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Evaluation of Systemic Inflammatory Response in Cardiovascular Surgery via Interleukin-6, Interleukin-8, and Neopterin

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ABSTRACT

Aim: The aim of this study was to evaluate the serum levels of interleukin-6 (IL-6), IL-8, and neopterin as a sign of systemic inflammatory response syndrome after open-heart surgery. In this study, we evaluated the influences on the levels of IL-6, IL-8, and neopterin of coronary artery bypass grafting (CABG) and valve replacement surgeries with and without the use of extracorporeal circulation (ECC).

Materials and Methods: This prospective study was performed in 30 patients. In this study, we evaluated patients who underwent valve replacement surgery (group 1, n = 10), CABG with ECC (group 2, n = 10), or CABG using the beating-heart technique (group 3, n = 10). With the Human Investigation Ethics Committee consent, blood samples were obtained from the patients before the surgery (T0) and after 1 hour (T1), 4 hours (T2), 24 hours (T3), and 48 hours (T4) of protamine injection. IL-6, IL-8, and neopterin levels were measured using commercial enzyme-linked immunosorbent assay kits.

Results: The demographic data and preoperative and operative characteristics of the patients were similar. Neopterin IL-6 and IL-8 levels significantly increased first at the fourth hour after the surgery. When compared to the levels before the surgery, this increase was statistically significant. Unlike the other 2 groups of patients, those who experienced CABG with the beating-heart technique (group 3) had decreased neopterin levels at the first hour after the surgery, but this decrease was not statistically significant. Neopterin levels increased later in the OPCAB group, but these increased levels were not as high as the neopterin levels of groups 1 and 2. Neopterin reached maximum levels at the 24th hour and, unlike groups 1 and 2, in group started to decrease at the 48th.

Conclusions: Complement activation, cytokine production, and related cellular responses are important factors during open-heart surgery. It is certain that ECC activates the complement systems, and activated complement proteins cause the production of several cytokines. In our study, neopterin levels in patients who underwent beating-heart method surgery were lower than those in the other groups, and these levels started to decrease at the 48th hour. These data suggest that the systemic inflammatory response was less activated in that patient group. The beating-heart method might be an important alternative in CABG surgery to minimize the complications and mortality related to surgery.

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INTRODUCTION

Although open heart surgery has been generally well tolerated, 3%-5% of patients experience a remarkably long and problematic recovery period, especially after extracorporeal circulation (ECC) [Butler 1993]. The systemic inflammatory response syndrome (SIRS) has been thought to be a major contributor to such complications [Kirklin 1989]. Cardiac operations with cardiopulmonary bypass (CPB) cause SIRS that has been implicated in postoperative organ dysfunction [Butler 1992]. Surgical trauma, contact of blood with the extracorporeal circuit, and lung reperfusion injury are causative factors, but the role of cytokines in these responses has not yet been established [Butler 1993].

CPB activates five plasma protein systems: contact, intrinsic coagulation, extrinsic coagulation, complement, and fibrinolytic. Blood cells activated by CPB are neutrophils, monocytes, platelets, lymphocytes, and endothelial cells. It has previously been shown that activation of the complement system and cytokines, especially interleukin 6 (IL-6), IL-8, and neopterin, could play an important role in the inflammatory response during ECC and could be used to predict the complications after cardiac surgery using ECC [Ramlawi 2009]. Plasma concentrations of nearly all cytokines peak several hours after CPB [Wan 1999]. Thus, the impact of circulating cytokines is largely in the early postoperative period [Schulze]. Increases in IL-6 and IL-8 levels are specifically noted in the literature as playing roles in the systemic inflammatory response [Butler 1992]. Since interleukin levels significantly increase after ECC, increasingly studies have suggested that interleukins, neopterin, and other cytokines are reliable markers for SIRS [Butler 1993b]. It has also been

Table 1. Demographic Data of Patients*

	Group 1	Group 2	Group 3
Sex	6 Men, 4 women	5 Men, 5 women	7 Men, 3 women
Age, years	60.40 ± 3.44	57.22 ± 2.57	53.32 ± 1.57
Postexertional angina status according to the Canadian Classification	3.21 ± 0.23	3.21 ± 0.13	3.31 ± 0.43
Body surface area	1.82 ± 0.09	1.71 ± 0.04	1.60 ± 0.06

*Data are presented as mean ± standard error of the mean.

shown that the inflammatory response during coronary artery bypass grafting (CABG) without using ECC is much stronger than that in CABG surgeries using ECC.

Some investigations have indicated that cytokines play a key role in the inflammatory cascade associated with CPB. Proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), IL-6, and IL-8, may contribute to myocardial dysfunction and hemodynamic instability after clinical CPB. Moreover, the myocardium is capable of synthesizing TNF-α, IL-6, and IL-8 during CPB [Wan 1999].

MATERIALS AND METHODS

Thirty patients hospitalized in the clinic of the Fırat University Medical Faculty Department of Thoracic and Cardiovascular Surgery who were to have valve replacement surgery (n = 10), CABG with ECC (n = 10) (ONCAB), or CABG using the beating-heart technique (n = 10) (OPCAB) were enrolled in the study. Demographic data are shown in Table 1.

In accordance with the rules accepted by the Human Investigation Ethics Committee, blood was drawn from the patients before the surgery (T0) and 1 hour (T1), 4 hours (T2), 24 hours (T3), and 48 hours (T4) after protamine injection. Serum was separated with centrifugation, and samples were stored at –20°C until analyzed.

IL-6, IL-8, and neopterin levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Neopterin; BRAHMS Diagnostica GmbH, 16761 Berlin; IL-6 ve IL-8, ELISA; CLB, Amsterdam, the Netherlands). Data from the groups were compared using Kruskal-Wallis and 1-way analysis of variance (ANOVA) tests. The Mann-Whitney U test was used to compare the data from different study groups. Correlations among parameters were evaluated by the Spearman rank order correlation test. Statistical analyses were carried out using SPSS version 10.0 software. $P < .05$ was accepted as the threshold for significance.

RESULTS

After the clinical, microbiological, and radiological tests were reviewed before surgery, patients who didn't have any infection symptoms or signs were enrolled in this study. Neopterin levels significantly increased first at the fourth hour after the surgery, and this increase continued at the 24th hour in both groups (n = 30) ($P < .05$). Unlike neopterin, IL-6 and IL-8 levels were increased at the 1st and 4th hour after the surgery. Compared to the levels before the surgery, this increase was statistically significant but started to decrease at the 24th hour after the surgery ($P < .05$) (Table 2).

In patients who had valve replacement surgery (n = 10), neopterin levels were significantly increased first at the fourth hour and this increase continued at the 48th hour after the surgery ($P < .05$). IL-6 and IL-8 levels were increased at the first and fourth hours, respectively, but then started to decrease gradually. Neopterin levels of the patients who had CABG with ECC (n = 10) started to increase after surgery, but this increase became significant at 14th and 48th hours ($P < .05$). IL-6 and IL-8 levels reached to their maximum levels at the fourth and first hours, respectively (Figure 1).

Unlike the 2 groups of patients who had on-pump surgery, patients who experienced CABG with the beating-heart technique (n = 10) had decreased neopterin levels at the first hour after the surgery, but this decrease was not statistically significant. Neopterin levels started to increase later, but this

Table 2. Neopterin, IL-6 and IL-8 Levels in All Patients (N = 30) at the End of the Scheduled Periods*

	T0	T1	T2	T3	T4
Neopterin, nmol/L	8.78 ± 2.40	9.55 ± 3.79	14.25 ± 5.90†	19.58 ± 7.73†	20.90 ± 11.99†
IL-6, pg/mL	0.98 ± 0.17	109.73 ± 68.7	144.04 ± 28.90	36.73 ± 6.08†	7.36 ± 1.18†
IL-8, pg/mL	8.51 ± 2.65	51.89 ± 11.46	70.34 ± 20.08	20.30 ± 11.01†	7.32 ± 3.79†

*Data are presented as mean ± standard error of the mean.

†$P < 0.05$

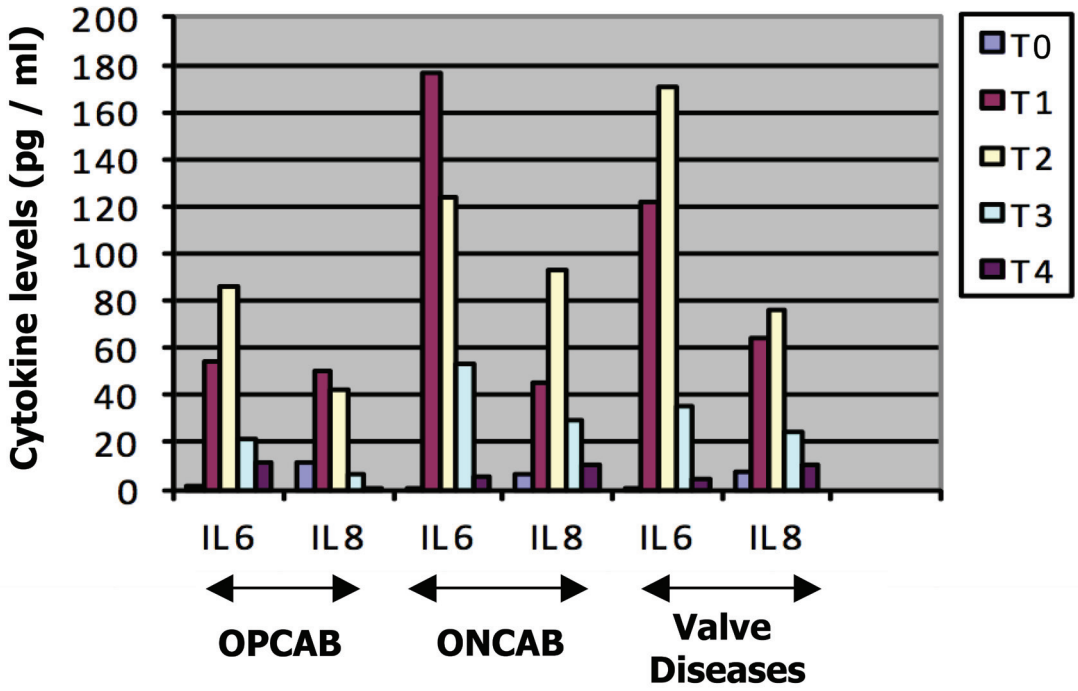

Figure 1. Cytokine levels before and after different cardiac surgery procedures.

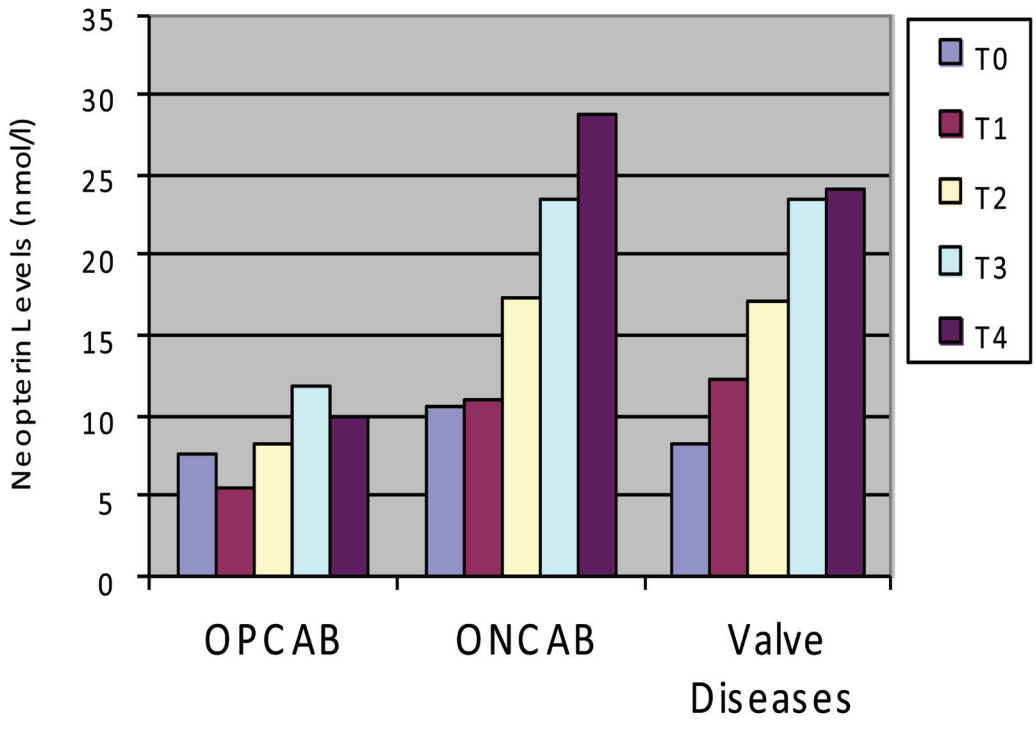

Figure 2. Neopterin levels before and after different cardiac surgery procedures.

increase in neopterin levels was not as high as that in the other 2 two groups. Neopterin levels in the off-pump group reached their maximum levels at the 24th hour, and unlike other groups started to decrease at the 48th hour (Figure 2).

DISCUSSION

It has previously been known that ECC, as a basic application of modern cardiovascular surgery, is associated with a systemic inflammatory response [Brkić 2006; Cremer 1996]. Although several factors have been held responsible for that effect, contact of the blood with nonphysiological artificial membranes and the activation of several cell types in the setting of ischemia and reperfusion are believed to play important roles in the development of this generalized inflammatory reaction [Viaro 2008]. SIRS is a clinical condition which may occur after the surgical procedures using ECC. Since SIRS is characterized by a hyperdynamic circulatory condition with decreased systemic vascular resistance and increased cardiac output, therapeutic approaches which aim to inhibit the inflammatory responses during ECC may be of great importance today. Patients who experience SIRS are at risk of lactic acidosis and multiorgan failure, and they also have increased infectious complications [Wan 1997a].

The use of ECC during open-heart surgeries is associated with the production of proinflammatory cytokines [Wan 1996]. These mediators, including TNF-α, IL-1, IL-6, IL-8, and neopterin, are endogenous proteins which are produced as a response to tissue damage, and they have several metabolic, hematological, and immunological effects [Hayashida 1999]. There is a prominent relationship between the degree of that response and multiorgan failure and mortality after the surgical procedures [Casey 1993]. It has recently been shown that proinflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, and neopterin play important roles in affecting cardiac functions [Raja 2007]. Hennein et al. reported that left ventricular wall movement disorders after coronary revascularization are related to production of IL-6 and IL-8 [Hennein 1994]. Decreasing the cytokine production during and after ECC might prevent the occurrence of cardiac dysfunction and respiratory distress syndrome after several surgical procedures. For all these reasons, therapeutic approaches, including pharmaceutical agents which minimalize the cytokine production, heparin-coated drugs, and hemofiltration, are of great interest today [Hayashida 1999].

Proinflammatory cytokine levels are related to the length of the ischemic period during ECC. Takayama et al. reported that although the increased levels of IL-6 are associated with cardiac dysfunction after ECC, the hemodynamic effects of IL-6 are still speculative [Takayama 2007]. Patrick et al. have shown that IL-8 caused neutrophil aggregation and could play an important role in lung damage related to pulmonary leucocyte secretion [Patrick 1996]. Proinflammatory cytokine production may contribute to multiorgan failure. Wan et al. reported that the heart muscle is the most important source for IL-6 and also IL-8 [Wan 1997b].

Our results showed that serum neopterin concentrations increased in both patients operated on with and those operated on without CPB. Also, our results showed that serum IL-6 and IL-8 concentrations increased in both groups of patients postoperatively and then decreased by 24 h after surgery. The immunological response to these kinds of surgical operations and our results were similar to those reported in the literature [Viaro 2008]. In our study, IL-8 levels significantly increased at the fourth hour, but there was not a significant difference between groups in IL-6 levels.

Compared with conventional CABG, OPCAB coronary revascularization is associated with reduced cytokine responses. The lower neopterin production seen after OPCAB procedures could be explained by the lower myocardial injury associated with the procedure. IL-8 is a crucial chemokine known to attract and activate neutrophils. It has previously been shown that during the acute-phase response neutrophil migration to the damaged area is stimulated via an infectious agent or physical trauma, and chemokines are the main players during this inducible response [Wan 1997a]. In animal models, the release of IL-8 is induced only after reperfusion of the ischemic myocardium [Ivey 1995]. In addition, some clinical studies have demonstrated that during reperfusion after a longer duration of ischemia the myocardium is a major source of IL-8 [Kutay 2006].

Decker et al. reported that IL-6 produced after cardiac surgery is the most important cytokine modulating the immune response and hematopoiesis. They have shown that IL-6 levels are compatible with the surgical traumatic process and thus IL-6 might be a sensitive marker for tissue damage [Decker 1997]. Donati et al. have asserted that before surgery routine measurement of IL-6 levels could be helpful for determining the risky patient groups and that measurement of high IL-6 levels after surgery could be an important predictor for complications related to surgery [Donati 1998].

Cytokines are cellular molecules which are sufficient to change target cell functions. Many important relations between immune cells are controlled via these molecular signals. Although IL-6 and IL-8 are important endogenous proteins which modulate the immunological, hematological, and metabolic responses to tissue damage [Cremer 1996; Wan 1997a], it has been shown that neopterin levels are more sensitive and specific than the other routine laboratory tests for predicting complications and also mortality after the surgery.

The relationship between inflammation and myocardial ischemia, as well as tissue damage, has been known for more than 50 years and is still an important research topic [Kirklin 1989; Butler 1993; Uyar 2013]. ECC and extracorporeal membrane oxygenation cause a systemic inflammatory response, which is characterized by the activation of chemotactic factors, oxygen-free radicals, and proinflammatory cytokines [Schulze 2009]. This systemic inflammatory response is related to the increase of the proinflammatory cytokines, including interleukins, as well as activation of the complement system. It has previously been shown that this inflammatory process might be related to multiorgan failure which is seen after ECC and causes several morbidities [Butler 1993]. Both with animal studies and in vitro research, it has previously been shown that as in septic shock, cytokines, including TNF-α, IL-6, and IL-8, are responsible for

leucocyte adhesion and vascular permeability [Uyar 2013]. The presence of free radicals may indicate that antioxidant therapy might be also useful during ECC.

In conclusion, although it is believed that complement activation is the major cause of adverse events occurring during and after ECC, this process is controlled by multiple complex factors. Complement activation, cytokine production, and related cellular responses are probably the most important factors during the process. It is certain that ECC activates the complement system and that activated complement proteins are the cause of increases in several cytokines. In our study, neopterin levels in patients who underwent OPCAB operations were lower than those in patients who underwent ONCAB bypass and started to decrease at the 48th hour. This effect showed us that the systemic inflammatory response was less activated in the OPCAB group. The OPCAB method might be an important alternative in CABG surgery to minimalize the complications and mortality related to surgery. It has been shown that IL-1 and TNF-α, which are known as the stimulating cytokines for IL-6 production, were increasing after ECC [Cremer 1996]. For all these reasons, adverse effects which are related to morbidity and mortality after cardiac surgery might be prevented by complete or partial inhibition of complement activation and/or cytokine production.

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