

## CLINICAL STUDY

## Cytokine levels in patients with a very low left ventricular ejection fraction after open heart surgery

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### Abstract

**Introduction:** Cardiopulmonary bypass and cardiac operations are obligatorily connected with systemic inflammatory reaction. Production of proinflammatory cytokines is responsible also for negative effects on the myocardial function.

**Objective of study:** Follow-up of the dynamic changes of proinflammatory and antiinflammatory cytokine levels in patients with left ventricular dysfunction during the first week after cardiac surgery.

**Patients and methods:** A total of nine patients with a very low left ventricular ejection fraction ( $22.75 \pm 0.65\%$ ) who had undergone cardiac surgery (for coronary artery bypass grafting or aortic valve reconstruction) were investigated during the first after week operation. The preoperative and postoperative plasma levels of tumor necrosis factor (TNF), interleukin-8 (IL-8) and interleukin-10 (IL-10) were estimated by means of ELISA technique.

**Results:** With respect to the preoperative levels, on the first postoperative day the levels IL-8 (from 9.36 to 16.65 pg/ml) ( $p < 0.05$ ) and of IL-10 (from 6.93 to 28.09 pg/ml) ( $p < 0.02$ ) significantly rose with a stepwise decrease down to the seventh day after surgery. From the third to seventh day an insignificant increase in TNF level was also noted.

**Conclusions:** The results have shown that open heart surgery in patients with a severe left ventricular dysfunction evoked a systemic inflammatory response demonstrated by early increase in proinflammatory cytokine IL-8 and was accompanied by increased level of antiinflammatory cytokine IL-10. Despite stepwise decrease in IL-8 levels, they did not reach the preoperative levels, not even on the seventh postoperative day. (Tab. 1, Fig. 3, Ref. 21.)

**Key words:** open heart surgery, patients with low left ventricular ejection fraction, tumor necrosis factor, interleukin-8, interleukin-10.

Open heart surgery realized by means of a cardiopulmonary bypass (CPB) and cardioplegic cardiac arrest induces systemic inflammatory response and this remains a major cause of postoperative morbidity and mortality (Cremer et al, 1996; Grunenfelder et al, 2000). Several authors have demonstrated that this inflammatory response is accompanied by an significant release of various kinds of cytokines during and especially shortly after weaning the CPB (Wan et al, 1996 a; Sablotzki et al, 1997). In developing this deleterious systemic inflammatory response due to ischemization and subsequent reperfusion the dominant role of proinflammatory cytokines, especially of tumor necrosis factor (TNF) and some interleukins has been well established (Speziale et al, 2000; Carvalho et al, 2001). The increased production of proinflammatory cytokines is also important with respect to their adversary effects on endothelial and myocardial cells. This kind of cytokines significantly influences the myocardial

contractility (Barry, 1994). Local release of functionally very active TNF may participate on the postischemic myocardial depression (stunning) resulting from CPB (Finkel et al, 1992), a direct relationship between the plasma levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) and the development of left ven-

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tricular wall dyskinesia has been described, too (Hennein et al, 1994). On the other hand, after weaning the CPB the antiinflammatory acting cytokine — interleukin-10 (IL-10) — is extensively released to maintain the balance between proinflammatory and antiinflammatory cytokines which is considered as a key condition for attenuation of postischemic reperfusion injury (Wan and Yim, 1999).

However, cytokine levels in patients undergoing open heart surgery have been estimated only shortly during surgery and a few hours postoperatively. Therefore, aim of our study was to determine in course of one week the levels of proinflammatory (TNF and IL-8) and antiinflammatory (IL-10) cytokine in patients with a severe left ventricular dysfunction undergoing open heart surgery.

### Patients and methods

A group of nine patients with a very low left ventricular ejection fraction (LVEF) were elected for open heart surgery (coronary artery bypass grafting and/or aortic valve reconstruction). After standard premedication the surgery, cardiopulmonary bypass and cold crystalloid antero/retrograde cardioplegia was performed according to a general scheme used routinely in our Institute (Pechan et al, 2001). Clinical characteristics of patients are shown in Table 1. Left ventricular ejection fraction of patients was measured on echographic examination before and in course of the first postoperative week. Patients were biochemically investigated immediately before operation and then at the first, third, fifth and seventh day after surgery. The Institutional Ethic Committee approved the study.

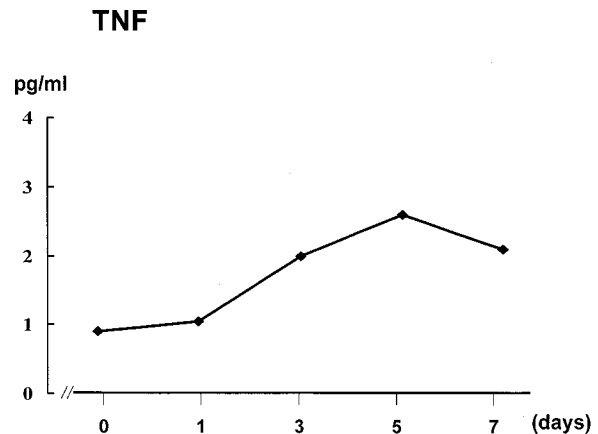
Twenty six clinically controlled patients without cardiovascular, hepatic or renal disturbances represent the control group.

Blood samples were taken up from the central venous line (in the control subjects from cubital vein) in evacuated blood collection tubes containing potassium salt of ethylenediaminetetraacetic acid. Samples were immediately cooled in cracked ice and then centrifuged (3,000 g for 5 min at 4 °C). Plasma was stored at -20 °C until assay.

**Tab. 1. Pre- and perioperative characteristics of patients.**

Patients (n=9)	8 males, 1 female
Original diagnosis	CAD - 6
NYHA classification	III - 5
Age (years) (range)	60.11±1.77 (52-67)
Left ventricular preoperatively ejection fraction postoperatively (range) (%)	22.56±0.73 (20-25) 36.67±22.95* (25-50)
Cardiopulmonary bypass (range) (min)	113.56±22.95 (71-280)
Aortic cross-clamping (range) (min)	58.33±8.56 (33-114)

Key: CAD — coronary artery disease, AoS — aortic valve stenosis  
\* — statistical significance: preoperative vs postoperative values =  $p < 0.001$



**Fig. 1. Plasma levels of tumor necrosis factor (TNF) in patients with a very low left ventricular ejection fraction before (day 0) and after open heart surgery.**

Cytokine plasma levels (TNF, IL-8 and IL-10) were measured by enzyme-linked immunosorbent assays using a commercially available kits (R&D Systems Inc., Minneapolis, U.S.A.). The measured values were calculated with respect to the actual hematocrit values of patients (evaluated standard hematocrit value=0.40).

Data are presented as arithmetic mean standard±error of the mean (SEM). Means were compared by Student t-test for paired samples and significance was assumed at a value of  $p < 0.05$ .

### Results

#### Effect of cardiac surgery

The result of cardiac surgery was beneficial for investigated patients. After operation their left ventricular function was substantially improved: left ventricular ejection fraction increased postoperatively by 60 % ( $p < 0.001$ ) (Tab. 1). In the majority of patients the postoperative course was uneventful, the only exception was death of one patient (preoperative LVEF=20 %, operation for aortic valve replacement) for cardiogenic shock due to an acute myocardial infarction at the seventh days after surgery.

#### Tumor necrosis factor (TNF)

In contrast to the very low, not detectable plasma levels of proinflammatory acting TNF in control group of investigated subjects, the preoperative level of TNF in patients with a very low LVEF was  $0.94 \pm 0.42$  pg/ml. On the third day after surgery the TNF plasma level revealed a tendency to an increase with a peak at the fifth day followed with a slight decrease at the day seven (Fig. 1). However, all these changes in pre- and postoperative plasma levels of TNF were not statistically significant.

#### Interleukin-8 (IL-8)

The plasma levels of another proinflammatory cytokine — IL-8 — compared with IL-8 levels of control subjects ( $1.47 \pm 0.35$

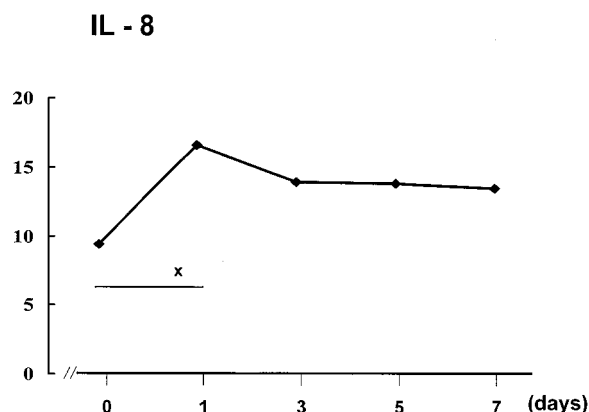


Fig. 2. Plasma levels of interleukin-8 (IL-8) in patients with a very low left ventricular ejection fraction before (day 0) and after open heart surgery. Statistical significance: x —  $p < 0.05$ .

pg/ml) — were significantly increased in patients group already before surgery ( $9.36 \pm 2.22$  pg/ml). On the first postoperative day, the IL-8 levels dramatically rose by more than 75 % ( $p < 0.05$ ) and then gradually insignificantly decreased up to the seventh day ( $13.53 \pm 1.68$  pg/ml) without reaching the preoperative levels (Fig. 2).

#### Interleukin-10 (IL-10)

The only analyzed antiinflammatory cytokine was IL-10, the preoperative plasma levels of which in patients with a very low LVEF were markedly higher ( $6.93 \pm 0.63$  pg/ml) in comparison with the levels of control subjects ( $0.65 \pm 0.26$  pg/ml) ( $p < 0.0001$ ). On the first postoperative day, the plasma level of IL-10 characteristically increased fivefold compared to the preoperative values ( $p < 0.01$ ) and in the next 6-day-postoperative period a significant decrease of the IL-10 levels have been observed ( $p < 0.01$ ) (Fig. 3).

#### Discussion

Cardiosurgical intervention (coronary artery bypass grafting, valve reconstruction or its replacement) with cardiopulmonary bypass is often the only solution for patients with severe progressive heart failure (Florian et al, 2001; Nemeč et al, 2001). This intervention, especially in elderly subjects, is associated with systemic inflammatory response syndrome (Kuneš et al, 1998). The basis of this syndrome is endothelial injury on the level of organ microcirculation, connected with increased vascular permeability, abnormal vasoreactivity and altered quality of endothelial surface associated with its decreased resistance against thrombogenic events and leukocyte adhesion (Cain et al, 1997). Because the studies published up to this time were oriented on the perioperative periods only, we followed the patients at the late reperfusion period, i.e. during a week after cardiosurgery.

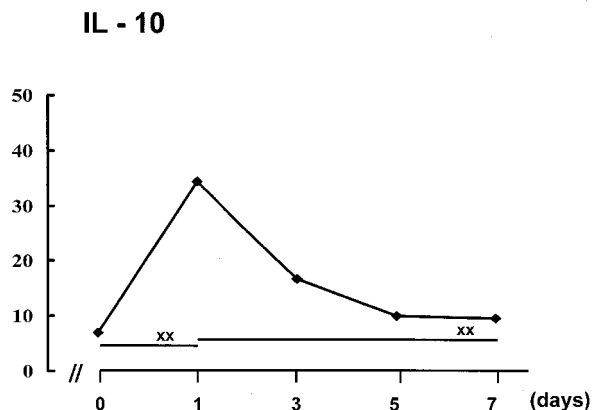


Fig. 3. Plasma levels of interleukin-10 (IL-10) in patients with a very low left ventricular ejection fraction before (day 0) and after open heart surgery. Statistical significance: xx —  $p < 0.01$ .

Systemic inflammatory response syndrome is pathogenetically a complex event as a result of ischemia-reperfusion injury during and after cardiac surgery with cardiopulmonary bypass. It is suggested that the biologically active polypeptides — cytokines — play an important role in the development of these inflammatory responses due to factors evoked predominantly by immunological reactions arising during and after cardiopulmonary bypass. In this process the proinflammatory cytokines, especially TNF, IL-6 and IL-8 play the dominant role (Wan et al, 1996 a). Several minutes after aortic declamping and the following end of cardiopulmonary bypass the marked increase of plasma levels of these proinflammatory cytokines was well documented by several authors (Hennein et al, 1994; Wan et al, 1996 a; Fransen et al, 2000) and the overproduction of this type of cytokines could be connected with the decreased contractility of myocardium as well as with its postischemic functional depression (stunning) (Finkel et al, 1992). Other authors (Hennein et al, 1994) observed in the postoperative period a direct relationship between raised levels of IL-6 and IL-8 and the dyskinesia of left ventricular wall. In present study we have found a marked increase of IL-8 level at the first postoperative day with stepwise non significant decrease during the next few days without a chance to reach in this time-period the control IL-8 values. On the other hand, at the first postoperative day such an increase in the plasma level of another very important proinflammatory cytokine — TNF — was not observed. It can be suggested that the plasma level of TNF had peaked already shortly after aortic declamping but at the first day after surgery the plasma level of this cytokine again declined as shown in the study of Wan et al (1996 b).

For a long time some authors suggest that the overproduction of proinflammatory cytokines is balanced by another group of these biologically active compounds — by increased synthesis of antiinflammatory cytokines, especially IL-10. This immunomodulatory acting cytokine directly inhibits the monocytes/macrophages mediated production of proinflammatory cytokines

on the transcription level (de Waal Malefyt et al, 1991; Gérard et al, 1993). In fact, some authors demonstrated that shortly after weaning off the cardiopulmonary bypass, the plasma level of IL-10 dramatically increases simultaneously with the rise in the levels of proinflammatory cytokines and common stepwise decrease 1–3 days after surgery (Wang et al, 1997; Brix-Christensen et al, 1998). We have found a significant rise of plasma level of IL-10 at the first postoperative day with successive stepwise decrease between third and fifth day after surgery. This observation agreed to the concept of a important role of anti-inflammatory acting IL-10 in eliminating the role of proinflammatory cytokines in maintaining and further development of inflammation as a negative feature of cardiac surgery.

In conclusion, the results of present study confirm the effort of the organism of patients to maintain the functionally important balance between pro- and anti-inflammatory factors after great cardiac surgery. Recovery of this balance was achieved during a few days after operation in patients with a very low left ventricular ejection fraction, too.

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