The reoperation for infective complications after major surgery of pancreas does not evoke additional cytokine response

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Abstract

Blood cytokines are accepted as semiquantitative markers of the operative tissue trauma and mediators of the host immune response. There is not enough data on the cytokine response following a secondary trauma in the same individual, as an early reoperation, which may influence the clinical course after surgical reintervention and predict the outcome. The reoperation, usually performed within the first week after primary surgery, is an additional burden for the immune system. The objective of this study was to evaluate how does the reoperation affect the level of serum blood cytokines. Does another rise of the proinflammatory or rather of the anti-inflammatory cytokines take place or is there a decrease as an effect of elimination of the source of local infection? Studies were carried out in 43 patients with pancreatic carcinoma before and after operation and reoperation. We measured serum levels of IL6, IL1ra and sTNFRI before and after first operations and after reoperations performed because of infective complications of the pancreatic cancer surgery. Although a high postoperative rise of serum IL-6, IL-1ra and sTNFRI levels in patients after pancreatectomy over the preoperative values was observed, there was no increase in cytokine concentration after re-operation performed because of infective complications. Albeit the serum cytokine levels are good markers of the immune reactivity of surgical patients to the first operative trauma and in certain cases early predictors of the postoperative infective complications, their diagnostic value after reoperations is questionable.

Key words: *infective complications, reoperation, cytokine response, major surgery*

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Introduction

Blood cytokines are accepted as semiquantitative markers of the operative tissue trauma and mediators of the host immune response. There is not enough data on the cytokine response following a secondary trauma in the same individual, as an early reoperation, which may influence the clinical course after surgical reintervention and predict the outcome. The primary postoperative immune response depends on the mass of traumatized tissues and their location. It is mediated by the proinflammatory cytokines, among others, interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF), and modulated by the naturally occurring antagonists of these cytokines as soluble TNF receptor (sTNFRI) and IL-1 receptor antagonist (IL-1ra) [1, 2]. The inflammatory complications and surgical reinterventions may further stimulate cytokine production leading to the development of SIRS [systemic inflammatory response syndrome]. This may be expected especially after major surgical procedures on the pancreas and colon.

Major surgery of the pancreas and colon brings about high production and release of cytokines [3-5]. In addition, postoperative complications as leaking anastomosis and local infection recruit granulocytes and peritoneal macrophages at

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the site of inflammation. These cells are the main source of cytokines released to the peritoneum and subsequently peripheral blood. We have previously found that infective complications at the site of colon anastomosis bring about a sharp increase in plasma sTNFRI already on day 1 and of IL-6 and CRP on day 3 after the operation [6]. Thus, serum cytokine levels may be a good early indicator of the development of postoperative complications after major surgery and justify an immediate surgical intervention [reoperation].

The reoperation, usually performed within the first week after primary surgery, is an additional burden for the immune system. The question arises how does the reoperation affect the level of serum blood cytokines. Does another rise of the proinflammatory or rather of the anti-inflammatory cytokines take place or is there a decrease as an effect of elimination of the source of local infection? Would a secondary increase in the proinflammatory or an insufficient anti-inflammatory cytokine response to reoperation lead to the development of organ dysfunction?

In this study we measured serum levels of IL-6, IL-1ra and sTNFRI after reoperations performed because of infective complications of the pancreatic cancer surgery. These cytokines were selected from an array of other measured cytokines, as they were found in our previous studies to be the most sensitive markers of the postoperative inflammatory complications [6]

Materials and methods

Studies were carried out in 43 patients of mean age of 64 years (Table 1). Seventeen of the 43 patients after pancreaticoduodenectomy developed serious postoperative complications, as intra-abdominal abscess and anastomosis dehiscence, requiring reoperation. In majority of patients laparotomy and abscess drainage was performed. Three patients underwent USG-controlled puncture of intra-abdominal abscess. There were two fatal cases on day 14 and 16 in a patient following Whipple operation with pancreaticojejunostomy rupture, intraabdominal abscess and MOF development. After reoperation total parenteral nutrition (TPN) was administered continuously in each patient with aminoacids, glucose, lipid emulsions, electrolytes, vitamins and oligoelements.

Table 1. Clinical data for patients after pancreatic cancer resection and reoperations

Clinical data		Patients with pancreatic cancer	Patients requiring reoperations
number of patients		43	17
age [years]		57 [46-67]	63 [48-67]
gender [M:F]		30:13	12:5
tumor location		head of pancreas	head of pancreas
tumor staging	I	15	4
	II	24	10
	III	4	3
	IV	–	
type of resection		Whipple procedure	Whipple procedure
duration of operation [min]		355 [240-365]	345 [280-435]
patients with postoperative blood transfusions		14	8

Blood samples and cytokine measurement

Blood samples were collected from the peripheral vein on the day preceding operation and on days 1, 3, 7, 10 and 14 thereafter. Serum samples were prepared and stored at -80°C until further use. The serum concentrations of IL-6, IL1-ra, and sTNFRI were measured by enzyme immunometric assay (Quantikine R&D Systems Europe Ltd, Barton Lane Abingdon, Oxon). Each sample was examined in duplicate. The lower limit of sensitivity of the assay for serum samples was 0,7 pg/ml for IL6, 22 pg/ml for IL-1ra, and 3,0 pg/ml for sTNFRI. As controls, serum concentrations of IL-6, IL-1ra and sTNFRI were measured in 16 healthy adult volunteers. In this group the IL6 concentration was 1.75±4.2 pg/ml, IL-1ra 327.6±435.2 pg/ml and sTNFRI 869±143.3 pg/ml.

All patients signed informed consent before entering the study. Protocol of the study was approved by Medical University Ethics Committee.

Statistical analysis

The postoperative cytokine levels after first operation were compared with the preoperative levels. Moreover, the levels of the group with complications requiring reoperation was compared with those with uneventful postoperative course. Then, the post-reoperation cytokine data were compared with the post-first-surgery data. Results are presented as means ±SD. The Mann-Whitney U test and the Wilcoxon signed rank test were used to analyze unpaired and paired samples respectively. P values of less than 0.05 were considered significant.

Results

Uneventful postoperative course

Cytokine levels in patients after pancreatic resections with uneventful postoperative course and complications have been shown on Fig.1-3. The IL-6 serum concentration increased from 206.8±386.6 before surgery to 250.4±578.7 pg/ml on day 3, to decrease to 49.0±40.8 pg/ml on day 7 and remain at this level until day 14. The same kinetics, of a peak on day 3 followed by a subsequent decrease, was

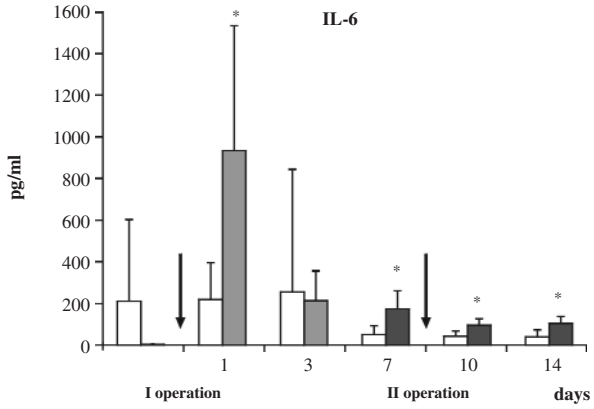

Fig. 1. The serum interleukin (IL-6) concentrations in patients before and after pancreatic resections with uneventful postoperative course (□) and with postoperative complications (▩) requiring reoperations (■) (n-17). * postoperative vs. preoperative (before I- operation) values, means ±SD, p<0.05

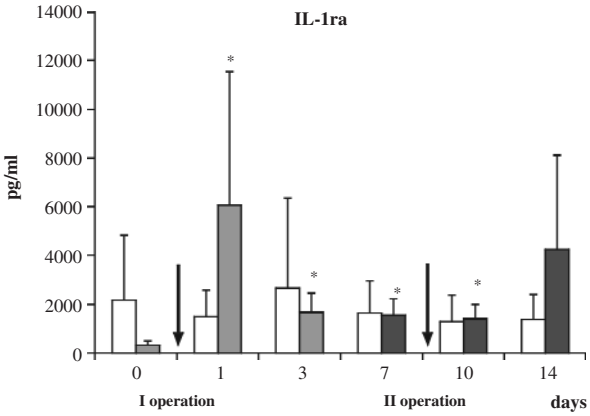

Fig. 2. The serum IL-1 receptor antagonist (IL-1ra) concentrations in patients after pancreaticoduodenctomy without (□) and with postoperative complications (▩) requiring reoperations (■) (n-17). *postoperative vs. preoperative (before I-operation) values, means ±SD, p<0.05

observed for IL-1ra. The highest level of IL-1ra of 2669±3700 pg/ml was observed on day 3 to decrease on day 7 to 1643.3±1310.0 pg/ml and on day 14 to 1366.5±1045 pg/ml. The differences were not significant. There was, however, a significant increase in sTNFRI level on day 1 and 3 to 3859.7±2436.0 pg/ml and 3316.6±1540.0 pg/ml, respectively (before vs. after surgery p=0.02). It was followed by 3247.9±979, 3276.2±1178 pg/ml on days 10 and 14.

Postoperative complications and re-operation

In the group of patients after pancreatic resections with postoperative complications, the IL-6 serum level rose from 3.4±2.2 pg/ml before operation to 917.7±591 pg/ml on day 1 and to 210±140 and 170±86 pg/ml on days 3 and 7 (p=0.01 on days 1-7 vs. before operation). It remained after re-operation, on days 10 and 14 after first surgery, at the level of 95.4±29 and 103.1±33 pg/ml, respectively. There was no difference with the preoperative values. The level of IL-1ra serum concentration increased from 322.5±190 pg/ml before surgery to 6063±4462, 1676.3±775 and 1543.1±690 pg/ml on day 1, 3 and 7 after surgery, respectively (before vs. after surgery, all p=0.01). The re-operation did not significantly change the IL-1ra level. It was 1419±573 and 4247.1±3854 after re-operation, on days 10 and 14 after first surgery. The serum sTNFRI rose from 1365.5±182 pg/ml before surgery to 4328.1±1714, 4618.7±1840 and 3998.4±906 pg/ml on days 1, 3 and 7, respectively (before vs. after surgery, all p<0.05). After re-operation, on days 10 and 14, sTNFRI level remained at 3571.8±976 and 3785.5±1005 pg/ml.

The levels of IL-6, IL-1ra and sTNFRI did not differ significantly between the groups with and without complications on days 1 to 7. After re-operation, on days 10 and

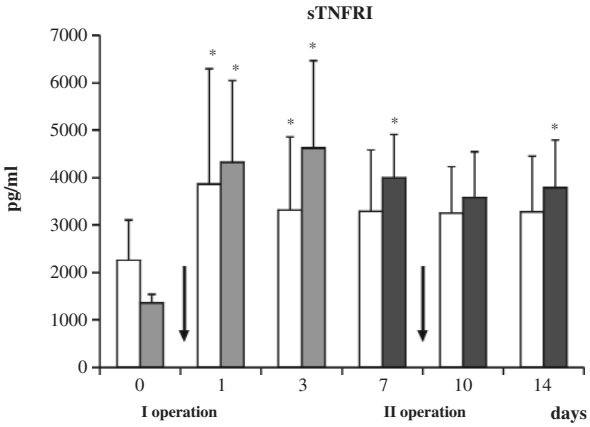

Fig. 3. Serum concentrations of soluble TNF receptor antagonists (sTNFRI) after pancreatic surgery without (□) and with complications (▩) requiring reoperations (■) (n-17). * postoperative vs. preoperative (before I-operation) values, means ±SD, p<0.05

14 after primary surgery, the cytokine concentrations did not differ significantly from those of day 7 (before re-operation), neither from those of day 10 and 14 in the group without complications.

Discussion

This study provided the following information: a) high postoperative rise of serum IL-6, IL-1ra and sTNFRI levels in patients after pancreatectomy over the preoperative values, and b) lack of increase in cytokine concentration after re-operation performed because of infective complications.

Surgical trauma evokes fast and diversified cytokine response. The most valuable parameters of the pro- and anti-inflammatory response to surgical trauma include the serum levels of IL-1, IL-1ra, TNFα, sTNFRI, as well as IL-6, IL-8 and IL-10 [3, 7-10]. The postsurgical infective complications are accompanied by an additional rise of serum sTNFRI, IL-1ra and IL-6 [1, 11, 12]. However, this rise depends on the operated organ and type of local infection.

Some patients developing postoperative infective complications require surgical re-interventions within the first days after elective surgery. This secondary surgical trauma should evoke a new wave of cytokinemia. The question arises what is the mechanism of control of cytokine synthesis and excretion after the second operation.

In this study we found that the first surgical procedures for cancer, as pancreatectomy, evoke a strong cytokine response reflected by high serum cytokine levels. In the group with an uneventful postoperative course the concentration of IL-6, IL-1ra and sTNFRI reached their peak on day 3 after surgery, to decrease slowly thereafter. Infective complications were accompanied by higher serum cytokine values, however, the differences were generally not statistically significant. Interestingly, we found that relaparotomy and revision of the infected site of the primary operation did not bring about any increase in the IL6, IL-1ra or sTNFRI levels. Neither was there any evident decrease in effect of removal of the source of infection.

Our observations are of importance for understanding the value of the post-injury cytokine levels in surgical patients. The concentrations of the pro- and anti-inflammatory cytokines are the indicators of the degree of surgical injury. They are also useful diagnostic parameters enabling early detection of infective complications and sometimes are predictors of the outcome of SIRS, sepsis and severe sepsis. Lack of an additional increase in serum cytokine levels in patients developing infective foci at the operated sites as well as lack of rise in the level such cytokines as IL-6 and sTNFRI after re-operation need elucidation.

During reoperation, the primary surgical wound is opened, the site of organ surgery is exposed and tissues around it are dissected. Thus, a large mass of tissues undergoes injury.

Each major abdominal operation is followed by two effects: a transient increase of cytokine concentration in the serum and an in vitro measured prolonged hyporesponsiveness of circulating leukocytes to lipopolysaccharide (LPS)-stimulated cytokine production [13] known as a form of "endotoxin tolerance". Severe injuries reduce monocyte responsiveness to LPS and decrease the secretion of proinflammatory cytokines [14]. A study that examined the relationship between cytokine production and posttraumatic sepsis found that LPS-stimulated synthesis of TNF and IL6 was reduced 2-4 hours after polytrauma [15]. A link between inflammatory events, particularly infections, and defects in signal transduction has been established [16]. Alterations in signal transduction pathways are present in LPS-pretreated human cell lines [17]. Endotoxin – tolerant human cells have impaired NF-kappa B [8], IRAK [19], ERK [20] and p38 kinase [21] activation. Human monocytes pretreated with low dose LPS have alterations in LPS-stimulated cytokine production [22-24]. The best characterized change is the reduction in TNF [21]. In addition, when stimulated with LPS, endotoxin-tolerant monocytes show reduced release of IL-6 [21], IL-12 [25], IL-8 [26] and chemokines [26].

Surgery alone may be a sufficient stimulus to induce a form of tolerance, because LPS-stimulated TNF activity in blood is depressed in surgery [27]. This may be the effect of IL-10 [28], although its serum levels may not be increased [29]. There is also elevation of IL-1ra and reduction in TNF α, IL-1β and IFNγ [30].

Deregulation similar to that seen in endotoxin tolerance may occur in critically ill patients, particularly with sepsis [31]. Low levels of LPS-stimulated whole blood TNF production are associated with poor clinical outcome [32]. Patients with sepsis reveal monocyte defects in production of LPS-stimulated TNF, IL-6, IL-1β [31] and IL-1ra [33]. This defect in cytokine production was most pronounced in patients with Gram-negative infections. Generally LPS-stimulated monocytes from trauma and SIRS patients produce significantly more TNF than cells from septic patients [24].

Some other factor may also be responsible for the low cytokine levels after reoperation. These may be depletion of the cytokine production sources. The immature forms of monocytes and granulocytes appearing in peripheral blood after trauma are low-producers of cytokines. Moreover, a decreased bacterial mass in effect of the antibiotic activity may bring about a decrease in the disintegration rate of granulocytes and macrophages and subsequently less cellular cytokines finding their way to the circulation.

The above mentioned observations may, to a certain extent, explain the mechanism of lack of increase of cytokine levels after reoperations. There might also be some additional iatrogenic factors affecting suppression of cytokine production. These are the post-re-operation pharmacological therapy, and specifically parenteral nutrition. In vitro production of the inflammatory mediators thromboxane 2/3 and TNFα by peripheral blood monocytes is diminished by infusion of omega – 3 lipid emulsions [34]. One of the accepted immunomodulatory strategies is enteral feeding supplemented with glutamine, omega-3 polyunsaturated fatty acid and nucleotides. It significantly reduces the number of SIRS days per patient and lowers the MOF (multiple organ failure) score on day 3 and thereafter [35].

In conclusion, although the serum cytokine levels are good markers of the immune reactivity of surgical patients to the first operative trauma and in certain cases early predictors of the postoperative infective complications, their diagnostic value after reoperations is questionable.

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