

The effect of enteral immune-enhancing diet (immunonutrition) on the apoptotic signaling pathways of peripheral blood lymphocytes in patients after pancreatic cancer surgery

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Abstract

Malnutrition results in disorders of programmed cell death influencing the patients susceptibility to infections after major surgery. The preoperative nutrition is a promising way of improving outcome after pancreatic surgery. The purpose of our study was to investigate the influence of enteral nutrition on the changes of apoptotic signaling pathways. The randomized studies were performed in patients operated on for pancreatic cancer receiving standard or enteral immunonutrition. The expressions of Fas/CD95 and TNFR1/CD120a in lymphocytes were assessed by flow cytometry before and on day 1, 3 and 7 after surgery. In comparison to the standard nutrition, the preoperative immunonutrition significantly increased the percentage of CD95 and TNFR1 positive lymphocytes after surgery. In conclusion, our findings suggest the up-regulation of proapoptotic signaling systems in lymphocytes of malnourished patients receiving immunonutrition after pancreatic cancer surgery. These changes in apoptotic signaling pathways proteins after immunonutrition may increase lymphocytes dysfunction.

Key words: apoptosis, pancreatic cancer surgery, immunonutrition.

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Introduction

The opinions regarding the effectiveness of immunonutrition in seriously ill patients are still controversial [1]. The results of meta-analysis of randomized studies have shown that in the group of seriously ill patients (systemic inflammatory response syndrome, grave infections, acute respiratory distress syndrome) the immunomodulating treatment with glutamine and fatty acids improves outcomes, which has not been confirmed after the enteral administration of arginine [2]. Another meta-analysis covering the perioperative results of immunonutrition aimed at enhancing immu-

nity in patients operated on for the tumors of the digestive tract has shown a decrease in the number of post-operative complications, reducing the length hospital stay and improving selected immune parameters such as: increase in the total number of lymphocytes, sub-population of CD4 lymphocytes, the concentration of IgG and decrease in the concentration of interleukin 6 (IL-6) [3]. Compared with the standard immunonutrition, the enteral administration of glutamine, arginine and/or fatty acids (N-3 PUFAs) in patients with acute pancreatitis shows no significant impact on decrease in the number of complications, the length of hospital stay and mortality rate [4]. Considering another review

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of study results the advantageous impact of enteral and parenteral administration of immunonutrition in patients with acute pancreatitis can be enhanced by administering inflammatory and immune response modulators [5]. The bibliography-based data on the immunomodulating effect of glutamine are unequivocal. In the group of patients after extensive surgical trauma adding glutamine has always resulted in a decrease of post-surgical complications, reduction in the length of hospital stay and even the reduction of mortality rate in the seriously ill patients [6]. Multi-center studies have shown that the administration of high doses of glutamine associated with antioxidants in seriously ill patients hospitalized in intensive care units results in a significant increase in morbidity and mortality [7]. The majority of studies that have been carried out to date shows that unsaturated fatty acids (N-3 PUFAs) have a significant regulative impact on immune response and outcomes in patients after surgery with serious infections including acute respiratory distress syndrome (ARDS) [8, 9].

The above-presented results show that improving outcomes in the group of patients with severe postoperative complications requires paying more attention to explaining the molecular mechanisms of immunonutrition. It has recently more clear that dietary polyunsaturated fatty acids (PUFAs) and some of their synthesized derivatives are able to modulate the molecular pathways involved in apoptosis [10]. Interestingly, decreased Bcl-2 anti-apoptotic protein level in pancreatic cancer cells incubated with omega-3 fatty acids enhanced media suggests mitochondrial directed apoptosis [11]. Glutamine can protect human T-cells from apoptosis by up-regulating glutathione, Bcl-2 and CD45RO anti-apoptotic protein expression in lymphocytes and down-regulation of the expression of caspase-3, Fas (CD95) and Fas ligand pro-apoptotic proteins [12].

To better understand the potential impact of immunonutrition on the main apoptotic signaling pathways the purpose of our study was to investigate the influence of enteral immunonutrition versus enteral standard nutrition on the changes of expressions of Fas/CD95 and TNFRI/CD120a receptors in lymphocytes of pancreatic cancer patients.

Materials and methods

Fifty patients operated on for pancreatic cancer were randomized (by using numbered sealed envelopes stratified by the surgeon) to receive either the enteral standard diet (group I – 24 patients, mean age 62.5 ± 9.2) or the immune-enhancing enteral diet (group II – 26 patients, mean age 61.8 ± 8.7). After full clinical diagnostic procedures (image and laboratory tests), all patients were operated on for resection of the head of the pancreas (Whipple's pancreaticoduodenectomy). Histopathological examination confirmed the diagnosis.

The present investigation did not include patients with early enteral or parenteral postoperative nutrition, early seri-

ous post-operative infectious complications, with unresectable pancreatic cancer, those who had transplantation of organs, patients treated with chemo- or radiotherapy or immunosuppressors, patients with autoimmune diseases, with diabetes type 1 (insulin-dependant), chronic respiratory insufficiency (chronic obstructive pulmonary disease), cardiovascular insufficiency, and kidney and liver diseases (biopsy-proven cirrhosis or a serum total bilirubin greater than 3.0 mg/dl). The control group comprised 30 healthy volunteers matched according to sex and age (mean age 58.2 ± 8.9).

Enteral nutrition

In the preoperative period standard enteral nutrition or immunonutrition was used as a supplementary diet for 5 days. The indication for pre-operative enteral nutrition treatment was the loss of body mass (greater than 6% within 2 months) and the extent of surgery (including the advancement of the tumor) [13]. Two enteral diets were used: an standard diet (Nutridrink[®], Nutricia Export BV, Zoetermeer, Holland) and an immune-enhancing diet. (FortiCare[®], Nutricia Export BV, Zoetermeer, Holland and Glutamine Plus[®], Fresenius Kabi). Each patient received 2 sachets (Glutamine Plus, $n = 17$) or 3 containers (FortiCare, $n = 9$ or Nutridrink, $n = 24$) of diets per day according to manufacturer's instruction. The immune-enhancing diets contain 20 g of glutamine, over 2 g of eicosapentaenoic acid (EPA) and 1.2 g of docosahexaenoic acid (DHA).

Flow cytometry

Blood samples were collected from the peripheral vein after (day 0) preoperative nutrition and on post-operative days 1, 3 and 7. For staining of surface proteins 100 μ l heparinized blood were incubated with 20 μ l of the following monoclonal antibodies: CD3 PE, CD95 FITC (Becton-Dickinson Immunocytometry Systems; Becton-Dickinson and Co, San Jose, Clifornia), CD120a PE (Immunotech, a Beckman Coulter Company 13009 Marseille, France) and their appropriate isotype-matched control antibodies. After 30 min incubation at room temperature, red cells were lysed by the addition of FACS Lysing Solution (Becton-Dickinson Biosciences, San Jose, California, USA) to the tubes. After 10 min, samples were washed in PBS with 1% bovine serum albumin (BSA; Sigma Chemical Co) and 0.1% sodium azide (NaN₃) and fixed with 1% paraformaldehyde (Sigma, Chemical Co). The stained cells were acquired using the LSR instrument (Becton-Dickinson) to detect two fluorescence parameters and two scatter parameters. Data were analysed using CellQuest ProSoftware – version 9.2 (Becton-Dickinson) and expressed as percentage of lymphocytes with positive surfaces or intracellular pro- and antiapoptotic proteins expressions (means \pm SD).

The protocol of the study was approved by the Medical University Ethics Committee.

Statistical analysis

Statistical analysis was performed using the Statsoft Statistica v.7.0 program. To compare the differences of lymphocyte percentage between groups and preoperative vs. postoperative results the Mann-Whitney or Wilcoxon signed-rank test with the Bonferroni correction was used. $p < 0.05$ was defined as significant.

Results

The immune-enhancing preoperative enteral nutrition included both glutamine and polyunsaturated fatty acids significantly increased the percentage of CD95+/CD3- and CD120a positive lymphocytes on day 3 after pancreas resection as compared to the group of patients receiving preoperative standard enteral nutrition (Figs. 1, 2). There were also a tendency to increased percentage of CD95+/CD3- and CD120a positive lymphocytes on day 7 after surgery in patients with preoperative immunonutrition ($p = 0.05$). Conversely, no significant difference was found in the percentages of CD95+/CD3+, CD95-/CD3+ positive lymphocytes between the group of patients with immunonutrition versus standard nutrition before as well as after pancreas surgery.

There were no significant difference between preoperative and postoperative levels of CD95+/CD3- and CD120a positive lymphocytes. Furthermore, no significant difference was found between the percentage of CD95+/CD3+ lymphocytes in comparison with control group (before and

after surgery) whereas CD95-/CD3+ positive cells were significantly decreased on day 1 after surgery ($16.38 \pm 10.3\%$ vs. $30.4 \pm 14.9\%$, $p = 0.002$). The percentage of lymphocytes with Fas (CD95+/CD3-) and TNFRI (CD120a) phenotypes were significantly higher before as well as after surgery as compared with control group (all $p < 0.05$).

Discussion

Major surgical trauma under general anesthesia induces an intracellular perturbation on peripheral lymphocytes, resulting in both up-regulation of death-signaling factors (Fas, FasL) and down-regulation of survival-signaling factors (Bcl-2). The increased apoptosis of CD8 lymphocytes, excluding CD4 cells, seemed to be associated with a greater risk of postsurgical infections [14]. Our findings strongly suggest that using preoperative enteral immunonutrition we can modulate apoptotic signaling pathways and maintain the lymphocyte subsets decreasing after pancreas resection as compared with standard nutritional support. In majority of patients with immunonutrition glutamine enriched diet was used. The small group of patients receiving unsaturated fatty acid does not permit on any separate conclusion. There were a significant elevation in percentage of Fas (CD95+/CD3-) and TNFRI (CD120a) lymphocytes after immunonutrition as compared with standard diet. Presented in our study changes in percentage of cells with death receptors family may enhance antitumor activity (for instance by Fas and TNF-R1 mediated cytotoxicity), but also higher lymphocytes susceptibility to apoptosis after such a kind of immunonutrition can be considered. These results confirmed the immunomodulatory effect of immune-

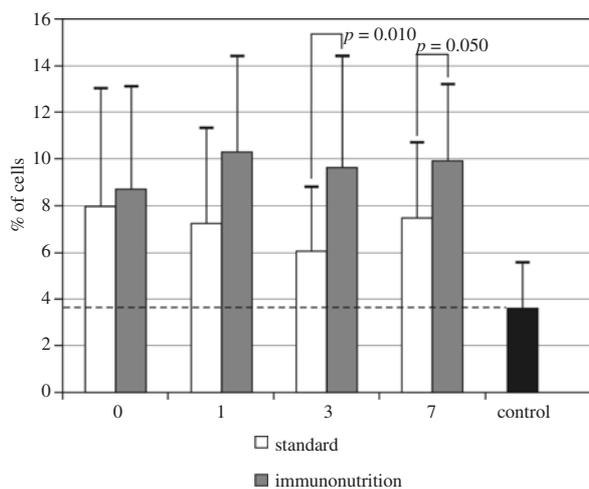


Fig. 1. Percentage of Fas (CD95+/CD3-) positive lymphocytes before and after surgery in pancreatic cancer patients received preoperative enteral standard (group I) and immune-enhancing nutrition (group II). Values are expressed as means \pm SD

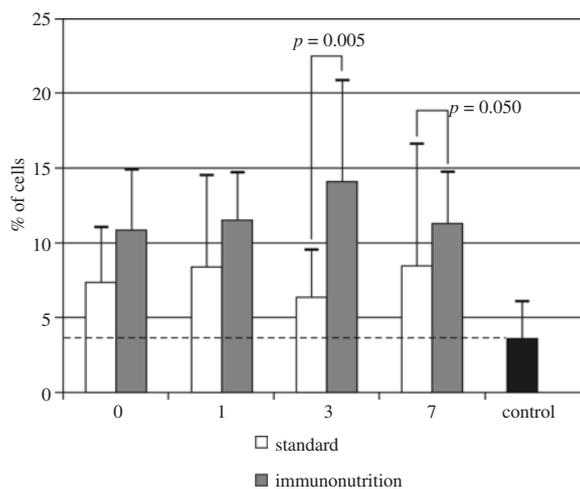


Fig. 2. Percentage of TNFRI (CD120a) positive lymphocytes before and after surgery in pancreatic cancer patients received preoperative enteral standard (group I) and immune-enhancing nutrition (group II). Values are expressed as means \pm SD

enhancing enteral nutrition, but on other hand may pointed to the proapoptotic influence of the pancreatic tumor.

In experimental study, the presence of glutamine increased lymphoproliferation as well as Bcl-2 and CD95 expression, but decreased CD95L and activation-induced T-cell death [12]. Authors of this study suggested that glutamine protects activated human T cells from apoptosis by up-regulating glutathione and Bcl-2 levels and down-regulating both caspases-3 and caspases-8 activities. The mechanism by which glutamine protects cells from apoptosis is still unclear, but these observations suggest that glutamine possesses anti-apoptotic property and supply of glutamine in malnourished patients is required for the immune system to function optimally.

In addition, our study revealed that up-regulation of proapoptotic signaling systems in lymphocytes of pancreatic cancer patients is very frequent. In pancreatic cancer patients the percentage of lymphocytes with Fas/CD95+/CD3- and TNF-R1/CD120a phenotypes were significantly higher before as well as after surgery (as compared with control group). One of the possible explanation of inappropriate changes in apoptotic signaling pathways proteins is related to immune system cells malnutrition in pancreatic cancer patients. Such a lymphocytes phenotypes especially after pancreatic cancer surgery may lead to an inappropriate cell apoptosis. The extensive apoptotic death of lymphocytes probably contributes to profound immunosuppression after pancreas resection. As a response to major surgery we showed that percentage of CD95-/CD3+ lymphocytes was decreased. Other studies revealed that parenteral and enteral administration of glutamine vs. standard feeding maintain lymphocyte numbers and function in patients at risk of immunosuppression or infection and improved patients outcome [15-17].

In conclusion, our findings suggest the up-regulation of proapoptotic signaling systems in lymphocytes of malnourished patients receiving immunonutrition after pancreatic cancer surgery. These changes in apoptotic signaling pathways proteins after immunonutrition may increase lymphocytes dysfunction. Further research needs to be undertaken to examine the difference between reaction of apoptotic signaling proteins to selected immunonutrients.

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