Anti-inflammatory response to early enteral immunonutrition in malnourished patients after pancreaticoduodenectomy

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Abstract

The aim of the study was to investigate whether the early enteral immunonutrition in comparison with standard enteral feeding affects the systemic production of pro- and anti-inflammatory cytokines in malnourished patients after pancreaticoduodenectomy. Prospective, randomized studies performed in 41 patients after pancreaticoduodenectomy receiving early enteral standard nutrition (group I, n=22) or enteral immunonutrition (group II, n=19). Cytokines and cytokine inhibitors (IL-1β, TNF-α, IL-6, IL-8, IL-10, IL-1ra, and sTNFRI) were determined before and on day 1, 3, 7, 10 and 14 after surgery using ELISA test. Patients treated with enteral immunonutrition showed elevated concentrations of IL-6 for longer periods (between day 1-14, $p<0.05$) and higher values than in patients receiving standard nutrition (on day 10-14: 150.5±85 and 142.3±77 pg/ml vs. 96.1±45 and 73.6±42 pg/ml, all p=0.01). The significantly higher concentrations of IL-1ra were also found in patients receiving immunonutrition (on day 7-14 respectively: 3909.2±1398.3; 4544.8±2215.2 pg/ml and 4238.2±2326.1 pg/ml in the immunonutrition-treated group vs. 2221.3±994.9; 2694.5±1223.6 and 2586.3±1105.7 pg/ml in patients receiving standard nutrition, all p= 0.01). The values for remaining cytokines did not significantly differ in the comparable groups of patients. The main factor increasing the level of cytokines on day 1 was the extensive operative trauma. The early enteral immunonutrition as compared with standard nutrition shows an immunomodulative effect on the changes in immune response after an extensive surgical trauma resulting in selective stimulation of IL-6 and IL-1ra production, but only the interleukin-1 receptor antagonist is the early sensitive marker of anti-inflammatory response to enteral immunonutrition in malnourished patients after pancreaticoduodenectomy.

Key words: *pancreatic cancer surgery, nutrion, cytokines.*

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Introduction

A surgical trauma increases the immune system suppression and deepens malnutrition occurring in approximately 50% patients with digestive system cancers [1, 2]. The immune disorders and malnutrition are worse in the early postoperative period, which considerably affects the process of wound healing, intestinal barrier function and the number of post-operative complications [3-6]. This period can feature an enhanced pro-inflammatory response (SIRS – systemic inflammatory response syndrome) and anti-inflammatory (CARS – compensatory anti-inflammatory response syndrome) to an extensive surgical trauma, which leads to immune functions breakdown [7, 8]. While pro-inflammatory cytokine production (IL-1, TNF, IL-6) is an essential part of response to surgical injury, the excessive

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production of these molecules may result in increased morbidity and mortality [9].

One of the ways to improve the immunity and to lower the number of post-operative complications in oncological patients after an extensive surgical trauma was the introduction of enteral immunonutrition. In patients suffering from neoplasms of the colorectum, stomach, or pancreas, the perioperative administration of a supplemented enteral formula (enriched with arginine, RNA, and omega-3 fatty acids) reduced the number of postoperative infections and the length of hospital stay [10]. After pancreaticoduodenectomy the established nutritional goal can be obtained by enteral feeding and the immunonutrition seems to improve outcome [11]. The rate of postoperative complications was lower in the immunonutrition-treated group than in the group treated with standard enteral formula or after total parenteral nutrition. The early post-operative enteral feeding may safely and effectively replace parenteral nutrition in patients undergoing pancreaticoduodenectomy. Other authors [12] included patients with esophageal, gastric and peripancreatic, or bile duct cancer undergoing resections and receiving early postoperative enteral feeding with an immune-enhancing formula and the results showed that there were no significant differences in the number of minor, major, or infectious wound complications, mortality and length of hospital stay between the groups. According to this data, the early enteral feeding with an immune-enhancing formula is not beneficial and should not be used in a routine fashion after surgery for upper gastrointestinal malignancies.

Despite the advantage of positive effects of immunonutrition on the treatment of surgical patients, the impact of this nutrition on immune system remains still unclear. The patients who underwent curative operations for gastric or pancreatic cancer with delayed hypersensitivity responses, phagocytic ability of monocytes and concentration of IL-2 receptors had all recovered more in the group receiving the enriched solution (with arginine, RNA and omega-3 fatty acids) on postoperative days 4 and 8, but there was no difference in the post-operative infection rates as compared with standard enteral diet or the group receiving entirely parenteral nutrition in the post-operative period [13]. A study performed by other authors showed [14] that in patients who have undergone major operations for gastrointestinal cancer the supplementation of postoperative early enteral nutrition with glutamine, arginine, and omega-3-fatty acids positively modulated postsurgical immunosuppressive and inflammatory responses. In this study the feedings started within 48 hours after operation and immune responses were determined by phagocytosis ability, respiratory burst of polymorphonuclear cells, total lymphocytes, lymphocyte subsets, nitric oxide, cytokines concentration, and inflammatory responses by plasma levels of C-reactive protein and prostaglandin E2 level. The post-operative levels of IL-6 and TNF-α were lower in the supplemented group. Other study showed that in patients with gastric carcinoma after 7 days of postoperative enteral nutritional support, the immunonutrition-treated group (enteral formula enriched with glutamine, arginine and omega-3 fatty acids) had higher levels of IL-2 than those in the control group who received standard nutrition, whereas IL-6 and TNF-α levels were significantly lower in the immunonutrition-treated group [15]. In patients after pancreaticoduodenectomy the immunonutrition enhances the immunometabolic response (phagocytosis ability of polymorphonuclear cells and plasma interleukin-2 receptors on day 8) and improves outcome compared to parenteral feeding [16]. Immunonutrition enhances the host response and induces a switch from acute-phase to constitutive proteins. Inverse correlation between IL-6 and pre-albumin levels was noted only in the immunonutrition group [17].

Any deeper insight into the possibility of immune disorders regulation after an extensive operative injury by using immunonutrition requires further investigation of changes in pro- and anti-inflammatory cytokine concentrations during immmunonutrition. The aim of our study was to compare the effect of early post-operative enteral immunonutrition and enteral standard nutrition on the concentration of cytokines and cytokine inhibitors (IL-1β, TNF-α, IL-6, IL-8, IL-10, IL-1ra, and sTNFRI) in malnourished patients after pancreaticoduodenectomy.

Materials and Methods

The prospective and randomized study covered 41 patients (mean age: 58.03±10.2) operated on for pancreatic cancer. All the patients after full clinical diagnostic procedures (image and laboratory tests) were operated on to resect the head of the pancreas (Whipple's pancreatoduodenectomy). The histopathological examination confirmed the diagnosis. Patients were randomized (by using numbered sealed envelopes stratified by the surgeon) to receive either an early standard (I group – 22 patients, Nutrison Nutricia) or immune-enhancing enteral diets (II group – 19 patients, Stresson Nutricia) (tables 1, 2).

The indication for the early post-operative enteral nutrition treatment was the pre-operative loss of body mass (>6% within 2 months) and the extension of surgery (including the advancement of tumor) questioning the possibility of receiving oral diet covering the calorific and protein demand within 7 days after the procedure [18]. The nutritional status was assessed before and on day 7 after the operation based on routine tests revealing a loss in body mass (for initial tests within recent 2 months), body mass index (BMI), albumin concentration and total lymphocyte count (TLC).

The investigations did not include the patients suffering from post-operative infectious complications, unrespectable pancreatic cancer, after transplantations of organs, the

Table 1. Patient characteristics and surgical parameters of the two groups (supplemented versus standard diet)

Characteristics	Supplemented diet		Standard diet		P
age (y)	59.8±6		54.2±4.1		NS
gender (M/F)	14/5		15/7		NS
location of cancer pancreas	19		22		
tumor staging (TNM classification) I II III	 9 8 2		 8 11 3		NS
type of operation pancreatoduodenectomy	19		22		
duration of surgery (min)	330±60		343±45		NS
operative blood loss (mL)	550±300		600±350		NS
transfused patients	6		7		
nutritional status	**before surgery**	**after surgery**	**before surgery**	**after surgery**	
weight loss (%)	6.52±2.1	9.14±2.8	6.34±3.4	9.23±3.2	NS
BMI (kg/m^2)	23.36±4.5	22.43±6.3	22.24±3.2	21.82±3.0	NS
albumin (g/L)	29.8±0.8	24.1±5.4	28.5±3.1	20.3±6.8	NS
TLC (cells/mm^3)	2151±253	1140±262	1900±624	930±145	NS

Table 2. Composition of diets

Variables	Composition of the diets/100mL	
	supplemented diet (n=19)	standard diet (n=22)
calories (kcal)	125	100
protein (g)	7.50	4.00
glutamine	1.34	-
arginine	0.89	-
fat (g)	4.2	3.9
LCT	2.0	0.4
MCT	1.5	1.2
EPA	0.079	-
DHA	0.028	-
n6:n3	3.5:1	5:1
L-carnitine (mg)	7.5	-
inositol	63	-
taurine	13	-
choline	46	37
Vit. A (μg RE)	91	82
Vit. E (mg α-TE)	13.0	1.3
Vit. C (mg)	25.0	10.0
osmolarity (mOsm/L)	410	260

patients treated with chemo- or radiotherapy, immunosuppresors, patients with autoimmune diseases, with diabetes type 1 (insulin-dependent), chronic respiratory insufficiency (chronic obstructive pulmonary disease), cardiovascular insufficiency and kidney and liver diseases (biopsy-proven cirrhosis or a serum total bilirubin of >3.0 mg/dL).

In both groups the post-operative nutrition was carried out by using a pump and a tube installed during operation in the distal small bowel loop. The rate of administering the diet was gradually increased from 30 ml/h for the first 24 to 48 hours then increased to full feeding depending on the passage of flatus and bowel action. All patients reached their nutritional goal within 72 h. The total feeding time for the entire group of patients amounted to mean 12.3±2 days. Daily supply of the main nutritional substances in standard enteral nutrition amounted on average to: 10.8±1.3 g nitrogen, 208±24.4 g glucose, 66±7.7 g fat (including 101.6±11.9 g of protein and 1693±198 kcal), whereas in enteral immunonutrition: 14.7±2.15 g nitrogen, 177±26 g glucose, 51.4±7.5 g fat, 16.4±2.4 g glutamine, 10.9±1.6 g arginine (including 91.8±13.5 g protein and 1529±224 kcal). The supply of calories and nitrogen did not differ significantly between both groups. Table 2 shows the composition of the diets. The tolerance for both formula diets was excellent.

All patients received antibiotics for prophylaxis (1.2 g augmentin and 2.0 g of cefoperazone), low-particle heparin and were intravenously given crystalline fluids as well as electrolites depending on actual demand.

Cytokine and cytokine antagonists measurement

In all patients blood samples were collected from the peripheral vein on the day preceding operation and on post-operative days 1, 3, 7, 10 and 14. Serum samples were prepared and stored at –80°C for further use. The serum concentrations of IL-1β, TNF-α, IL-6, IL-8, IL-10, IL-1ra, and sTNFRI (p55) were determined using commercially available enzyme immunoassay kits (Quantikine R&D Systems Europe Ltd, Barton Lane Abingdon, Oxon). Samples were prepared and tested in duplicate according to the manufacturers' instructions. The lower limit of sensitivity of the assay for serum samples was 1 pg/ml for IL-1β, 4.4 pg/ml for TNF-α, 0.7 pg/ml for IL-6, 10 pg/ml for IL-8, 3.9 pg/ml for IL-10, 22 pg/ml for IL-1ra, and 3.0 pg/ml for sTNFRI.

The patients signed a written consent after the details of the protocol were fully explained. Protocol of the study was approved by the Medical University Ethics Committee.

Statistics

The data were presented as a mean taking into account the ± deviation and analyzed by using the analysis of variance (ANOVA). To evaluate the statistical significance of differences between the pre-operative and post-operative cytokine levels Dunnett's test and Mann-Whitney *U* test for comparisons of cytokine levels in standard and supplemented group were used. Chi-squared test and Fisher exact test were used to compare discrete variables. *P* values of less than 0.05 were considered significant.

Results

In the compared groups of patients (group I vs. group II) the pre-operative concentrations of cytokines and their inhibitors did not show any significant difference. In the group treated with early enteral immunonutrition (group II) in comparison with pre-operative values the results showed a significant increase in the levels of IL-6 and sTNFRI from post-operative day 1 to 14, and for IL-1ra from day 3 to 14 ($p<0.05$), whereas concentrations of IL-1β, TNF-α, IL-8 and IL-10 did not significantly change. In group II we noted the elevated concentrations of IL-6 lasting for longer periods and significantly increased values ($p=0.01$) as compared with group I on day 10 and 14 after operation (table 3).

In patients receiving standard nutrition (group I), as compared with the pre-operative test results, the significant increase referred to IL-6, IL-8, IL-10 (only in day 1, $p=0.01$), IL-1ra and sTNFRI (between day 1-14, $p<0.05$). The post-operative changes in concentration of IL-1β, TNF-α, were in this group statistically insignificant. While comparing both groups of patients (group I vs. group II) the significantly higher concentrations of IL-1ra were found in patients receiving early immunonutrition (on day 7-14, for all $p=0.01$) (table 3, figure 1).

Discussion

Pancreaticoduodenectomy is one of the most invasive operations in the upper abdominal surgery with a high incidence of postoperative complications [19-21]. The immune disorders occurring together with the surgical injury as well as the malnutrition that deepens after operation

Table 3. Changes in kinetics of serum cytokine and cytokine antagonist levels (pg/ml) before and after surgery in patients with standard and supplemented diet

Variables	Diet	Pre-operative	POD-1	POD-3	POD-7	POD-10	POD-14
IL-1β	standard	3.6±1.8	3±2.1	3.8±2.6	2.9±0.8	4.92±3.5	4.8±1.6
	supplemented	5.8±2.3	3.6±1.7	2.8±1.5	1.4±0.9	4.58±2.6	3.5±1.9
IL-1ra	standard	458.4±365.6	3198.7±1979.3[a]	3582.7±2044.1[a]	2221.3±994.9[a]	2694.5±1223.6[a]	2586.3±1105.7[a]
	supplemented	1069.5±940	2792.6±1416	4106.2±2022.9[a]	3909.2±1398.3[a,b]	4544.8±2215.2[a,b]	4238.2±2326.1[a,b]
IL-6	standard	55.2±45.02	355.3±143.2[a]	189.1±153.6	99.9±42.7	96.1±45.0	73.6±42.5
	supplemented	13.1±8.8	280.7±64.9[a]	233.9±159.6[a]	81.4±40.6[a]	150.5±85.8[a,b]	142.3±77.6[a,b]
IL-8	standard	20±19.07	66±38.7[a]	39±29.7	26.5±18.8	47±36.1	35.5±19.2
	supplemented	85±40.1	105±53.8	140±58.1	85±34.6	110±63.9	105±44.7
IL-10	standard	7.5±6.5	17.7±10.9[a]	8.3±7.7	20.2±14.7	7±6	18±9.3
	supplemented	14.3±5.3	17.5±11.5	18.1±6.4	24.3±15.4	45±31.7	24±13.3
TNF-α	standard	5.6±2.5	5.9±3	4.6±3.7	9.7±8.4	7.4±5.4	6.4±4.7
	supplemented	3.1±2.1	4.1±3.8	2±1.2	2.5±1.1	8±5.3	7.5±4.2
sTNFRI	standard	1888.3±818.2	3478.6±1177.8[a]	3821.4±2139.8[a]	3035.7±1224[a]	3561±2023.6[a]	3481.4±2219.5[a]
	supplemented	1531.2±716.9	3256.4±1144.2[a]	3491.2±1385.7[a]	3297.5±1053.2[a]	3178.7±929.2[a]	3285.6±1694.8[a]

[a] – $p<0.05$ pre-operative versus post-operative levels and [b] – $p<0.05$, standard versus supplemented group; POD – post-operative day.

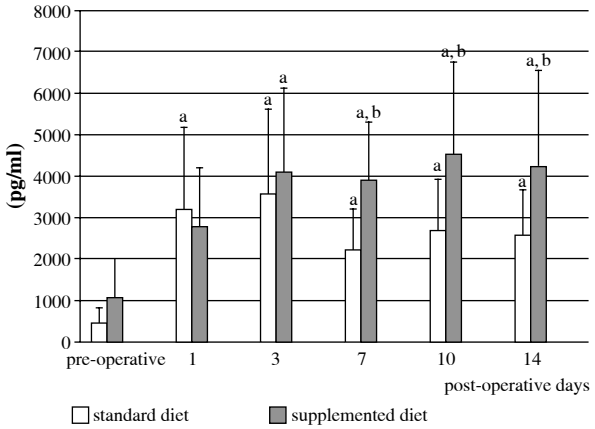

Fig. 1. Changes in kinetics of interleukin-1 receptor antagonist levels (pg/ml) before and after surgery in patients with standard and supplemented diet. [a] – $p<0.05$ pre-operative versus post-operative levels and [b] – $p<0.05$, standard versus supplemented group

usually worsens outcome. The attempt to correct the post-operative nutritional disorders by introducing immunonutrition is a promising way of improving outcome, but still little is known about the mechanisms of correcting the post-operative immune disorders by using this sort of nutrition. The most controversial is the effect of immunonutrition on the post-operative cytokine level, which is very important for the immune response to surgical trauma.

The current study investigated whether the early enteral immunonutrition as compared with standard enteral feeding affected the systemic production of pro- and anti-inflammatory cytokines. The results show that the main factor changing the level of cytokines and their inhibitors (IL-6, IL-8, IL-10, IL-1ra and sTNFRI) on day 1 after pancreaticoduodenectomy is the operative trauma. The similar dynamics of changes in cytokine and their inhibitor concentration in peripheral blood was found in patients suffering from cancer after resection of colonic cancer who had not received immunonutrition [22]. As we can notice from the presented study, the assessment of IL-6, IL-1ra and sTNFRI concentration values is significant in monitoring the immune disorders and early diagnostic of SIRS/CARS in malnourished patients after pancreatic cancer surgery. The changes in concentration of remaining cytokines (IL-1β, TNF-α, IL-8, IL-10) are short-term or statistically insignificant, which reduces their diagnostic usefulness. The explanation might be the short half-life and inactivation by other cytokine (for example IL-6) or cytokine inhibitors (for example IL-1ra or sTNFR). The IL-1β and TNF-α blood levels in trauma patients and after surgery have not been found elevated in a number of studies [23, 24]. Maybe assessing the concentration of those cytokines during or at the end of surgery would be useful in diagnosing the disorders of immune response to extensive surgical trauma [25].

The results of our investigations confirm the emphasized by other authors modulative effect of immunonutrition on changes in immune response to surgical trauma in the post-operative periods. Unlike any previous investigations that did not cover the wide range of pro- and anti-inflammatory cytokines in malnourished patients after pancreatic cancer surgery, in the currently presented study the early enteral immunonutrition as compared with standard nutrition had no significant effect on the post-operative concentration of majority of assessed cytokines and their inhibitors (IL-1β, TNF-α, IL-8, IL-10 and sTNFRI). Other studies revealed that in patients who underwent major thoracic and abdominal surgery (included only 3 patients after pancreaticoduodenectomy) treated with enteral diet containing arginine, omega-3 fatty acids and RNA (IMPACT) the elevated concentrations of IL-1β and IL-2 were found as late as on day 16, whereas the decreased concentration of IL-6 depending on the sample collection time were noted on day 8 or between day 3-7 [26]. In the last study the interleukin concentrations were measured either without stimulation of a mitogen or after phytohaemagglutinin (PHA) stimulation and glutamine was not used in immunonutrition. In patients after gastrectomy for cancer, the perioperative versus post-operative administration of an enteral immune-enhancing diet (without glutamine) ameliorated the host defense mechanisms and controlled the inflammatory response (lower levels of IL-6 on postoperative day 1, 4, and 8 were detected) [27]. The postoperative systemic IL-6 and IL-8 responses in small group of patients with colorectal cancer who received standard TPN preoperatively were greater than in patients who received an enteral diet. Preoperative nutrition via the enteral route may provide a better regulation of cytokine responses after surgery than parenteral nutrition [28]. In patients with cancer of the stomach or colo-rectum, the perioperative nutrition with a supplemented enteral diet (arginine, omega-3 fatty acid and RNA) without glutamine modulates cytokine production (higher interleukin-2 receptors alpha and lower IL-6, and IL-1 soluble receptors II were noted) [29]. Studies of other authors[30] showed that early enteral immunonutrition (IMPACT) without glutamine after colorectal cancer surgery was not associated with increased post-operative complications nor was it related to any change in cytokine profiles, but only IL-6 and TNF-α plasma levels were measured. The differences in results of the above mentioned investigations can be caused by many factors, including among others differences in the number of patients and in the level of surgical trauma, time and the way of diet administration, the nutritional mixture composition, nutritional status and differences in selection of immune parameters or in selection of test methods for immu-

ne changes. The short "diagnostic windows" especially in the case of assessing the dynamics of changes in the level of pro-inflammatory cytokines (IL-1β and TNF-α) are another problem making it difficult for immunonutrition to have an impact on immune system. In our studies the effect of nourishment status, the extension of surgery, advancement of cancer, supply of nitrogen and calories in both groups (group I vs. group II) were almost the same.

In the group of patients receiving immunonutrition (group II) we found a longer-term (up to day 14) significantly elevated concentration values of IL-6, but the significantly higher values as compared with group I (this group showed a significant increase only on day 1) were found not before day 10-14. The post-operative changes in concentration of this cytokine on later post-operative days can be associated with the effect of enteral immunonutrition. The significant increase in concentration of IL-6 on days 1-3 is an example of immune response to an extensive surgical trauma in malnourished patients with pancreatic cancer. Prolonged and excessive elevations of circulating IL-6 levels in patients after major surgery have been also associated with complications [31] and IL-6 appears to be an early marker of subsequent postoperative sepsis in patients undergoing surgery for cancer [32], but in our study patients with postoperative infections complications were excluded. Unlike the investigation results of other researchers, where immunonutrition was mainly enriched with arginine and non-saturated fatty acids, in our studies the nutritional mixture included also glutamine. In an experimental study the addition of glutamine to cultured rat macrophages stimulated with LPS increased IL-6 mRNA [33], but the production of TNF-α, IL-1β and IL-6 by human blood monocytes appears to be slightly affected by glutamine availability [34]. Parenteral administration of glutamine after colorectal surgery increased the mitogen-stimulated proliferation of blood lymphocytes, but did not affect ex vivo TNF or IL-6 production [35]. There were also no effects of the glutamine dipeptide on the production of TNF-α or IL-6 by LPS-stimulated whole blood after major abdominal surgery [36]. In the examined group of patients (group II) the stimulation of IL-6 production, which is produced mainly by monocytes and macrophages, enteral immunonutrition could increase the immunosuppressive response to surgical trauma. Interleukin 6 is a multifunctional cytokine whose functions include also the modulation of anti-inflammatory response after surgical trauma. For example, IL-6 enhances the synthesis of glucocorticoids that possess immunosuppressive properties and also directly inhibits the expression of TNF and IL-1. In addition, IL-6 stimulates macrophage expression of IL-1 receptor antagonist and soluble TNF receptor [37], which bind to the pro-inflammatory cytokine (IL-1, TNF). The administration of recombined human interleukin-1 antagonist receptor (rhIL-1ra) extended the survival time of mouse with experimentally induced septic shock [38]. Soluble cytokine receptors and receptor antagonists are sequentially released after trauma and serum levels of sTNF RI/RII and IL-1ra are found elevated [39]. It has been also confirmed by our studies covering the both groups after pancreaticoduodenectomy.

In comparison with standard nutrition, the significantly higher concentrations of IL-1ra (between day 7 and 14) were found only in patients receiving enteral immunonutrition (group II). The results obtained can be explained by the stimulating effect of immunonutrition (mainly glutamine) on the immune system of bowels (GALT), whereas the increase of IL-1ra concentration on day 1 after surgery should be associated with the response to surgical trauma. A study of Pruitt et al. [40] revealed the highest peak plasma IL-1ra concentrations (at 2-4 h) in patients undergoing thoraco-abdominal aneurysm repair and bowel resection. Similarly, O'Nuallain [41] has shown that IL-1ra can be induced as an early-response cytokine following major trauma in the absence of an infection. The highest IL-1ra level was detected in patients 4 hours after the commencement of an abdominoperineal colon resection and within 24 hours reached the pre-operative values.

In our study it is difficult to assess the effects of glutamine and arginine apart from some effects of the other nutritional additives used in immunonutrition like taurine, L-carnitine (enzyme that is required for fatty acid transport into the mitochondria) or inositol (essential for cell signaling), which is not added to standard enteral nutrition formula used in group I. On the other hand, supplementing glutamine may be a way to provide the patient with glutamine as well as arginine, since it has been suggested that citrulline, a product of intestinal glutamine metabolism, can be taken up by the kidney to serve as a precursor for arginine synthesis. It is also possible that glutamine and arginine works better in conjunction with other nutrients. For example, glutamine serves as a precursor of the endogenous antioxidants taurine and glutathione. Taurine (as taurine chloramine) offers protection against oxidant damage and may exert a potent anti-inflammatory effect by suppressing TNF-α, IL-6, PGE2 and NO production in endotoxin and IFN-α stimulated macrophages in culture [42]. Because glutamine-enriched enteral feeding reduces the incidence of infectious complications and restores body-fluid distribution, it was hypothesized that glutamine enrichment might have an important effect on adequate plasma taurine concentrations, which are also needed to restore the osmolar disturbances observed after trauma. First of all, glutamine as a nitrogen donor for the synthesis of purines and pyrimidines is the major energy source for the immune system and cells of the small intestine, such as enterocytes. Glutamine maintains the integrity of the gut mucosa. After the enteral administration of glutamine the number of lymphocytes T increases in Peyer's glands [43], whereas after parenteral administration the concentration of SIgA, IL-4 and IL-10 in intestinal mucous

membrane [44]. Some previous studies have shown that glutamine depletion increases spontaneous apoptosis and oxidant-induced cell death in intestinal epithelial cell lines [45] and glutamine prevents cytokine-induced apoptosis in human colonic epithelial cells [46]. Intestinal requirements for glutamine appear to increase during catabolic conditions associated with decreased plasma glutamine concentrations and increased cytokine generation by gut mucosal cells [47]. These changes can worsen in malnourished patients after an extensive surgical trauma. The most prominent effect of supplemental arginine is in abrogating trauma-induced immunosuppresion and improvement of wound healing. In the mechanism of arginine operation we should remember esepecially about the key role of nitric oxide and the increased activity of lymphocytes T (increased production of IL-2 and expression of the receptor for IL-2 on lymphocytes), whereas in the studies of innate immunity the increased activity of NK cells and macrophages [48, 49].

The stimulation of intestinal immune system cells by applying immunonutrition to increase the production of IL-1ra in an early period after an extensive surgical trauma can have a positive effect on the regulation (decrease) of the post-operative inflammatory response. At the same time we have to ask the following question: how long should we maintain the effect (stimulation of IL-1ra production) and to what extent it can be increased by immunonutrition, for instance either by raising the doses of glutamine or arginine, or by introducing the immunonutrition as early as in the pre-operative period. Our previous studies of malnourished patients operated for esophageal cancer showed that the application of standard parenteral and enteral pre-operative nutrition for the period of 10 days resulted in significant increase of IL-6 and IL-1ra concentration in peripheral blood even before surgery, but it does not affect the concentration of sTNFRI and pre-operative improvement of nutrition status [50]. The recent results of studies performed in patients operated on for colonic cancer suggest that both pre- and post-operative immune disorders (Th1/Th2 imbalance) can be corrected by applying short--term (five-day-long) pre-operative immunonutrition [51]. It is known that in the group of patients suffering from colonic cancer malnutrition occurs less frequently than in patients with pancreatic or esophageal cancer.

In conclusion, our study has clearly indicated that the anti-inflammatory mechanisms are early activated in malnourished patients after pancreaticoduodenectomy. The high levels of serum IL-6, IL-1 antagonists and TNF after pancreatic resection suggest the tissue level involvement of these cytokines in post-operative hyper-inflammation. An early enteral immunonutrition in comparison with standard nutrition has an immunomodulative effect on the changes in immune response after an extensive surgical trauma, consisting in selective stimulation of IL-6 and IL-1ra production. Among all investigated cytokines and their inhibitors IL-6, IL-1ra and sTNFRI are sensitive markers of surgical trauma, but only IL-1ra is the sensitive marker of post-operative anti-inflammatory response to enteral immunonutrition in malnourished patients with pancreatic cancer. Further research needs to be undertaken to examine the interaction between nutrients and to establish the levels and time of intake required to optimize immune responsiveness in malnourished patients after pancreatic cancer surgery.

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