

EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Markers of Inflammation and Intracellular Metabolism of Neutrophils in Patients with Acute Coronary Syndrome and Normal Troponin T Levels

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Markers of inflammation and intracellular metabolism of neutrophils were studied in 58 patients with acute coronary syndrome with different levels of troponin T. In patients with repeated coronary events within a year, more pronounced endothelial dysfunction associated with activation of oxygen-dependent metabolism of neutrophils and lipid peroxidation process was revealed at admission to the hospital.

Key Words: *coronary artery disease; troponin T; endothelial dysfunction; neutrophils; free radical oxidation*

Modern diagnosis of acute coronary syndrome (ACS) including unstable angina and myocardial infarction is based on the data of electrocardiography and cardio-specific enzyme assay of the blood. Among various markers of myocardial necrosis, troponin T showed the highest sensitivity and specificity. Its determination had improved the methodology for calculating the risk of clinical complications of coronary heart disease (CHD) [11]. However, it was found that the level of biochemical inflammation markers can increase in patients with ACS and normal troponin T content [7]. Increased count of peripheral blood neutrophils (PBN), an important factor of ischemic and reperfusion myocardial injury during CHD, is also a prognostic marker of risk for unfavorable outcome after myocardial infarction [4,10].

The purpose of this study was to examine the levels of IL-6, TNF- α , von Willebrand factor (vWF),

and intracellular metabolism of PBN in patients with ACS with different levels of troponin T.

MATERIALS AND METHODS

The study included 58 patients with ACS (age 34-85 years, mean 62.5 ± 10 years). Of them, 31 (53.4%) patients had unstable angina and 27 (46.6%) had myocardial infarction. The patients were followed for 12 months from the date of entry into the study. Cases of resumption of severe rest angina (unstable angina), repeated or new nonfatal myocardial infarction, and cardiac death were recorded. Repeated coronary events occurred in 34 (58.7%) patients. Determination of troponin T was performed in 36 (62.1%) patients with ACS, in 21 of them (58.3%) the level of troponin T was <0.1 ng/ml. During the year, repeated coronary events were recorded in 11 (52.4%) patients with normal troponin T value, of them repeated anginal attacks at rest were recorded in 5 patients (45.4%), new myocardial infarction in 4 patients (36.4%), and coronary death in 2 (18.2%).

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Analysis of peripheral blood was performed at admission to the cardiology intensive care unit. PBN were isolated from heparinized blood on double density Ficoll-Verografin gradient 1.077 and 1.092 g/ml. Spontaneous and stimulated nitroblue tetrazolium test (NBT) indicating production of superoxide anion radicals by PBN was carried out by quantitative spectrophotometric method as described previously [8]. For evaluation of PBN C3-receptor activity, phytohemagglutinin from kidney beans (*Phaseolus vulgaris*) was used. Activity of myeloperoxidase in PBN was quantified spectrophotometrically using 0.04% solution of ortho-phenylenediamine [5]. Glutathione reductase activity in PBN was determined spectrophotometrically by oxidation of NADPH [3]. Malonic dialdehyde (MDA) in the serum was measured by an indirect method based on MDA reaction with thiobarbituric acid [1]. Serum concentration of vWF antigen was determined by ELISA method using commercial DAKO reagents and international standards (NIBSC). Serum levels of IL-6 and TNF- α were estimated by ELISA using commercial ProCon IL-6 and TNF- α kits (Proteinovyi Kontur Co Ltd). The data were processed by parametric and nonparametric methods using Statistica 8.0 software (Stat Soft, Inc.). The data of research were presented as the mean and standard deviation ($M \pm SD$). Differences between the groups were considered significant at $p < 0.05$.

RESULTS

At admission, patients with ACS and elevated levels of troponin T in blood had significantly higher levels of aspartate aminotransferase (AST) (117.4 ± 86.5 vs. 41.6 ± 18.2 U/liter; $p = 0.0004$), creatine kinase (158.6 ± 165.7 vs. 20.6 ± 8.28 U/liter; $p = 0.009$), C-reactive pro-

tein (44.5 ± 46.5 vs. 4.6 ± 7.5 mg/liter; $p = 0.002$), peripheral blood leukocyte count (8.8 ± 2.7 vs. $6.5 \pm 1.8 \times 10^9$ in normal; $p = 0.003$) compared to patients with normal levels of troponin. In patients with ACS with unfavorable outcome within the year, higher levels of vWF (3.2 ± 1.2 vs. 2.3 ± 0.9 U/ml; $p = 0.004$), stimulated NBT test (123.2 ± 19.6 vs. 106.1 ± 26.3 nmol reduced NBT; $p = 0.02$), MDA (73.1 ± 22.7 vs. 60.3 ± 18.7 μ mol/liter; $p = 0.04$), and relative PBN content in the peripheral blood (64.9 ± 11.2 vs. $59.2 \pm 9.6\%$; $p = 0.04$) were revealed at admission compared to patients with favorable outcome. In patients with ACS and normal values of troponin T, in whom recurrent coronary events developed within a year of follow-up, the values of vWF, stimulated NBT-test, and MDA at admission were significantly higher (Table 1).

It was proven that endothelial dysfunction not only plays an important role in the development of cardiovascular diseases, but also serves as a marker for the risk of fatal outcome in CHD [2,12]. Oxidation stress is one of the major causes of endothelial dysfunction; its development in patients with ACS is related to the formation of free oxygen radicals by activated PBN [4,9]. It was established that damage or destruction of endothelial cells intensifies synthesis and release of vWF. In this study, in patients with normal levels of troponin T and unfavorable outcome of ACS, increased levels of vWF were associated with increased formation of superoxide radical anion by PBN stimulated via C3-receptors. It is known that both CD11b/CD18 and CD11c/CD18 act as receptors of iC3b, a product of C3b-complement component cleavage. PBN use CD11b/CD18 and CD11c/CD18 as the major intercellular adhesion molecules involved in adhesion of leukocytes to the endothelium. It is assumed

TABLE 1. Biochemical and Cytochemical Parameters in Patients with ACS and Normal Levels of Troponin T ($M \pm SD$)

Parameter	Favorable outcome ($n=10$)	Unfavorable outcome ($n=11$)
AST, U/liter	42.9 ± 23.1	40.2 ± 11.6
Creatine phosphokinase, U/liter	16.6 ± 100.2	24.6 ± 111.5
C-reactive protein, mg/liter	2.7 ± 5.7	0.7 ± 9.2
IL-6, pg/ml	36.6 ± 30.2	47.8 ± 55.1
TNF- α , pg/ml	15.1 ± 28.1	63.7 ± 119.1
vWF, U/ml	2.3 ± 0.8	$3.9 \pm 1.6^*$
Spontaneous NBT-test, nmol reduced NBT	99.0 ± 19.4	111.6 ± 12.3
Stimulated HCT-test, nmol reduced NBT	100.9 ± 12.3	$134.1 \pm 17.1^*$
Myeloperoxidase, SED	11.2 ± 5.9	14.6 ± 10.5
Glutathione reductase, nmol \times liter $^{-1} \times$ sec $^{-1}$	63.3 ± 58	47.8 ± 29.4
MDA, μ mol/liter	56.5 ± 15.4	82.4 ± 24.3^o

Note. $^*p < 0.009$, $^o p < 0.002$, $^p p < 0.01$ as compared with the patients with the favorable outcome.

that fixation of iC3b on endothelial cells may cause adhesion of PBN [6]. The increase in MDA content in the blood of patients with normal levels of troponin T and unfavorable outcome suggests that intensification of free radical lipid peroxidation and accumulation of products impairing structural organization and functioning of biopolymer molecules is associated with activation of PBN. In patients with normal troponin T content, stimulated NBT-test, an indicator of intensification of free oxygen radical generation by PBN, directly correlated with values of vWF ($R=0.52$; $p=0.02$) and MDA ($R=0.43$; $p=0.04$).

Thus, analysis of parameters of oxygen-dependent metabolism of PBN by NBT-test and levels of vWF antigen in the serum can be used for predicting the risk of unfavorable outcome in patients with ACS and normal content of troponin T.

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