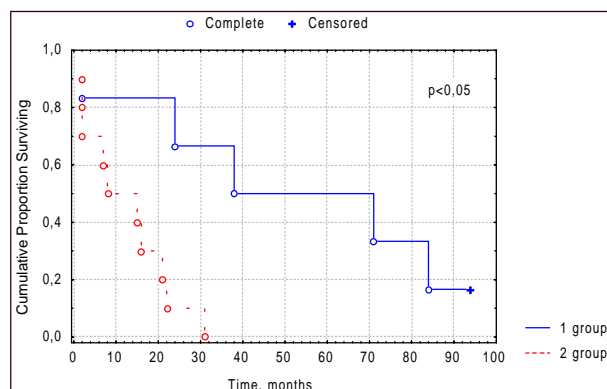


Graph 3. Dependence of overall survival of gastric cancer patients on the number of CD₈⁺ lymphocytes before surgery

Group 1 – CD₈⁺ lymphocytes ≤ 0.3·10⁹/l, group 2 – CD₈⁺ lymphocytes > 0.3·10⁹/l.

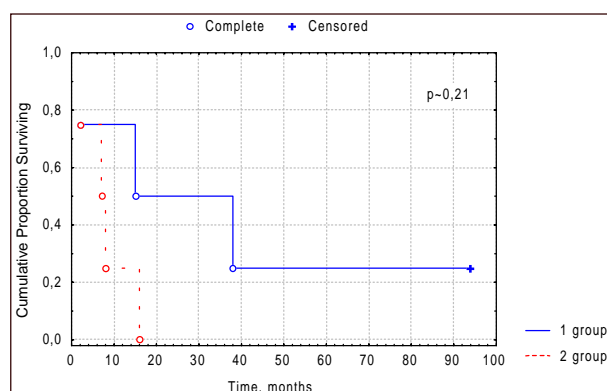
percentage and absolute number) 14 days after gastric cancer surgery (2nd analysis) were compared to the related parameters in research before treatment (1st analysis). The parameters of cellular immunity were not changed significantly after gastric cancer surgery.

The Cox analysis has shown that the higher absolute number of lymphocytes may be associated with a longer survival of gastric cancer patients in stage III and IV ($P = 0.023$). The survival of cancer patients was also analysed by gradually testing different cut-off numbers of total lymphocytes and lymphocyte subsets. The analysis showed that the overall survival of patients with III–IV gastric cancer stage and pre-surgical CD₃⁺ lymphocyte levels > 0.8·10⁹/l was significantly higher ($P = 0.0201$, log-rank test) compared to patients with the pre-surgical CD₃⁺ lymphocyte levels < 0.8·10⁹/l (Graph 1), and their 3-year survival was 71% and 7% respectively. The Cox analysis has confirmed this tendency for CD₃⁺ lymphocyte ($P = 0.006$). The overall survival of patients with pre-surgical CD₄⁺ lymphocyte levels > 0.25·10⁹/l was significantly higher ($P = 0.014$, log-rank test) compared to patients with pre-surgical CD₄⁺ lymphocyte levels < 0.25·10⁹/l (Graph 2), so the 3-year survival of patients



Graph 4. Dependence of survival of gastric cancer patients on MDA concentration after surgery

Group 1 – MDA ≤ 8 nmol/ml, group 2 – MDA > 8 nmol/ml.



Graph 5. Dependence of survival of gastric cancer patients on DK concentration after surgery

Group 1 – DK ≤ 6 nmol/ml, group 2 – DK > 6 nmol/ml.

with stage III–IV gastric cancer was 46% and 0% respectively. The overall survival of patients with pre-surgical CD₈⁺ lymphocyte levels > 0.3·10⁹/l was significantly higher ($P = 0.018$, log-rank test) compared to patients with pre-surgical CD₈⁺ lymphocyte levels < 0.3 (Graph 3), so the 3-year survival was 54.5% and 0% respectively. The Cox analysis confirmed these tendencies: CD₄⁺ lymphocytes ($P = 0.011$), and CD₈⁺ lymphocyte ($P = 0.0075$).

The parameters of lipid peroxidation products (malondialdehyde and diene conjugates) were studied in gastric cancer patients before and after operation. A significantly lower level of MDA concentration was observed after surgery (9.2 nmol/ml), while before surgery it was higher (13.2 nmol/ml) ($P = 0.0257$). The decrease of DK concentration after surgery was insignificant – 6.2 and 7.3 nmol/ml respectively ($P = 0.068$). Analysis of the parameters of the lipid peroxidation process in relation to survival indicates that after surgery the value of the MDA parameter, which has a positive influence on the survival of gastric cancer patients, was 8 ≤ nmol/ml ($P = 0.0107$; Graph 4). According to the Cox analysis, irrespective of disease stage, patient's sex and age, there was a tendency

that the survival depended on MDA values of the 1st and 2nd analysis ($P = 0.061$ and 0.098 respectively). DK concentration had almost no influence on the survival of gastric cancer patients after surgery (Graph 5), although the 2nd analysis of DK had a tendency ($P = 0.068$) to exert a positive effect on the survival.

DISCUSSION

Thus, analysis of the obtained data has shown that the parameters of cellular immunity (total leukocyte, monocyte, total lymphocyte, and CD_3^+ , CD_4^+ , CD_8^+ , immunoregulation index (CD_4^+ / CD_8^+), CD_{20}^+ , CD_{16}^+ percentage and absolute number) were only insignificantly changed after gastric cancer surgery. This fact demonstrated the absence of the influence of surgical treatment on cellular immunity parameters of gastric cancer patients, or these parameters returned to the initial level within 14 days.

Although we do not completely understand the mechanisms that underlie the specific immunologic alterations, it is clear that both functional and quantitative defects of immunity develop with cancer, especially in advanced stages.

Analyzing the dependence of survival on immunological indices of gastric cancer patients, a significant dependence of survival was determined on the absolute pre-treatment number of lymphocyte subpopulation (CD_3^+ lymphocyte levels $>0.8 \cdot 10^9/l$, $CD_4^+ >0.25 \cdot 10^9/l$, $CD_8^+ >0.3 \cdot 10^9/l$). The Cox analysis has shown that the higher absolute number of lymphocyte subpopulation may be associated with a longer survival of gastric cancer patients in stage III and IV. Other authors underlie that immunosuppression is associated with CD_3^+ , CD_4^+ , CD_8^+ T-cell level depression, and it worsens the prognosis for patients with stage III gastric cancer. The 5-year disease-free survival rates of patients with stage III gastric cancer were poor at lower values of CD_3^+ and CD_4^+ T-cells (11, 12).

Data on the lipid peroxidation process in gastric cancer patients show that the significantly decreased level of the lipid peroxidation product – malondialdehyde after operation has a positive influence on the survival of gastric cancer patients in stage III–IV. The Cox analysis shows that the survival depends on MDA values before and after operation, but it is necessary to note that it depends more on the MDA concentration before operation. The level of lipid peroxidation products, diene conjugates, had almost no influence on the survival; only 2nd analysis had a tendency to exert effect on the longer survival of gastric cancer patients in stage III–IV.

CONCLUSIONS

1. The parameters of cellular immunity before surgery and 14 days after surgery statistically did not differ.

2. This study suggests that higher levels of the absolute number of T lymphocyte subsets before surgery have a beneficial effect on the overall survival of gastric cancer patients at advanced stages of the disease.

3. Malondialdehyde concentration decreased after surgery in gastric cancer patients in stage III and IV. Lower levels of the parameters of lipid peroxidation after surgery may play a positive role in the survival of gastric cancer patients.

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T-LIMFOCITŲ SUBPOPULIACIJŲ IR LIPIDŲ PEROKSIDACIJOS REIKŠMĖ LIGONIŲ, SERGANŲ VĖLYVOSIOMIS SKRANDPIO VĖPIO STADIJOMIS, GYVENIMO TRUKMEI

Santrauka

Darbo tikslas – įvertinti ląstelinio imuniteto ir lipidų peroksidacijos rodiklių pokyčius gydant skrandžio vėžiu ser-

ganėius ligonius operaciniu būdu, taip pat tirtø rodikliø pokyėiø reikõmæ ligoniø gyvenimo trukmei.

Medžiaga ir metodai. Iøtyrėme 14 ligoniø, serganėiø III skrandžio vėpio stadija, ir 8 ligonius – IV skrandžio vėpio stadija.

Nustatyti ðie lãstelinio imuniteto rodikliai: leukocitø, neutrofilø, monocitø, bendrøjø limfocitø, limfocitø subpopuliacijø (CD_3^+ , CD_4^+ , CD_8^+ , CD_{20}^+ , CD_{16}^+) procentinis ir absoliutus skaiėius, taip pat lipidø peroksidacijos produktø – dijeniniø konjugatø (DK) ir malono dialdehido (MDA) – koncentracija ligoniø periferiniame kraujyje priėð operacijà ir prãėjus 14 dienø po operacijos

Rezultatai. Analizės duomenimis, mûsø tirtieji lãstelinio imuniteto rodikliai, prãėjus 14 dienø po operacijos, statistiškai nepakito. Galima manyti, kad operacija neturėjo ãtakos lãstelinio imuniteto rodikliams arba ðie atsistatė. Taėiau ilgiau gyveno tie ligoniai, kuriems priėð operacijà buvo nu-

statytas CD_3^+ limfocitø absoliutus kiekis $>0,8 \cdot 10^9/l$ ($P = 0,0201$, log-rank testu), CD_4^+ – $>0,25 \cdot 10^9/l$ ($P = 0,018$, log-rank testu), CD_8^+ – $>0,25 \cdot 10^9/l$ ($P = 0,0201$, log-rank testu). Kiti lãstelinio imuniteto rodikliai ligoniø gyvenimo trukmei ãtakos neturėjo.

Sumažėjusi lipidø peroksidacijos produkto MDA koncentracija ($P = 0,0107$), prãėjus 14 dienø po operacijos, prailgino skrandžio vėpiu serganėiø ligoniø gyvenimo trukmæ.

Išvados. Ligoniai, sergantys vėlyvosiomis skrandžio vėpio stadijomis, kuriems priėð operacijà buvo nustatytas CD_3^+ limfocitø absoliutus kiekis $>0,8 \cdot 10^9/l$, CD_4^+ – $>0,25 \cdot 10^9/l$, CD_8^+ – $>0,25 \cdot 10^9/l$, gyvena ilgiau.

Lipidø peroksidacijos produkto MDA koncentracijos sumažėjimas po operacijos prailgino skrandžio vėpiu serganėiø ligoniø gyvenimo trukmæ.

Raktaþodžiai: skrandžio vėþys, chirurginis gydymas, imuninė sistema, lipidø peroksidacija, gyvenimo trukmė