Changes in the serum interleukin-8 concentration after coronary by-pass surgery predict the occurrence of the post-cardiac injury syndrome

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Abstract

The post-cardiac injury syndrome (PCIS) is an important cardiologic problem associated with heart surgery. The pathogenesis of the PCIS is poorly understood, but immunologic mechanisms are considered to be the most important. Accordingly, we investigated the profiles of pro- and anti-inflammatory cytokines [interleukin (IL) 8, IL-1β, IL-6, IL-10, tumor necrosis factor α (TNF-α) and IL-12p70], before and after the heart surgery in patients with or without the PCIS, and in the healthy controls. The surgery caused significant increase of all the aforementioned cytokines; in addition, the pre-operative values were significantly higher than in control patients. Moreover, when the relative changes of the IL-8 concentration (e.g. ratios between the concentration after the surgery to the concentration before the surgery) were compared between PCIS-positive and PCIS-negative patients (PCIS-positive: mean ration 1.7 ±0.64 vs. PCIS-negative: 1.0 ±0.11) the difference between the indexes was statistically significant ($p < 0.001$). This is a novel finding suggesting that changes in the serum IL-8 concentration after the heart surgery may predict the occurrence of the PCIS.

Key words: *post-cardiac injury syndrome, PCIS, pericarditis, IL-8, heart surgery.*

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Introduction

According to the generally accepted view, myocardial tissue damage, regardless of the specific cause, results in the release of intracellular constituents from the injured cardiomyocytes, which are recognized by innate immune system, thereby leading to the inflammatory response. An inflammatory complication occurring after a heart injury has been known for several decades as the post-cardiac injury syndrome (PCIS), originally described by William Dressler in 1950s [1]. He observed the occurrence of pericarditis accompanied by fever, elevated inflammatory markers, and pleural exudate (Dressler syndrome). He described the symptoms as being nagging, yet associated with a good prognosis, and proposed an auto-aggression mechanism as a putative cause.

The PCIS appears within several days to weeks after surgery or myocardial infarction, with the frequency ranging from 10% to 40% [2, 3]. It occurs significantly more often in patients after corticosteroids treatment, and affects patients with pericarditis in the past [3-5]. In the period preceding PCIS there may occur elevated body temperature, the chest pain, leukocytosis and elevated erythrocyte sedimentation rate, otherwise there are no specific symptoms. The diagnosis of PCIS is based on clinical evaluation, e.g. there is a lack of specific laboratory tests. However there are observations about the involvement of cellular and humoral immunity in the pathogenesis of PCIS [3, 6-10]. The prognosis of PCIS leads to prolonged hospitalization, possibility of relapses or the cardiac tamponade. The treat-

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ment of PCIS include nonsteroidal anti-inflammatory drugs, colchicine or corticosteroids [3,11].

The aim of our study was to find whether increased plasma concentrations of pro-inflammatory cytokines in patients undergoing coronary bypass surgery predispose to development of PCIS; in addition, we asked whether the maintaining of the elevated levels of the cytokines over 5 days post-surgery might be a direct cause of PCIS. Finding cytokine or cytokines responsible for post-surgical inflammatory reaction would make possible to apply molecules with specific antagonistic property.

Material and methods

Patients

Twenty patients qualified to coronary by-pass surgery and 12 adult healthy subjects as the control were included to this study. In the group of patients there were 17 men and 3 women. The mean age of patients was 65.6 ±7.83 SD years (range 51-83). The mean BMI was 28.1 ±3.95 SD (range 19.6-34.8). Only patients which underwent coronary by-pass surgery without pump were included in the study.

Before surgery all patients had normal value of CRP, and normal leukocytes count. Post-cardiac injury syndrome was diagnosed in 15 out of 20 patients after surgery according to the presence of pericardial or/and pleural effusion.

The control group consisted of 12 healthy age-matched volunteers selected from the hospital employees (6 men and 6 women).

Venous blood samples (1-2 ml) were obtained from patients and immediately placed into tubes without anticoagulant. After blood clotting, serum was separated by centrifugation and transferred into clean tubes, then stored at –80°C for cytokine analysis. The study protocol complied to the guidelines for the conduct of research involving human subjects as established by the Bioethics Committees on Human Research at the Institute of Cardiology. Patients and volunteers gave their informed consent to participate in this study.

Measurement of the cytokines concentrations

The BD Cytometric Bead Array Human Inflammatory Cytokines Kit (Becton-Dickinson) was used to quantitatively determine IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70 concentration in samples of serum or plasma. The preparation of beads, standards, specific antibodies, reagents and serum samples as well as protocols for flow cytometer setup, data acquisition and analysis, were performed according to Becton-Dickinson original instruction that was attached to the kit. Briefly, six bead populations with distinct fluorescence intensity (FL4 see Fig. 1) have been coated with PE-conjugated antibodies (FL2) specific for the above mentioned cytokines. Fifty µl of mixed capture beads coated by specific antibodies were transferred to each assay tube and mixed together with 50 µl recombinant standards or patients' serum samples diluted according to the manufacturer's recommendations. Then the tubes were incubated for 1.5 h at room temperature, protected from light. After incubation, the samples were washed with 1 ml wash buffer, and centrifuged for 5 min at 200 g. To the 100 µl residual

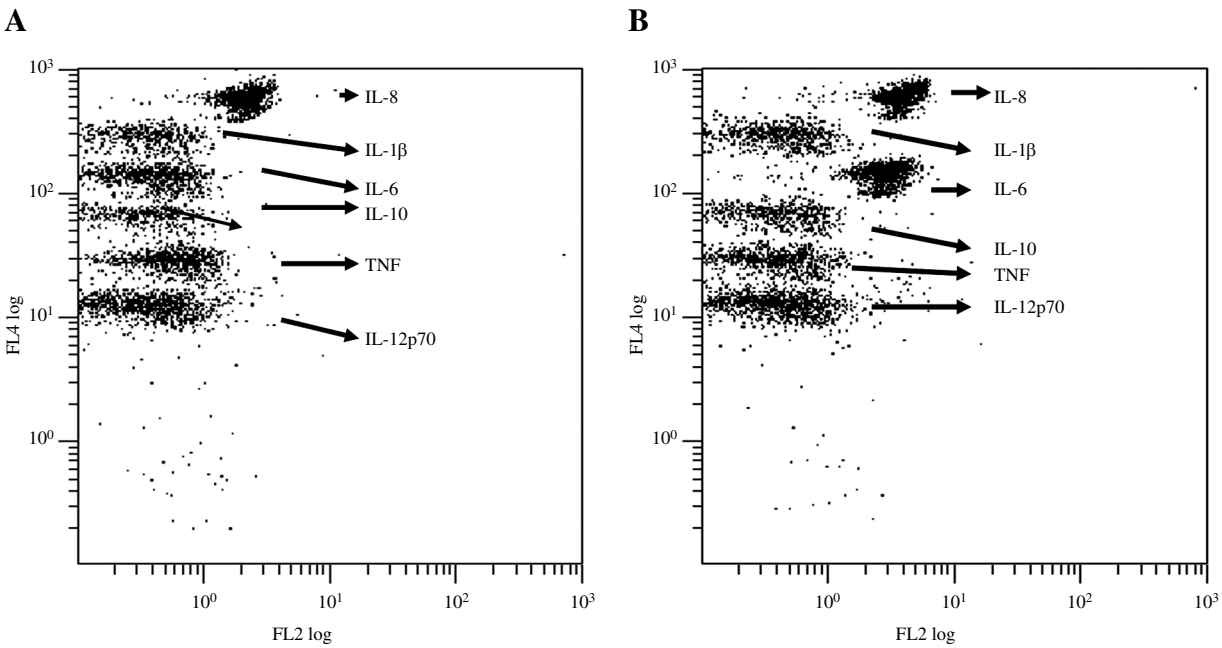

Fig. 1. Cytometric flow cytograms with detection of cytokines. A and B: patient's sera from PCIS+ group before and after coronary by pass surgery, respectively. FL2 log and FL4 log represent signal-intensity in the two different channels that respectively detect the cytokine identity and measure the cytokine concentration

volume that was leaved in each tube, PE Detection Reagent (50 µl/test) was added. After 1.5 h incubation with light protection, the samples were washed once again with 1 ml of wash buffer, centrifuged (5 min, 200 g), mixed with 300 µl of wash buffer, and subjected to the cytometric analysis using Beckman Coulter Flow cytometer Cytomix FC500 and FCAP Array software (SoftFlow, Hungary).

Statistical analysis

This was performed using Students *t*-test for results with Gaussian distribution, non-parametric Mann-Whitney U test for two groups of non-dependent results, nonparametric signed test for two groups of dependent results, and χ^2 test with Yates correction for small number of cases.

P value < 0.05 was considered as statistically significant. All calculations were performed using Statistica 7.1 software (StatSoft).

Results

In the present study we measured IL-8, IL-1β, IL-6, IL-10, TNF-α and IL-12p70 concentration in sera of patients on the day of surgery, and on the 5th day after the surgery. The typical image obtained from flow cytometer is shown in Fig. 1. Patients' results were compared to the results obtained in healthy subjects studied using the same method. Within the group of 20 patients there were two subgroups of patients, e.g. with (n = 15) and without (n = 5) the hallmarks of PCIS. Comparison of results before and after surgery was performed for the two subgroups separately.

In patients' sera taken before, and 5 days after the surgery, the concentration of all the aforementioned cytokines were significantly higher in comparison to the healthy control group (Table 1).

On the 5th day after the surgery, the concentrations of the cytokines in group with PCIS significantly increased in comparison to the results obtained before coronary by-pass surgery (Table 2).

In contrast, in patients without PCIS, the serum concentrations of IL-8, IL-1β, IL-10, TNF-α and IL-12p70 on the day 5 after the surgery were on comparable levels to those observed on the day of the surgery. In this group, on the day 5th, only IL-6 concentration was significantly increased. Mean

Table 1. Cytokines concentration in healthy subjects and in patients before and after by-pass

Cytokine	Samples	PCIS+ (n = 15)		Samples	PCIS– (n = 5)	
		pg/ml ±SD	*P* value before and after vs. control		pg/ml ±SD	*P* value before and after vs. control
IL-8	control	19.6 ±7.81		control	19.6 ±7.81	
	before	36.7 ±24.31	0.002	before	51.9 ±17.53	0.01
	after	59.5 ±38.22	0.0001	after	54.2 ±18.24	0.008
IL-1β	control	6.9 ±3.48		control	6.9 ±3.48	
	before	18.9 ±3.91	0.0001	before	19.7 ±3.65	0.001
	after	24.3 ±5.14	0.00001	after	28.7 ±23.91	0.008
IL-6	control	10.3 ±4.49		control	10.3 ±4.49	
	before	23.6 ±6.7	0.002	before	27.6 ±5.44	0.001
	after	46.7 ±20.65	0.0001	after	49.1 ±20.4	0.001
IL-10	control	9.2 ±5.39		control	9.2 ±5.39	
	before	26.1 ±7.07	0.001	before	31.9 ±9.38	0.001
	after	34.2 ±4.78	0.001	after	29.9 ±11.55	0.003
TNF	control	10.8 ±5.84		control	10.8 ±5.84	
	before	27.5 ±6.43	0.0001	before	29.7 ±11.69	0.002
	after	37.7 ±5.8	0.00002	after	33.6 ±11.8	0.001
IL-12p70	control	10.5 ±6.31		control	10.5 ±6.31	
	before	30.7 ±6.42	0.003	before	31.3 ±10.14	0.003
	after	35.1 ±5.94	0.00001	after	19.3 ±15.52	0.001

PCIS+ and PCIS–: patients results and without post cardiac injury syndrome, respectively; P – level of statistical probability calculated using non parametric U Mann-Whitney test for two independent samples. In control group were studied 12 adult healthy subjects

Table 2. Comparison of cytokines concentration in patients serum before and after coronary by-pass surgery

Cytokine		Patients with PCIS $n = 15$ mean ±SD	Patients without PCIS $n = 5$ mean ±SD	p2
IL-8	B	36.7 ±24.21	51.9 ±17.53	0.26
	A	59.5 ±38.22	54.2 ±18.24	0.69
	p1	**0.0006**	0.5	
IL-1β	B	18.9 ±3.91	19.7 ±3.65	0.60
	A	24.3 ±5.14	28.7 ±23.91	0.69
	p1	**0.02**	0.5	
IL-6	B	23.6 ±6.7	27.6 ±5.44	0.25
	A	46.7 ±20.65	49.1 ±20.4	0.89
	p1	**0.0003**	**0.04**	
IL-10	B	26.1 ±7.07	31.9 ±9.38	0.33
	A	34.2 ±4.78	29.9 ±11.55	0.57
	p1	**0.002**	0.68	
TNF	B	27.5 ±6.43	29.7 ±11.69	0.63
	A	37.7 ±5.8	33.6 ±11.8	0.35
	p1	**0.001**	0.22	
IL-12p70	B	30.7 ±6.42	31.3 ±10.14	0.72
	A	35.1 ±5.94	19.3 ±12.52	0.80
	p1	**0.02**	0.68	

PCIS: post cardiac injury syndrome, B and A: results obtained before and 5 days after surgery, respectively. P: 1 – level of statistical probability calculated using non-parametric Wilcoxon's test for two dependent samples and 2 – U Mann-Whitney test for independent samples

serum concentrations before and after surgery were on comparable level in the both studied groups (Table 2).

Comparison of individual changes in cytokines concentration in patients from both groups revealed that in patients with PCIS, the differences between IL-8 concentration on the 5th day after the surgery and the concentration before the surgery were significantly higher, in comparison to patients without PCIS (range of ratios – 1.15 to 3.25 vs. 0.91 to 1.16 respectively) ($p < 0.001$; Fig. 2).

In 13 out of 15 PCIS patients in group with PCIS, the concentration of IL-8 increased more than 20% but in 5 patients without PCIS such a high increase was not observed. Chi square analyzes with Yates correction revealed that the chance of developing PCIS is significantly lower in patients without the increase in IL-8 concentration of more than 20% (arbitrarily taken) in comparison to the concentration before surgery (Chi square with Yates corr. = 8.86; df = 1; $p < 0.029$).

Significantly decreased mean ratio of the results after the surgery to the results before the surgery was found also

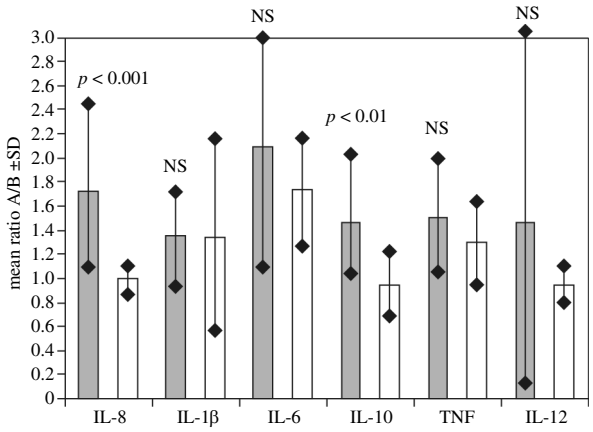

Fig. 2. Relative changes in cytokines concentration in patients after (A) coronary by-pass in ratio to results before surgery (B). Gray and white bars: patients with and without post cardiac injury syndrome (PCIS), respectively. *P* value calculated by Student's *t*-test. NS – non statistically significant

for IL-10 concentration in group without PCIS. Similar analysis performed for IL-10 as for IL-8 did not revealed any statistical relationships.

Discussion

The cause of PCIS is not completely understood. Currently, the autoimmune mechanism, with the involvement of both cellular and humoral immune responses, is postulated. The involvement of the immune system is consistent with a) the delayed onset of the symptoms after surgery or myocardial infarction, b) dramatic improvement after anti-inflammatory treatment, c) the tendency to recur, often associated with the discontinuation of steroid administration, and d) lack of correlation between the magnitude of symptoms and the level of myocardial damage markers [5].

It was hypothesized that the surgery leads to the release and presentation of cardiac antigens, leading to the production of various, cardiac-specific or unspecific antibodies. As a result, the formation of immune complexes can be detected in the pericardium, lungs, and pleura, e.g. in the vicinity of the "injury" site. The immune complexes can be responsible for the initiation of the inflammatory reactions via complement system activation, leading to the occurrence of specific inflammatory symptoms [5, 6].

The hallmark of any inflammatory reaction is a release of various cytokines that support a molecular cross-talk among subpopulations of inflammatory cells, and between inflammatory cells and endothelium and platelets. In the present work, we measured the serum concentrations of IL-1β, IL-6, IL-8, TNF-α as well as IL-10, IL-12p70, before coronary by-pass surgery and on the day 5 after the surgery. This particular set of cytokines was chosen based on the following considerations: a) IL-1β, IL-6, IL-8, and TNF-α are the major pro-inflammatory cytokines released from

phagocytic cells, b) IL-12 is an important cytokine released from antigen-presenting cells, able to induce of Th1-associated inflammatory responses but also involved in autoimmune tissue destruction. Interleukin-10 is important inhibitor of inflammation, derived from activated T and B lymphocytes and monocytes.

Previously, some changes in the level of cytokines, as a result of the surgical procedure and the cellular activation were reported [7, 8, 12, 13]. However, these published results revealed only temporary changes in the level of cytokines with peak of concentration at the time of surgery. After 24-48 hrs post-surgery their concentrations returned to initial level [13-15]. Franke *et al.* postulated a biphasic immune response triggered by cardiac surgery. The first phase is evoked by innate system with increases of pro- and anti-inflammatory cytokines. Duration of this phase is short and on the day 3 system returned to base line. On postoperative day 5th, adaptive immune system started to be activated [13]. In our study, serum cytokines concentration estimated on the day 5th after surgery were still significantly higher in group of patients which after cardiac surgery developed PCIS (Table 2). It is possible, that in these patients, except of innate system, adaptive immune system undergoes additional activation, because of serum IL-10 and IL-12 concentrations were still higher in comparison to results before surgery. Slightly increased IL-6 concentration in patients on the day 5 after surgery is compatible with observations published by others [16]. Significantly decreased IL-10 and IL-8 as well as slightly lower concentrations of others cytokines on the day 5 allow to suggest that immunological reaction in group without hallmark of PCIS expires.

When patients were exposure on different surgical trauma increase IL-8 was found characteristic only for cardiac surgery [13, 17, 18]. The peak of level IL-8 was on time of surgery and returned to beginning value on the day 5 after surgery [13-15]. We observed slop down IL-8 concentration only in patients which did not developed PCIS. Thus, we suggest that low level of IL-8 may predict postoperative course without complications. Our both group of patients were treated in the same way in time of surgery and perioperatively. Therefore, the elevated IL-8 concentration only in group which developed PCIS suggests that IL-8, the most potent chemotactic factor, recruit and activate of leukocytes and the influx of the phagocytic cells into the area of myocardial damage might be a crucial event in the pathogenesis of PCIS. Recently, the role of macrophages and monocytes following myocardial infarction is an object of research interest [19].

Accordingly, the 20% increase in the concentration of IL-8 on the day 5 after the surgery compared to the day of the surgery, appears to distinguish the PCIS group from the group without PCIS. However, too small number of patients without PCIS do not allow for calculation of confidence limit useful as diagnostic tools for clinical and laboratory practice. For this reason the study should be continued.

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