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Increased Neopterin in Patients With Chronic and Acute Coronary Syndromes

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Objectives. **The aim of our study was to determine neopterin levels in patients with chronic and acute coronary syndromes.**

Background. **In chronic and acute coronary syndromes the release of different cytokines activates cellular defense. Infiltration of neutrophils and monocytes/macrophages is detected in the vessel wall as well as in the myocardium. Neopterin, which is a by-product of the guanosine triphosphate–biopterin pathway, is a marker for those activated macrophages.**

Methods. **We studied 123 subjects: 1) 21 consecutive patients (17 men, 4 women; mean age ± SD 66 ± 15 years, range 31 to 87) with acute myocardial infarction (AMI); 2) 62 consecutive patients (50 men, 12 women; mean age 61 ± 8 years, range 43 to 81) with signs and symptoms of clinically stable coronary artery disease (CAD); and 3) 40 healthy blood donors (28 men, 12 women; mean age 35 ± 13 years). Neopterin levels were determined with a commercially available enzyme-linked immunosorbent assay method.**

Results. **In patients with AMI before thrombolytic therapy, neopterin levels were significantly higher than levels in patients with CAD and control subjects (13.7 vs. 8.6 and vs. 6.8 nmol/liter, $p < 0.0001$). Values also differed significantly between patients with CAD and control subjects ($p < 0.0001$). Neopterin levels in patients with AMI were measured seven times during a 72-h period. Within-group comparison showed significant differences over this period ($p < 0.00001$). The lowest value (11.4 nmol/liter) was observed after 4 h and differed significantly from the initial value and values after 24 and 72 h ($p < 0.05$). After 72 h, neopterin increased to 14.9 nmol/liter, a value significantly different from all values other than the initial one. There was no correlation between neopterin and creatine kinase (CK); CK, MB isoenzyme; or lactate dehydrogenase as markers for the extent of the myocardial infarction during the observation period.**

Conclusions. **Our data support the hypothesis of an activation of monocytes and macrophages in patients with an acute or chronic coronary syndrome. Neopterin as a marker for macrophage activation is significantly increased in patients with chronic CAD and more pronounced in patients with AMI shortly after the onset of symptoms.**

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Atherosclerosis seems to be a chronic inflammatory process that can develop to an acute clinical event by the induction of plaque rupture (1). The risk of plaque rupture depends more on the number and the activation status of macrophages, the principal inflammatory cells in atherosclerotic plaques, than on plaque size (2). Inflammatory cells are capable of releasing lytic enzymes that may be responsible for the weakening of the fibrous cap and subsequent rupture of the atherosclerotic plaque (3). Neopterin, a by-product of the guanosine triphosphate pathway, is produced by activated macrophages and serves as a marker for the activation status of monocytes/macrophages. In addition to significant elevation of urinary or serum pteridine levels in several clinical conditions (4), increased serum neopterin levels were found in patients with pronounced peripheral atherosclerosis (5) and in patients with carotid atherosclerosis (6). Moreover, urinary neopterin levels seem to increase in patients with acute myocardial infarction (AMI) within the first week (7).

No data are available on neopterin in patients with acute and chronic coronary syndromes. We therefore compared neopterin levels in patients with AMI with those in patients with stable coronary artery disease (CAD) and healthy control subjects. Additionally, we examined whether neopterin increases during AMI, serving as a marker for inflammation, and whether this variable is correlated with noninvasive indexes for the extent of myocardial necrosis, that is, creatine kinase (CK); CK, MB isoenzyme [CK-MB]; or lactate dehydrogenase (LDH).

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Abbreviations and Acronyms

AMI = acute myocardial infarction
ANOVA = analysis of variance
CAD = coronary artery disease
CK = creatine kinase
CK-MB = creatine kinase, MB isoenzyme
HDL = high density lipoprotein
LDH = lactate dehydrogenase
LDL = low density lipoprotein
mRNA = messenger ribonucleic acid
TNF = tumor necrosis factor

Methods

Patients. Group 1 comprised 21 consecutive patients (17 men, 4 women; mean age ± SD 66 ± 15 years [range 31 to 87]) who were admitted to the coronary care unit with AMI <6 h after the onset of symptoms. Inclusion criteria were typical chest pain and ST segment elevation ≥0.1 mV in at least two contiguous electrocardiographic leads. We excluded patients with the usual thrombolytic contraindications, those with previous myocardial infarction at the same site and those with previous coronary artery bypass surgery. All 21 patients were treated with either front-loaded recombinant tissue-type plasminogen activator (n = 15), as previously described (8), or streptokinase, 1.5 million U over 60 min (n = 6). Additionally, they received aspirin, 100 mg; diazepam, 10 mg orally; pethidine, intravenously or subcutaneously, or both; heparin, intravenously, according to partial thromboplastin time; and nitroglycerin infusions when the systolic blood pressure was ≥90 mm Hg. Blood samples were taken before and 2, 4, 6, 8, 12, 24 and 72 h after thrombolytic therapy from a separate cannula in the forearm. Separation of the serum was done immediately and samples were stored at −70°C.

Group 2 comprised 62 consecutive patients (50 men, 12 women; mean age ± SD 61 ± 8 years [range 43 to 81]) who underwent coronary angiography because of signs and symptoms of clinically stable CAD. None of these patients had a history or clinical signs of generalized atherosclerosis or carotid artery atherosclerosis. Blood samples were taken from a separate cannula in the forearm after a rest period of 12 h in the morning before coronary angiography. Separation of the serum was done immediately and samples were also stored at −70°C.

Forty healthy blood donors (28 men, 12 women; mean age 35 ± 13 years [range 20 to 58]) served as a control group. Blood samples and serum separation were performed as described for Groups 1 and 2.

Measurements. Serum CK, CK-MB and LDH were measured by an autoanalyzer (Hitachi 704, Boehringer Mannheim, Mannheim, Germany). Neopterin levels were determined by using a commercially available enzyme-linked immunosorbent assay method (IBL, Germany).

Lipid profile in patients with CAD was measured by an autoanalyzer with enzymatic methods (total cholesterol, triglycerides—autoanalyzer Hitachi 717; HDL cholesterol—enzymatically after precipitation by a phosphatunstic acid and magnesium ions), LDL cholesterol was calculated by the Friedewald formula.

Statistical analysis. For analysis, we used the statistical package "SigmaStat for Windows version 1.0." Continuous variables between groups were analyzed by the unpaired *t* test or the Mann-Whitney rank sum test in case of not normally distributed groups. In the case of dichotomous variables, the chi-square test was used. Data within groups were compared with the one-way repeated measures analysis of variance (ANOVA) on ranks; differences between groups were analyzed by the Student-Newman-Keuls test. Correlations were tested by the Spearman rank correlation, and linear regression analysis was done; p values < 0.05 were considered significant. Unless otherwise indicated, data are presented as mean value ± SD or as median with 25th and 75th percentiles (midrange) specified.

Table 1. Patient Characteristics of the Three Groups

	Patients		
	With AMI (n = 21)	With CAD (n = 62)	Control Subjects (n = 40)
Age (yr)	66 ± 15	61 ± 8	35 ± 13
Male/female	17/4	50/12	28/12
Delay from (min)	164 ± 86	—	—
MI site (ant./inf.)	12/9	—	—
rt-PA/streptokinase	15/6	—	—
CK-MB (U/liter)*	115 ± 82		
LDH (U/liter)*	826 ± 498		

*Mean value ± SD of the highest individual values. Other values are expressed as mean value ± SD or number of patients or subjects. AMI = acute myocardial infarction; ant. = anterior; CAD = coronary artery disease; CK-MB = creatine kinase, MB isoenzyme; Delay = delay from onset of symptoms; inf. = inferior; LDH = lactate dehydrogenase; MI = myocardial infarction; rt-PA = recombinant tissue-type plasminogen activator.

Results

Comparisons among groups. The two patient groups were similar with respect to gender and age; subjects in the control group were significantly younger ($p < 0.001$) (Table 1). A box plot graph of neopterin values is shown in Figure 1. Patients with AMI (before therapy) showed the highest neopterin levels (mean 13.7 ± 3.4 nmol/liter [$p < 0.0001$ vs. levels in patients with CAD (8.6 ± 2.2 nmol/liter) and vs. levels in control subjects (6.8 ± 1.8 nmol/liter)]). A significant difference was also observed between values in patients with CAD and the control group ($p < 0.0001$).

Patients with (Group 2) stable CAD were classified into two subgroups: Group 2A comprised 27 patients (19 men, 8 women; mean age 60 ± 5 years [range 52 to 69]) who had only minimal changes or one-vessel coronary artery disease in the coronary angiogram (minor CAD). Group 2B comprised 35 patients (31 men, 4 women; mean age 60 ± 9 years [range 43 to 81]) with two- or three-vessel CAD (severe CAD). Patients

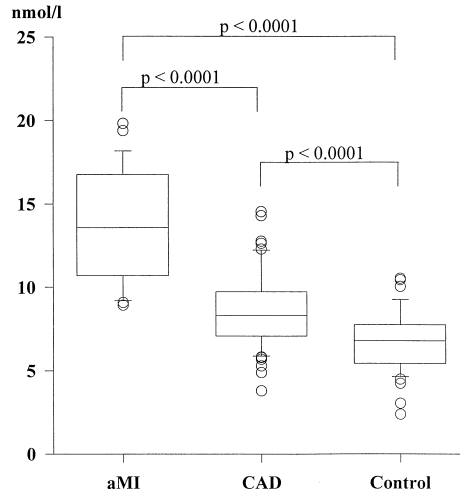


Figure 1. Box plot graph of neopterin values in the three groups (values of patients with AMI are those before thrombolytic therapy). Values shown are mean values **(circles)** and median **(center rule in box)** with 25th percentile **(lower rule of box)**, 75th percentile **(upper rule in box)**, 10th percentile **(lower short bars)** and 90th percentile **(upper short bars)**.

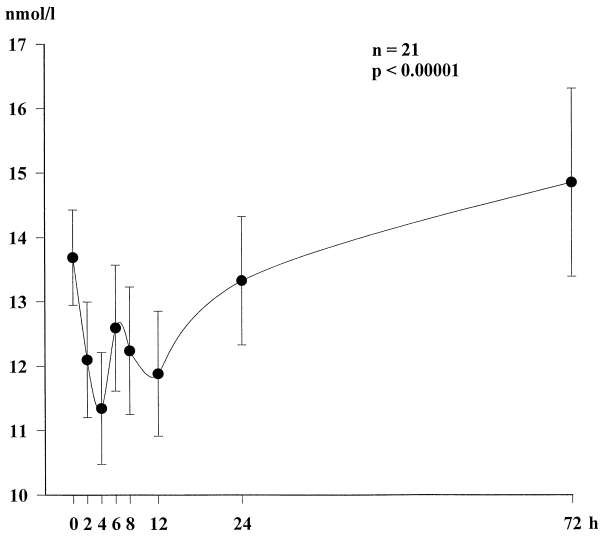


Figure 2. Time course of neopterin over a 72-h observation period in patients with AMI. Values are given as mean **(circles)** ± SEM **(bars)**.

with severe CAD tended (p = NS) to have higher neopterin levels (8.9 ± 2.4 nmol/liter) than patients with minor CAD (8.3 ± 1.9 nmol/liter). Serum lipids were also studied in patients with minor and severe CAD. No significant difference was found between these two subgroups with respect to total cholesterol (minor CAD 241 ± 46 mg/dl vs. severe CAD 237 ± 37 mg/dl; p = NS); high density lipoprotein (HDL) cholesterol (48 ± 12 vs. 50 ± 13 mg/dl; p = NS) triglycerides (176 ± 117 vs. 165 ± 85 mg/dl; p = NS) and low density lipoprotein (LDL) cholesterol (159 ± 39 vs. 156 ± 35 mg/dl; p = NS). Smoking status did not differ significantly between the two subgroups (minor CAD 18 smokers, 9 nonsmokers; severe CAD 24 smokers, 11 nonsmokers).

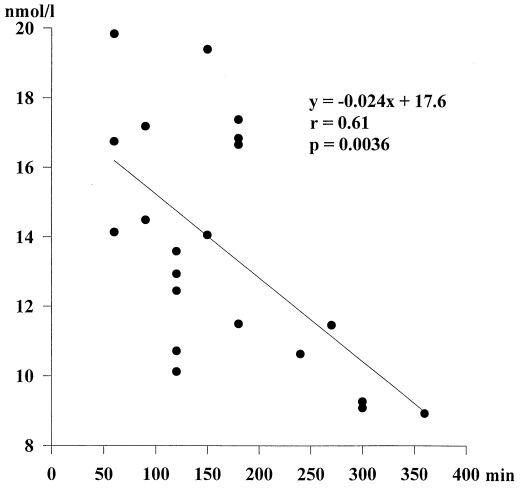
AMI group. Patients with AMI were also followed up over a 72-h period. Within-group comparison showed significant differences over time (one-way repeated measures ANOVA, $p < 0.00001$ [Fig. 2]). The lowest level (11.4 nmol/liter) was observed after 4 h and was statistically significant different from the initial level before therapy and from the levels after 24 and 72 h (Student-Neuman-Keuls test, $p < 0.05$). After 72 h neopterin increased to 14.9 nmol/liter, which was significantly different from all levels except the initial one. Correlation between the duration of symptoms and the first measured neopterin level before therapy is shown in Figure 3. There was a significantly inverse correlation between the time of admission to the coronary care unit and the initial neopterin level ($n = 21$; $y = -0.024x + 17.6$; $r = 0.61$; $p = 0.0036$). No correlation was found between neopterin and CK, CK-MB or LDH as markers for the extent of the myocardial infarction at any time during this observation period. There was also no correlation between initial neopterin levels and the individual maximal level of CK, CK-MB or LDH (Table 1) (neopterin vs. maximal CK, $r = -0.221$, p = NS; neopterin vs. maximal CK-MB, $r = -0.269$, p = NS; neopterin vs. maximal LDH, $r = -0.043$, p = NS).

Discussion

Our data clearly demonstrate that neopterin, a marker for activation of the monocyte/macrophage system, is significantly elevated in patients with acute and chronic coronary syndromes.

Chronic CAD. The increase in neopterin in patients with stable CAD was independent of the number or extent of coronary vessel involvement. The trend to higher neopterin levels in the group with severe CAD in comparison with the group with minor CAD was not statistically significant. Similarly, our pilot study (9) found no difference in neopterin levels

Figure 3. Linear regression between the duration of symptoms in the 21 patients with AMI before admission to the coronary care unit and the first measured neopterin values.



between patients with minor and severe CAD. One possible explanation for these findings is that the power of both studies was too small to detect significant differences. Another explanation is that, because the affected coronary vessels are small, the number of activated macrophages in patients with minor or severe CAD is too small to be detected by increased peripheral blood neopterin. However, patients with clinical signs of CAD had significantly higher neopterin levels than healthy blood donors. Obviously, in patients with pronounced atherosclerosis in large vessels like the carotid artery or in patients with diffuse atherosclerosis, the chronic activation of monocytes and macrophages is measurable by neopterin (5,6).

AMI. In patients with AMI as the most fatal consequence of a plaque rupture, neopterin levels were significantly higher than in patients with chronic CAD and control subjects. This elevation was present before the start of intervention such as thrombolysis or adjuvant therapy. After therapy, neopterin first decreased and then increased, but levels were always approximately two times higher than levels in control subjects and patients with CAD. The neopterin increase reflects the pronounced activation of monocytes/macrophages as the cause or consequence of the plaque instability. Perhaps, rupturing plaques release components from oxidized LDL that stimulate the migration and activation of monocytes/macrophages in the arterial wall (10,11). Activation of the monocyte/macrophage system is consistent with the increase of different unspecific markers of inflammation in patients with AMI and unstable angina. For example, serum interleukin-6 levels, a major cytokine mediator of the acute phase response, was found (12,13) to be significantly higher in patients with AMI than in a control group. C-reactive protein and serum amyloid A protein, two very sensitive yet unspecific markers of inflammation, were also found (14–17) to be increased in patients with an acute coronary syndrome. Such inflammation variables may also have a prognostic importance, as shown by Liuzzo et al. (18). In addition to inflammation associated with plaque rupture, acute phase markers of inflammation may also be elevated because of the presence of necrotic myocardial cells due to reperfusion injury after initiation of thrombolysis or due to abrupt closure of the infarct-related artery. This hypothesis is supported by the elevated cytokine gene expression for tumor necrosis factor (TNF)-alpha reported by Herskowitz et al. (19) in a rat model of myocardial infarction/reperfusion; these authors found an early increase of TNF-alpha messenger ribonucleic acid (mRNA) expression in rat hearts both with and without reperfusion. Their observation suggests that cytokine gene expression may be primarily induced in myocardial cells in response to ischemia. In patients with a transmural myocardial infarction, increased secretion of TNF-alpha in the peripheral blood was observed (20) with a peak within 24 h after the onset of therapy. Moreover, our group (unpublished data) found an activation of soluble TNF receptors 1 and 2 as a sign of an early inflammatory response in a series of 15 patients with AMI. These two soluble TNF receptors peaked 2 h after initiation of therapy, possibly indicating reperfusion success. Additionally, a positive correlation between these two markers of inflammation and the extent of myocardial damage as reflected by the elevation of serum LDH was observed. However, in the present study no correlation was found with neopterin and CK, CK-MB or LDH as markers for the extent of myocardial damage.

Time course in AMI. In patients with AMI, neopterin levels changed significantly within 72 h (Fig. 2). The lowest level (11.4 nmol/liter) was observed after 4 h and was significantly different from the initial level and from levels after 24 and 72 h. After 72 h, neopterin increased to 14.9 nmol/liter, a value significantly higher than all except the initial one. The reason for the decrease after 4 h remains speculative, but it seems to be a general phenomenon rather than a consequence of treatment (i.e., aspirin, heparin, thrombolytic therapy). This view is supported by the clear inverse correlation between the first measured neopterin levels and the duration of symptoms (i.e., the delay between onset of symptoms and admission and blood withdrawal in the coronary care unit) (Fig. 3). This observation probably illustrates that neopterin levels started to decrease at or shortly after the onset of symptoms. One possible explanation for the decrease may be the putative role of neopterins as endogenous antioxidants. It has been reported (21–23) that 7,8-dihydroneopterin and, to a lesser extent, neopterin scavenge free radicals, and the decrease may be due in part to their destruction by radical products generated by reperfusion. The subsequent increase of neopterin during AMI is consistent with the observation of Melichar et al. (7), who found an increase in urinary neopterin during week 1, but initially elevated neopterin values in urine were not demonstrated in their study. The neopterin increase in our study corresponds with the increase in the white blood cell count in the days after infarction (24) and cytokine mRNA expression in an animal model (19). It may also be that the late rise in neopterin is an indirect consequence of thrombolytic therapy (25).

Conclusions. Our data suggest a graduated activation of the monocyte/macrophage system that is independent on the clinical situation of patients with CAD. During the period of stable CAD, the chronic inflammatory process with only a few macrophages involved produces only slightly elevated neopterin levels. During the process leading to plaque rupture, macrophages become successively activated and, as a consequence, plasma neopterin levels start to rise. The subsequent decrease of neopterin during the first hours of AMI remains speculative. One possible explanation may be the action of neopterins as antioxidants and their consumption by free radicals produced during reperfusion. The second increase after 24 and 72 h seems to express a monocyte/macrophage–mediated reaction. Effects of the complement system or therapy may also contribute to this reaction. However, neopterin is not a marker for the extent of infarction because of its lack of correlation with CK-MB and LDH.

These findings may have clinical importance in distinguishing between patients with an acute or chronic coronary syndrome.

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