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Neopterin Kinetics after Cardiac Surgery with or without Cardiopulmonary Bypass

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A B S T R A C T

Cardiac surgery (CS) with cardiopulmonary bypass (CPB) induces systemic inflammatory response by activating plasma proteins and blood cells. Activated monocytes/macrophages produce inflammatory marker neopterin (NP). The aim was to explore the NP kinetics in first 24 hours after CS according to the CPB use. Significant difference between groups was found for NP levels 12 and 24 hrs after CS, being higher in on-pump group. Strong association was found between NP levels 12 hrs after CS and the length of ICU stay for on-pump group (r=0.744, p<0.001). Strong association was found between preoperative NP levels and the length of ICU stay for those on-pump patients with elevated preoperative NP (r=0.855, p=0.001; linear regression equation y=0.50x – 5.14, p<0.001). Preoperative NP levels higher than 10 nmol/L in on-pump group could predict prolonged ICU stay and outpoint patients at higher risk for developing postoperative complications and, therefore, help to determine the necessary therapeutic interventions.

Key words: *cardiac surgery, cardiopulmonary bypass, neopterin, systemic inflammatory response*

Introduction

Cardiac surgery with cardiopulmonary bypass (CPB) induces systemic inflammatory response (SIR) by activating plasma proteins (complement, coagulation, and fibrinolysis) and blood cells (neutrophils, monocytes/macrophages, and lymphocytes). In uncomplicated cases, SIR is temporary event representing a physiologic reaction to tissue injury. However, if this response is exaggerated, it may lead to substantial morbidity, including myocardial dysfunction, respiratory failure, renal and neurological dysfunction, bleeding disorders, altered liver function, and, ultimately, increased mortality[1].

Activated monocytes/macrophages produce various inflammatory markers including neopterin (NP), a pteridine derivate synthesized from guanosine triphosphate[2]. Its production is mainly induced by interferon-gamma from T lymphocytes and co-stimulated by both TNF-alpha and endotoxins[3]. NP levels rise in the early stage of different diseases and increased levels are usually in correlation with severity of the disease[4]. Therefore, measuring NP levels is helpful for follow-up pathological states associated with the activation of cell-mediated immunity[5].

Dynamics of NP after cardiac surgery with the use of CPB has been analyzed by Adamik and his co-workers. They reported levels of NP at 24 and 48 hours after surgery, along with procalcitonin, to be good predictor of mortality of sepsis and postoperative complications secondary to CPB[6]. However, kinetics of NP within the first 24 hours is unknown.

Therefore, the main focus of this study was to explore the NP kinetics in first 24 hours after cardiac surgery according to the use of CPB. The secondary aim of this study was to test hypothesis that NP, as a marker of activated monocytes/macrophages, can be used as an important factor for monitoring patient's postoperative status.

Materials and Methods

Patients

Between April and December 2002, seventy eight consecutive patients undergoing elective cardiac surgery were enrolled in the study and divided in two groups according to the type of surgical procedure performed: 42

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TABLE 1
BASIC CLINICAL CHARACTERISTICS OF PATIENTS WHO UNDERWENT ELECTIVE CARDIAC SURGERY WITH (ON-PUMP) OR WITHOUT (OFF-PUMP) THE USE OF CARDIOPULMONARY BYPASS

	Off-pump	On-pump	p value*
Number of patients	42	36	–
Male sex, n (%)	32 (76)	25 (69)	0.679
Age, years (median, range)	59 (56–66)	65 (62–68)	0.140
Diabetes mellitus, n (%)	12 (29)	20 (56)	0.028
Arterial hypertension, n (%)	29 (69)	16 (44)	0.050
Hyperlipidemia, n (%)	23 (55)	10 (28)	0.029
EuroScore (median, range)	2 (0–12)	3 (0–7)	0.899
Myocardial infarction, n (%)	23 (55)	11 (31)	0.055
Cerebrovascular insult, n (%)	1 (2)	1 (3)	0.543

* Student's t-test

TABLE 2
OPERATIVE CHARACTERISTICS OF PATIENTS WHO UNDERWENT ELECTIVE CARDIAC SURGERY WITH (ON-PUMP) OR WITHOUT (OFF-PUMP) THE USE OF CARDIOPULMONARY BYPASS

	Off-pump (n=42)	On-pump (n=36)	p value*
Total operating time (min)	200 (90–370)	200 (105–360)	0.966
Cardiopulmonary bypass time (min)	–	72 (18–178)	–
Aortic cross clamp time (min)	–	45 (0–106)	–
The length of stay at Intensive care unit (days)	2 (1–6)	2 (1–12)	0.145
Total hospital stay (days)	15 (6–44)	16 (6–29)	0.670

*Student's t-test, variables are presented as median with range

patients undergoing cardiac surgery without the use of CPB (off-pump group) and 36 patients undergoing cardiac surgery with the use of CPB (on-pump group): 25 patients undergoing coronary artery bypass surgery, 4 patients undergoing aortal valve replacement and 7 patients undergoing mitral valve replacement. Patients undergoing reoperations, combined procedures or emergency surgery, those with acute or chronic renal failure requiring hemodialysis and known inflammatory diseases requiring antibiotics or steroids were not included. Preoperative patient's characteristics are presented in Table 1 while their intraoperative characteristics and both length of ICU stay and total length of hospital stay are presented in Table 2.

The Hospital ethics committee approved the study, and informed written consent was obtained from each patient.

Intraoperative patient management

Same anesthesia protocol was used for all patients. In the on-pump group, just prior to establishing the cardiopulmonary bypass, patients were heparinized with 3 mg/kg of heparin (Belupo, Koprivnica, Croatia), to achieve an activated coagulation time higher than 480 seconds. CPB was performed with a membrane oxygenator and roller pump system. Details of the extracorporeal circulation and perfusion procedures have been published previously[7]. After discontinuation of CPB, heparin was neutralized with protamine sulphate (ICN, Budapest, Hungary).

In the off-pump group, patients were heparinized with 1 mg/kg. Myocardial stabilization was achieved using the vacuum stabilizers (Octopuss; Starfish-Medtronic, Inc., Minneapolis (MN), USA). Details on surgical procedure have been published previously[8]. After all anastomoses were completed, heparin was fully reversed with protamine sulphate.

Blood sampling

Blood samples were taken from each patient at four predefined time points. First blood sample was taken between 6 and 8 am one day before the surgery. Next samples were drawn 12 and 24 hours after the surgery. The last sample was obtained on third postoperative day between 6 and 8 am. Blood samples were drawn from peripheral venous line (BD Vacutainer System; Becton, Dickinson & Co., Plymouth, UK). After withdrawal, blood was spun down for 15 minutes (3000*g* at 4 °C) and serum was stored at –86 °C until it was assayed.

Neopterin measurements

The serum NP concentrations were measured by commercial ELISA kit (Quantikine; R&D Systems, Vienna, Austria) in duplicate. According to the manufacturer's information, sensitivity of the assay was 10 nmol/L. The intra-assay variability coefficients were 1.1–3.1%, while the inter-assay variability coefficients were 5.6–

6.1%. The investigator performing the assay was blinded to all clinical data and outcomes.

Statistical analysis

Data were summarized using proportions, median and range, and mean and standard deviation, as indicated. The associations between variables were assessed by Pearson's r, while the predictions were made on the basis of linear regression analysis. The neopterin dynamics were first analyzed by repeated-measures ANOVA, followed by either independent-samples or paired-samples Student's t-test with Bonferroni's correction for multiple testing. The alpha level was set at 0.05. All the analyses were performed using SPSS 13 for Windows (SPSS Inc., Chicago, IL).

Results

Preoperative NP concentrations were age dependent, being higher in elderly people ($r=0.396$, $p<0.001$), but not gender-related. Significantly more patients in the on-pump group had diabetes mellitus, while in off-pump group more patients had hyperlipidemia (Table 1). However, no association was found between preoperative NP values and any of the preoperative characteristics (data not shown). Furthermore, postoperative NP concentrations were not associated with the operative characteristics (data not shown).

The NP levels were significantly influenced by the type of operation, being higher in the on-pump group (ANOVA, $F_{1,45}=4.152$, $p=0.047$). Specifically, significant difference between the groups was found for concentrations at 12 and 24 hours after the surgery ($p<0.05$) (Figure 1). To determine if NP kinetics after the surgery was associated with preoperative NP values, Pearson correlation coefficients were calculated. Good associations were found between preoperative NP values and all postoperative time points in both groups (Table 3).

Moderate correlation was found between preoperative NP concentration and the length of ICU stay for on-pump group ($r=0.526$, $p=0.001$) while no such association was detected for off-pump group. NP concentrations 12 hours after the surgery showed very good correlation with the length of ICU stay for on-pump group ($r=0.744$, $p<0.001$) while the correlation in the off-pump group was slightly weaker ($r=0.556$, $p=0.001$). Furthermore, NP concentrations 24 hours after the surgery showed poorer but significant associations for both groups ($r=0.455$, $p=0.010$ for on-pump, and $r=0.387$, $p=0.016$ for off-pump group). The linear regression analysis indicated that the length of ICU stay could be predicted by NP concentrations 12 hours after the surgery (regression equation $y=0.17x + 0.69$, $p<0.001$), only for the on-pump group.

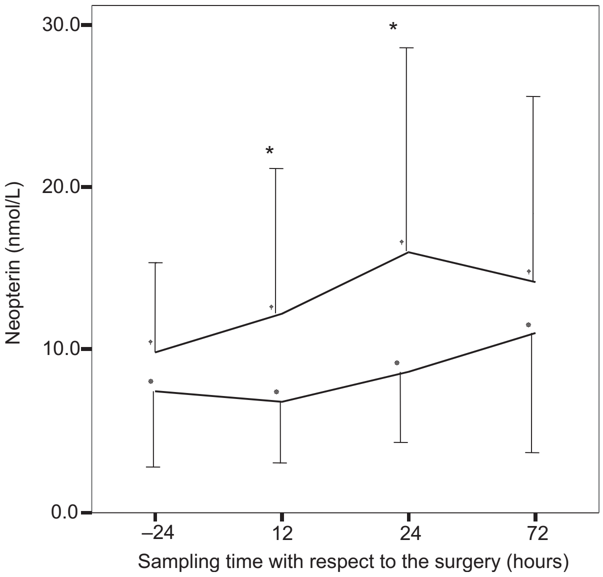

Fig. 1. Neopterin kinetics (X±SD) in 78 patients who underwent elective cardiac surgery with (on-pump) or without (off-pump) the use of cardiopulmonary bypass. Circles – on-pump patients (n=36), squares – off-pump patients (n=42). Asterisk indicates p<0.05 for on-pump vs. off-pump group in the same time point, Student's t-test with Bonferroni's correction.

Due to the wide range of preoperative NP levels in our study (1.6–31.4 nmol/L), patients were additionally compared on the basis of a cut-off value, set to 10 nmol/L, as a generally accepted upper value of normal NP range[4]. There were 7 patients (17%) in off-pump group and 12 patients (34%) in on-pump group with elevated preoperative NP levels (Figure 2). Strong association was found between preoperative NP levels and the length of ICU stay only for on-pump patients with elevated preoperative NP ($r=0.855$, $p=0.001$). The linear regression analysis showed that the length of ICU stay could be predicted by preoperative NP concentrations (regression equation $y = 0.50x – 5.14$, $p<0.001$) in this subgroup of patients.

TABLE 3
ASSOCIATION OF PREOPERATIVE NEOPTERIN (NP) VALUES WITH NP VALUES AT VARIOUS TIME POINTS AFTER THE SURGERY IN PATIENTS WHO UNDERWENT ELECTIVE CARDIAC SURGERY WITH (ON-PUMP) OR WITHOUT (OFF-PUMP) THE USE OF CARDIOPULMONARY BYPASS

NP values after surgery	Preoperative NP value			
	Off-pump (n=42)		On-pump (n=36)	
	r*	p	r*	p
12 hrs	0.572	0.001	0.670	<0.001
24 hrs	0.485	0.002	0.498	0.004
72 hrs	0.393	0.021	0.644	<0.001

*Pearson's correlation coefficient

Discussion

Our study was the first to describe NP kinetics within first 24 hours after cardiac surgery, with respect to the use of CPB. Elevated postoperative NP levels were indirectly related to the development of postoperative com-

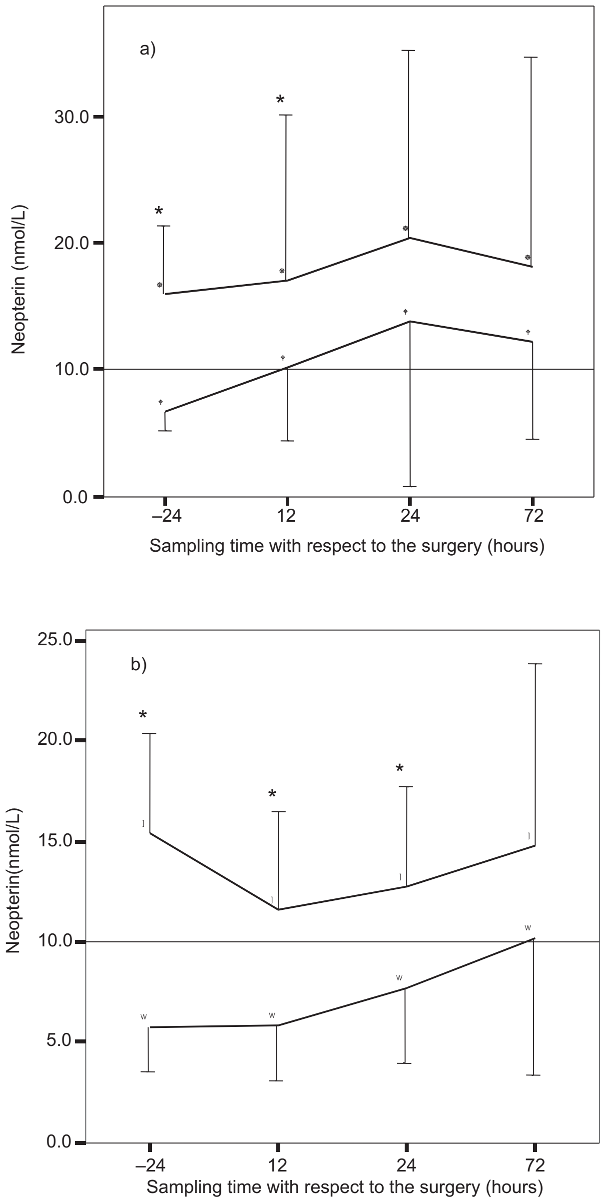

Fig. 2. *Neopterin (NP) kinetics (N ± SD) in patients who underwent elective cardiac surgery with (on-pump, n=36) (a) or without (off-pump, n=42) the use of cardiopulmonary bypass (b). Patients were compared with respect to the NP cut-off value of 10 nmol/L (represented with reference line): squares – patients with preoperative NP values higher then 10 nmol/L, circles – patients with preoperative neopterin values lower then 10 nmol/L. Asterisk indicates p<0.05 for patients with preoperative NP levels higher then 10 nmol/L vs. patients with equal or lower NP levels in the same time point, Student's t-test with Bonferroni's correction.*

plications by predicting prolonged ICU stay. Furthermore, preoperative NP cut-off value set at 10 nmol/L could be used to predict the length of ICU stay after cardiac surgery performed with the use of CPB.

Early postoperative NP kinetics was similar in both on-pump and off-pump group, although absolute levels were significantly higher in the on-pump group, particularly at 12 and 24 hrs after the surgery (Figure 1). Postoperative NP increase in the on-pump group could present a cumulative consequence of both blood contact with CPB tubes (which activated monocytes/macrophages[9,10]) and surgical stress. This event indicates more activated cell-mediated immune response which suggest progress of certain postoperative complications, probably associated with infection. Slight NP increase in the off--pump group is a result of operative trauma *per se*, also observed in the study of Johansson-Synnergren et al.[11] and explained it as less inflammatory-associated complications in off-pump patients.

Examining the absolute numbers of preoperative NP values, wide range was observed (1.6–31.4 nmol/L). There are plenty of evidence that macrophages are activated in diabetes mellitus, due to the chronic hyperglycemia[12,13]. Since there were more patients in on-pump group with diabetes mellitus, this could be one possible reason for elevated preoperative NP in this patient group. Furthermore, it was noticed that in atherosclerosis and its complications (e.g. hyperlipidemia), NP was present in blood as a result of macrophage activation[14–16]. This could influence on NP levels in off-pump patients since there was higher incidence of hyperlipidemia. However, no association between hyperlipidemia and diabetes mellitus with preoperative NP levels was found in our study.

Since our primary hypothesis was that NP can be used as a predictor of postoperative complications, after failing to relate any specific complication to NP levels (data not shown), we have analyzed the relationship between postoperative NP levels and the length of ICU stay, as a non-specific and indirect factor reflecting patient's status. Linear regression analyses have pointed out that the length of ICU stay could be predict with NP levels 12 hours after surgery only for patients in on-pump group. Therefore, patients with elevated early postoperative NP levels should be treated with extra attention and monitoring, due to higher risk for developing certain postoperative complications. Due to a small study sample as well as different surgical procedures performed, exact postoperative complications that are associated with elevated NP still have to be determined.

Several studies have shown that using NP cut-off value, set to 10 nmol/L, was useful method for detecting normal and elevated concentrations[4,17]. After introduction of this cut-off level in our study, the shape of neopterin kinetic cur

ve remain similar within groups. However, absolute levels were higher for those patients with preoperative NP values higher then 10 nmol/L. Elevated preoperative NP levels remain significantly elevated postoperatively till 12 hrs for on-pump group and 24 hrs for off-pump group. This evidence showed us that even preoperative elevated NP levels could identify patients at higher risk for prolonged ICU stay.

Similar to the work of Adamik et al.[6], our study points out that NP is an important determinant of SIR. However, there were only few studies exploring the role of NP after cardiac surgery[6,10,18,19], probably due to the fact that

NP was mainly considered to be a marker of sepsis[20,21] and prognostic marker in malignant diseases[22–24]. Measurement of NP values after cardiac surgery could be advocated by several arguments: first, the NP value not only reflects the effect of a single cytokine but also allows assessing the cumulative effect of immunological network on the population of monocytes/macrophages[25], secondly, NP is inert and its biological half-life is only determined by renal excretion, which is in contrast with the biological half-life of cytokines that stimulate NP secretion (i.e. interferon-gamma and TNF-alpha) influenced by target and/or soluble receptors. Moreover, increased NP concentrations after cardiac surgery are probably related to endothelial damage[20], which is consistent with recent findings of endothelial damage as a central feature in CPB related procedure[26] that can be distinguished from off-pump procedure.

Limitations of our study include small sample size and wide age range (39–77 years). Since preoperative NP levels were age dependent, it is necessary to analyze relationship between NP and the length of ICU stay in more specifically designed patient groups. Additionally, this study was not designed to study clinical endpoints and, therefore, future studies are necessary to determine exact postoperative complications associated with higher preoperative NP levels.

In summary, our study describes NP kinetics early after cardiac surgery in respect of the use of CPB. CPB was found to increase postoperative NP levels more than off-pump surgery, especially in patients with higher NP levels preoperatively. Preoperative NP levels higher than 10 nmol/L in on-pump group predicted prolonged ICU stay and therefore, could outpoint patients at higher risk for developing postoperative complications. This is important because the early identification of patients at risk of developing postoperative complications may help in determining therapeutic interventions.

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