

## Immunomodulation by perioperative administration of *n*-3 fatty acids

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It has been increasingly reported that administration of *n*-3 fatty acids is beneficial in patients with inflammatory processes. This effect is most likely caused by different biological characteristics, including an immunomodulating effect of the products derived from *n*-3 fatty acids through eicosanoid metabolism. The aim of this study was to investigate the effect of perioperative administration of *n*-3 fatty acids on inflammatory and immune responses as well as on the postoperative course of patients with extended surgical interventions of the abdomen. In particular, the effect of *n*-3 fatty acids on interleukin-6 release and on granulocyte/monocyte function (HLA-DR expression) was studied. There was a downregulation of the inflammatory response, and, simultaneously, a smaller postoperative immune suppression in the *n*-3 fatty acid group. In addition, we observed shorter postoperative periods in the intensive care unit and on the regular medical wards as well as lower rates of severe infections. The results suggest that perioperative administration of *n*-3 fatty acids may have a favourable effect on outcome in patients with severe surgical interventions by lowering the magnitude of inflammatory response and by modulating the immune response.

### *n*-3 Fatty acids: Immunomodulation: IL-6: Abdominal surgery

#### Introduction

Extended interventions in abdominal surgery can be associated with severe complications such as the systemic inflammatory response syndrome (SIRS) and sepsis, which are the main causes for fatal postoperative outcome. Regulation of the immune response plays an important role in the pathogenesis of such postoperative conditions, i.e. proinflammatory mechanisms are balanced by anti-inflammatory processes. The reason for these immunoregulatory mechanisms is to maintain or re-establish homeostasis, which is essential to avoid organ dysfunction, multiorgan failure or even death (Döcke *et al.* 1997; Heidecke *et al.* 2000; Weimann *et al.* 1998).

In addition to basic treatment efforts such as sanitation of the septic focus, use of antibiotics and surgical intensive care in general, there have been therapeutic attempts aimed at controlling the development of SIRS and sepsis such as administration of immunomodulating agents. Initial studies have shown favourable effects of administration of granulocyte colony-stimulating factor (G-CSF) or interferon gamma (IFN- $\gamma$ ), and plasma separation on the immune system and the outcome of

patients with sepsis or SIRS (Lundblad *et al.* 1996; Murray, 1996; Reeves *et al.* 1999). In addition, the advantageous effect of immunonutrition in critically ill patients has been reported using substances such as glutamine, arginine, purine nucleotides or *n*-3 fatty acids (Grimminger *et al.* 1997; Schricker *et al.* 1997; Weimann *et al.* 1998).

Polyunsaturated fatty acids are essential for synthesis of cellular membranes and as precursors of modulators of biochemical processes. In particular the lipid mediator system is important in mediating the inflammatory response. Lipid mediators include products of the oxidation of arachidonic acid such as thromboxanes, prostaglandins and leucotrienes, as well as platelet-activating factor (PAF). In the case of SIRS or sepsis, synthesis of arachidonic acid metabolites is increased and leads to numerous effects on the microcirculation (vascular tone and permeability) and on the innate inflammatory response (activation of polymorphic neutrophils [PMN] or chemotaxis).

Arachidonic acid metabolites lead to vasoconstriction, bronchoconstriction, platelet activation, increased vascular permeability, activation of the inflammatory processes and

**Abbreviations:** SIRS, systemic inflammatory response syndrome; G-CSF, granulocyte colony stimulating factor; IFN- $\gamma$ , interferon gamma; PCT, procalcitonin; TNF, tumour necrosis factor; HLA, human leucocyte antigen.

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suppression of cell-mediated immune function. *n*-3 Fatty acids compete with *n*-6 fatty acids for metabolism, resulting in products such as eicosapentaenoic acid derivatives, which are believed to be less inflammatory than those produced from arachidonic acid (Grimminger *et al.* 1993, 1997; Hayashi *et al.* 1998; Kinsella & Lokesh 1990; Schricker *et al.* 1997; Weimann *et al.* 1998). Advantageous effects on the course of severe inflammatory diseases such as sepsis, ulcerative colitis, psoriasis, acute respiratory distress syndrome, and after organ transplantation as well as after surgical interventions have been observed following increased *n*-3 fatty acid supply (Grimminger *et al.* 1993; Kinsella & Lokesh 1990).

While the majority of studies have investigated *n*-3 fatty acid as an additional component of parenteral nutrition, we have studied the specific immunomodulatory effect of *n*-3 fatty acids administered perioperatively as a novel concept to modulate hyperinflammation following extended surgical interventions. We aimed to use preoperative administration of *n*-3 fatty acids to positively influence hyperinflammation and the simultaneously generated counter-regulation (compensatory anti-inflammatory response syndrome; CARs), which are induced by surgical intervention.

### Materials and methods

Twenty-four individuals with elective but extended surgical interventions ( $\geq 3$  h of duration) of the abdomen (stomach, pancreas) with (i) no evidence of malnutrition prior to surgery, (ii) no signs and symptoms of acute or chronic infection, and (iii) at least 5 days of no oral nutrition postoperatively were enrolled in the study. Antibiotics were only administered as perioperative prophylaxis as appropriate or if infections occurred postoperatively. Postoperatively, only fluids and electrolytes were infused and on the fourth postoperative day, low-caloric ( $\leq 1400$  kcal) parenteral nutrition was permitted. The main aim was to investigate the effect of perioperative administration with *n*-3 fatty acids on the magnitude of postoperative immune and inflammatory responses. Therefore, the patients were randomized into two groups. Group I (study group) received 100 ml Omegaven® (Fresenius AG, Bad Homburg, Germany) providing 10 g of fish oil (consisting of approximately 30–70% of *n*-3 fatty acids) on day (d) –1, 0 and on postoperative d1–d5 in addition to (i) the regular infusion (for appropriate maintenance and substitution of fluid volume and electrolytes) on postoperative d1 to d3 and (ii) parenteral nutrition with 180 g of glucose, 90 g amino acid (AKE 1100®, Fresenius AG, Bad Homburg, Germany) and 50 g fat (Lipovenös®, Fresenius AG, Bad Homburg, Germany) on postoperative d4 and d5. Group II (control

group) received the same perioperative infusion protocol but containing no *n*-3 fatty acids. In addition to the clinical course (rate of infectious complications, duration of intensive care unit [ICU] and total hospital stay), white blood cell count, and serum C-reactive protein (CrP), interleukin (IL)-6, procalcitonin (PCT), tumor necrosis factor-alpha (TNF- $\alpha$ ) concentrations were measured. PCT was measured using a Lumitest-PCT kit from Brahms (Berlin, Germany). IL-6 was quantified with a chemoluminescence enzyme assay on immulite (DPC Biermann, Bad Nauheim, Germany). Human leucocyte antigen (HLA)-DR expression on monocytes was analyzed by flow cytometry (FACs calibur, Becton Dickinson, Heidelberg, Germany) using fluorescein isothiocyanate labelled anti-CD<sub>14</sub> (Becton Dickinson, Heidelberg, Germany) and phycoerythrin labelled anti-HLA-DR (Coulter, Hamburg, Germany). TNF- $\alpha$  production by monocytes stimulated with lipopolysaccharide (LPS; DPC Biermann, Bad Nauheim, Germany) was measured using an *ex vivo* stimulation kit (Milenea®; Coulter, Hamburg, Germany) by stimulating 100  $\mu$ l heparinized blood with 50 and 500 pg LPS (DPC Biermann, Bad Nauheim, Germany) and following measurement of TNF- $\alpha$  on the immulite. Respiratory burst of granulocytes was measured after stimulation of 100  $\mu$ l heparinized blood with four different bacteria (*E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* [patient isolates]) at 37°C using flow-cytometry and the respiratory burst Phagotest® Kit from Becton Dickinson (Heidelberg, Germany). Data were tested for statistical significance using the software SPSS (Version 9.0). For the statistical evaluation of the data repeated measures ANOVA and *t*-tests were used. The study was conducted according to the principles and guidelines of declaration of Helsinki for biomedical research and the protocol was approved by the Institutional Review Board. Consent was obtained from each patient prior to study enrolment.

### Results

There were no significant differences in characteristics of the two groups (Table 1). One patient in the control could not be evaluated because of lack of adequate documentation.

White blood cell count and CrP concentrations were not different between the two groups during the 5-day postoperative period. The peak of CrP was reached on the third postoperative day. The mean was 194 mg/l (range, 87–433 mg/l) in group I and 182 mg/l (range, 95–234 mg/l) in group II. Serum PCT and TNF- $\alpha$  concentration were not different between the groups. Although a decrease in TNF- $\alpha$  release by monocytes was observed, there was no statistical

**Table 1.** Patient characteristics in the two groups

	Study group	Control group
Sex ratio (f:m)	3:9	3:8
Age <sup>a</sup>	57.4 years (range, 45–70 years)	61.6 years (range, 44–79 years)
Duration of surgical intervention <sup>a</sup>	332 min (range, 240–460 min)	315 min (range, 215–480 min)
ASA classification <sup>a</sup>	2.3	2.4

<sup>a</sup> Mean.

difference between groups during the first 3 days post-operatively. Later, TNF- $\alpha$  release became normal in the control group, but decreased further in the study group, although the two groups were not different from each other (Fig. 1a/b). Respiratory burst activity of granulocytes after bacterial stimulation decreased significantly post-operatively ( $P < 0.01$ ), but was not different between groups (Fig. 2a–d). In contrast, significantly different levels of serum IL-6 were seen in the two groups (Fig. 3). On the fifth postoperative day, this difference was less marked (Fig. 3). Monocytic HLA-DR expression, a marker of immune competence, was significantly diminished in the control group, but not in the *n*-3 fatty acid group, on postoperative days 3 and 5 (Fig. 4).

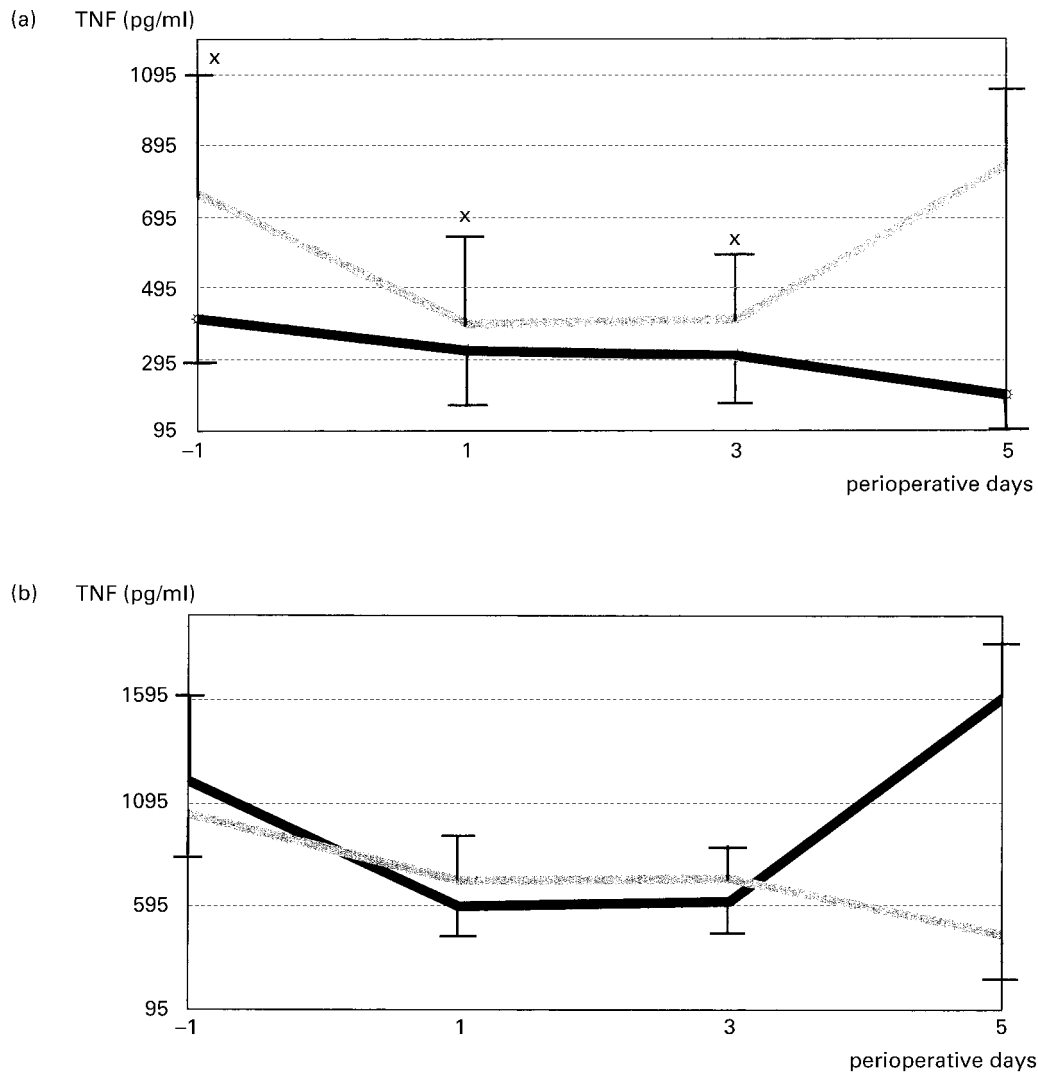
The ratio of input and output of fluids, which was measured to elucidate capillary leakage due to SIRS, was not different between the two groups. In the study group there were five infectious complications in four patients (one pancreatic abscess, one pneumonia, one infection of

the central venous line, two wound infections) whereas in the control group, five infections in three patients were observed (three pneumonias, one infection of the urinary tract, one virus-induced skin infection). In each group, one patient died from multiorgan failure, which occurred postoperatively as a consequence of severe pneumonia. With regard to other than infectious complications, one myocardial infarction occurred on the ninth postoperative day in a patient in the control group.

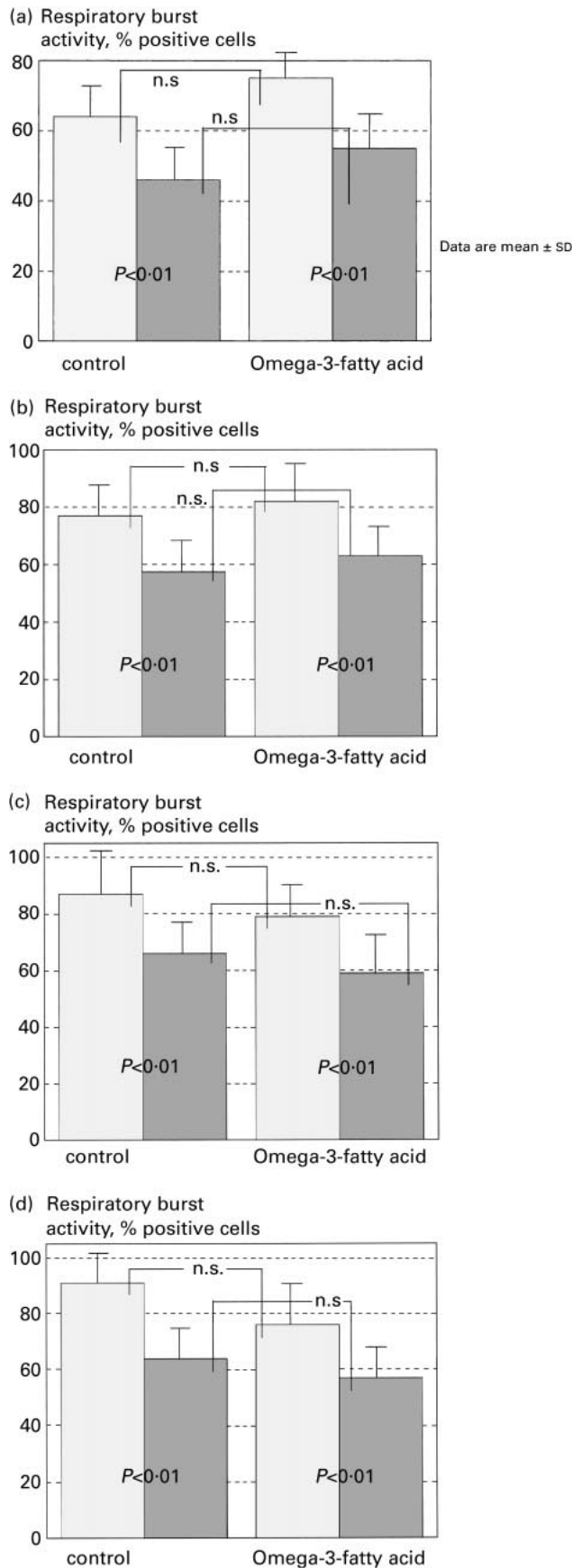
Average, postoperative stay on the ICU (4.1 v. 9.1 days) and clinical course in total (17.8 v. 23.5 days) were shorter in patients in the study group than in the control group (Fig. 5). The postoperative stay on the medical wards was significantly shorter ( $P < 0.05$ ) in the study group compared with the control group.

## Discussion

Recently, numerous novel findings about perioperative



**Fig. 1.** TNF- $\alpha$  release (pg/ml) after LPS stimulation (a) 50 pg; (b) 500 pg, of blood samples from the *n*-3 fatty acid and control groups. Fig. 1a refers to ■ as Omega-3-fatty acid and refers to ■ as control. Fig. 1b refers to ■ as Omega-3-fatty acid and refers to ■ as control. Data are mean  $\pm$  SD,  $x = P > 0.05$  (n.s.).



immune mechanisms due to a surgical intervention have been elucidated. Disturbances of physical integrity due to surgical interventions lead to a general inflammatory response associated with a stimulation of the innate immune system. The hallmark of this response is the activation of the acute phase through cytokines such as IL-6 and IL-1 $\beta$ . Simultaneously, compensating mechanisms with anti-inflammatory activity are generated, in particular, if there is a threatening over-response initially. Markers of this compensatory activity are IL-10, IFN- $\gamma$ , IL-1 receptor antagonist and other soluble receptor antagonists, suppressed monocyte HLA-DR expression and suppressed respiratory burst of granulocytes (Döcke *et al.* 1997; Heidecke *et al.* 2000).

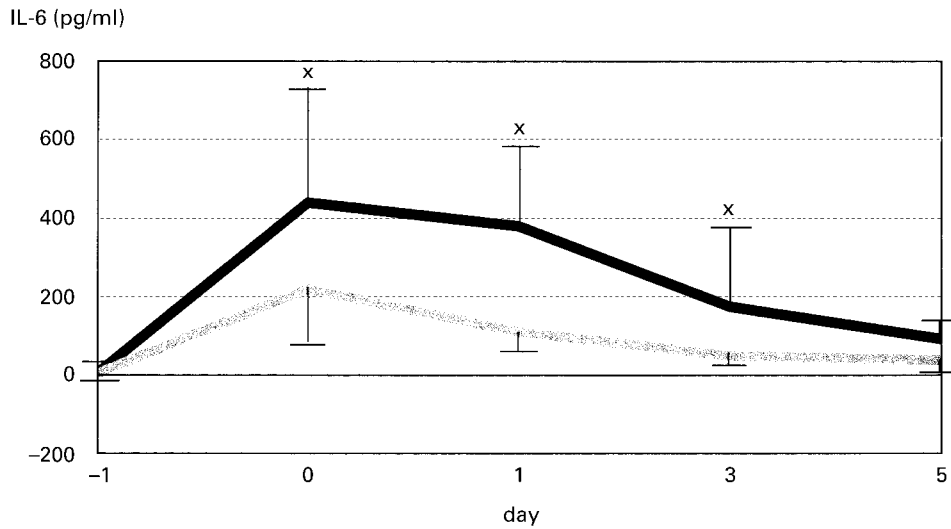
In the cascade of inflammatory and immune responses there are steps which offer treatment options in patients with SIRS or sepsis and following multiorgan failure. There are two promising options: (i) lessening the excessive inflammatory response with glucocorticoids or non-steroidal antirheumatics or (ii) lessening the immune suppression with IFN- $\gamma$ , G-CSF, glutamine, or toxin elimination.

In this study, we aimed to investigate the anti-inflammatory potential of *n*-3 fatty acids in patients who had undergone major abdominal surgery. The novel concept was to administer *n*-3 fatty acids perioperatively in contrast to previous studies, in which *n*-3 fatty acids were given as part of the total parenteral nutrition, since (i) the intervention induces an inflammatory response and (ii) in certain patients, as well as after extended interventions, there is a biphasic course of immune response characterized by an initial stimulation followed by a depression.

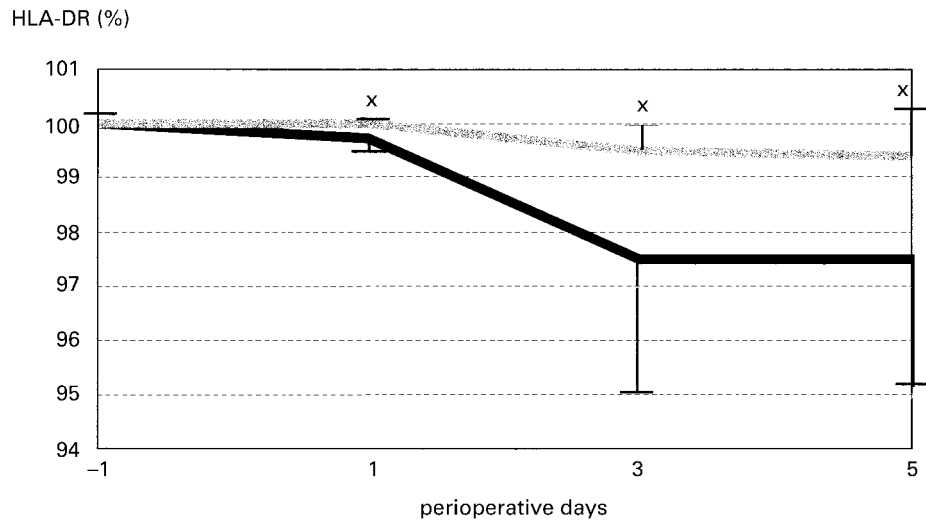
In the *n*-3 fatty acid group, we found significantly decreased IL-6 levels indicating a diminished inflammatory response. This is in general accordance with reports by others (Chavali *et al.* 1998; Hayashi *et al.* 1998; Wachtler *et al.* 1998; Wigmore *et al.* 1997). Hayashi *et al.* (1998) showed lowered levels of IL-10, an anti-inflammatory cytokine, in burned rats. Simultaneously, there was a significantly lowered reduction of HLA-DR expression on monocytes, an indicator of a sustained compensatory potential required to balance immune response (Hayashi *et al.* 1998). In the current study, administration of *n*-3 fatty acids did not prevent the suppression of granulocyte respiratory burst induced by surgery. In addition TNF- $\alpha$  production was altered to the same extent in both groups until the third postoperative day, although it is unclear why TNF- $\alpha$  production normalized faster in the control group than in the study group on the fifth postoperative day.

The lower magnitude of the inflammatory response post-surgery with administration of *n*-3 fatty acids should have a favourable impact on the postoperative clinical course. Both duration of stay on the surgical ICU (not significant) and in total (significant) were different between the groups. The longer ICU and hospital stay in the control group was

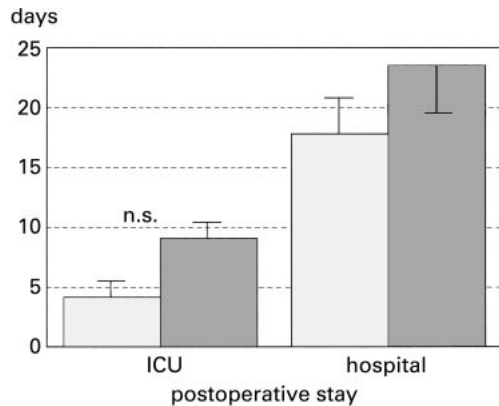
**Fig. 2.** Respiratory burst activity after bacterial stimulation (a) *E. coli*; (b) *Staphylococcus aureus*; (c) *Pseudomonas aeruginosa*; (d) *Streptococcus*, of granulocytes from patients in the *n*-3 fatty acid and control groups. □ refers to preoperatively and ■ refers to 3rd postop. day.



**Fig. 3.** Serum IL-6 levels in patients in the *n*-3 fatty acid (■) and control (●) groups. Data are mean ± SD,  $x = P < 0.05$ .



**Fig. 4.** HLA-DR expression on monocytes from patients in the *n*-3 fatty acid (■) and control (●) groups. Data are mean ± SD,  $x = P < 0.005$ .



**Fig. 5.** Duration of postoperative stay at the surgical ICU and in the hospital in total in patients in the *n*-3 fatty acid and control groups (■). □ refers to study group. Data are mean ± SD.

caused by the five postoperative infectious complications, in particular, by the three pneumonias. In the study group, the number of infections was the same, but there was only one pancreatic abscess and four slighter skin and wound infections. However, there was no difference in mortality between groups.

The results of this study suggest a distinct impact of perioperative administration of *n*-3 fatty acids on postoperative immune response in patients who had undergone major abdominal surgery. This immunomodulation was associated with a shorter stay on the ICU and in total in hospital and a positive, but not significant effect on the rate and severity of postoperative complications. From an immunological point of view, perioperative administration of *n*-3 fatty acids seems to be a reasonable approach in patients at risk of SIRS and sepsis during the phase of hyperinflammation. Further investigations are required to

evaluate the effect of *n*-3 fatty acids on complication rate and mortality in patients with such severe conditions.

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