Autoantibodies to Myosin Light Chains in the Blood as Early Marker of Myocardial Injury after Aortocoronary Bypass Surgery

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Serum concentration of autoantibodies to myosin light chains was measured after resumption of the bloodflow recovery in patients who underwent hypothermic aortocoronary bypass surgery. The patients were divided into 3 groups according to postoperative hemodynamic parameters and degree of myocardial injury. The studies showed significant differences in the concentrations of autoantibodies to myosin light chains between the groups. High correlation was shown between the duration of aorta clamping and concentration of autoantibodies to myosin light chains. Some factors characterizing initial severity of the disease can modulate blood concentration of autoantibodies to myosin light chains.

Key Words: *autoantibodies to myosin light chains; myocardium; reperfusion; ischemia; mitral/aortal diseases*

Metabolism of the myocardium after cardioplegic ischemia is routinely assessed by ECG and echocardiography, clinical status and need in inotropic drugs, level of biochemical markers of myocardial injury (lactate, creatine phosphokinase, myoglobin, troponin, *etc.*) and by morphological studies of biopsy specimens. Some authors claim that creatine phosphokinase, cardiac form of creatine phosphokinase, myoglobin, and lactate dehydrogenase fractions are ineffective for the diagnosis of ischemic injury to the myocardium in patients after cardiac surgery because of low specificity of these markers. More specific markers are recommended [5] for this purpose, for example, myosin light chains (MLC) [4].

Light chains (LC) are myosin subunits essential for its normal functional activity. A specific feature of myocardial MLC important from the diagnostic viewpoint in various diseases is that these chains have special isoforms in different compartments of the heart: atria (LC1a and LC2a) and ventricles (LC1v and LC2v). That is why detection of MLC isoforms in the blood can indicate not only necrotic processes in the myocardium, but also their location. We previously showed the presence of free LC2 fractions in atrial and ventricular myocardium during myocardial infarction, dilatation and ischemic cardiomyopathy, and rheumatic heart disease [3], and a decrease in the content of LC2 in combined mitral-aortic heart disease and coronary disease [1] indicating their migration into the blood. Other authors showed the appearance of cardiac MLC in the blood in patients with myocardial infarction and heart failure [6,8-10]. Available data and our results indicate the diagnostic significance of MLC.

We evaluated clinical efficiency of MLC as markers of myocardial ischemia using enzyme-linked immunosorbent assay (ELISA).

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MATERIALS AND METHODS

The study was carried out in 56 patients operated on for cardiac valve disease, including that combined with coronary disease, at A. N. Bakulev Center in 2006-2007. The operations were carried out under conditions of hypothermic artificial circulation (AC). Myocardial protection was realized by pharmacological cold cardioplegia with Custodiol solution [5]. The patients were divided into 3 groups according to postoperative parameters. Group 1 (n=34) consisted of patients with stable postoperative hemodynamics. No intraoperative myocardial injuries were observed in this group, or these injuries were minor and did not manifest during the postoperative period. In group 2 (n=15), coronary bloodflow recovery was followed by myocardium stunning, manifesting in diastolic dysfunction of the myocardium and arrhythmia. Intraoperative injuries to the myocardium were minor in this group, changes in the myocardium were reversible. In group 3 (n=7), the immediate postoperative period was complicated by severe heart failure. Echocardiogram and ECG showed changes characteristic of myocardial infarction.

Clinical characteristics of the patients are presented in Tables 1 and 2.

The mean duration of aorta clamping in group 1 was 69.1±6.6 min, in group 2 116.8±13.3 min, in group 3 157.0±15.3 min; duration of AC was 131±32, 158±28, and 220±81 min, respectively.

Normal composition of myocardial MLC was studied in human heart unfit for transplantation because of immunological incompatibility. The LC were isolated from left ventricular myosin as described previously [7].

The severity of intraoperative injury to the myocardium before AC (initial data) and after resumption of coronary bloodflow was evaluated by the concentrations of autoantibodies to myocardial MLC and troponin-B in venous blood 3, 6, 9, 12, 18, 24, and 36 h after the intervention. Autoantibodies to myocardial MLC were measured by ELISA [2].

RESULTS

Before surgery, the concentrations of autoantibodies to MLC in groups 1, 2, and 3 were 1.388±0.044, 1.415±0.083, and 1.561±0.175 μg/ml, respectively (Table 3). The concentration of autoantibodies to MLC started to increase by the 3rd hour after resumption of coronary bloodflow in all three groups, and increased significantly (by on average

TABLE 1. Clinical Preoperative Characteristics of Patients

Parameter	Group 1	Group 2	Group 3	Total
Total number of patients	34 (60.7%)	15 (26.8%)	7 (12.5%)	56 (100%)
Mean age	48.0±5.2	47.8±4.3	45.5±9.5	47.7±3.4
Sex (m/f)	14/20	8/7	6/1	28/28
Coronary disease	4 (11.8%)	3 (20%)	3 (42.9%)	10 (17.9%)
Essential hypertension	8 (23.5%)	4 (26.7%)	3 (42.9%)	15 (26.8%)
Repeated surgery	4 (11.8%)	7 (46.7%)	—	11 (19.6%)
2A circulatory insufficiency	27 (79.4%)	13 (86.7%)	6 (85.7%)	46 (82.1%)
2B circulatory insufficiency	4 (11.8%)	1 (6.7%)	1 (14.3%)	6 (10.7%)
Functional class 3	18 (52.9%)	8 (53.3%)	4 (57.1%)	30 (53.6%)
Functional class 4	7 (20.6%)	5 (33.3%)	3 (42.9%)	15 (26.8%)
Atrial fibrillation	15 (44.1%)	8 (53.3%)	5 (71.4%)	28 (50%)

TABLE 2. Etiology of Cardiac Valvular Diseases

Diagnosis	Group 1	Group 2	Group 3	Total
Congenital heart disease: 2-leaflet aortic valve	6 (17.6%)	1 (6.7%)	2 (28.6%)	9 (16.1%)
Rheumatism (inactive period)	14 (41.2%)	10 (66.7%)	5 (71.4%)	29 (51.8%)
Infective endocarditis	7 (20.6%)	4 (26.7%)	1 (14.3%)	12 (21.4%)
Active infective endocarditis	6 (17.6%)	3 (20%)	1 (14.3%)	10 (17.9%)
Myxomatosis	1 (2.9%)	—	—	1 (1.8%)
Rupture of chordae	4 (11.8%)	1 (6.7%)	—	5 (8.9%)

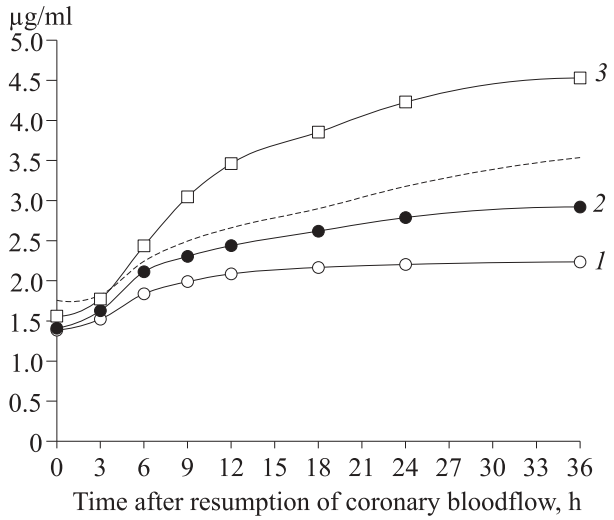

Fig. 1. Blood concentration of autoantibodies to MLC in patients of group 1 (*1*), 2 (*2*), and 3 (*3*). Interrupted line: threshold values for irreversible changes in the myocardium.

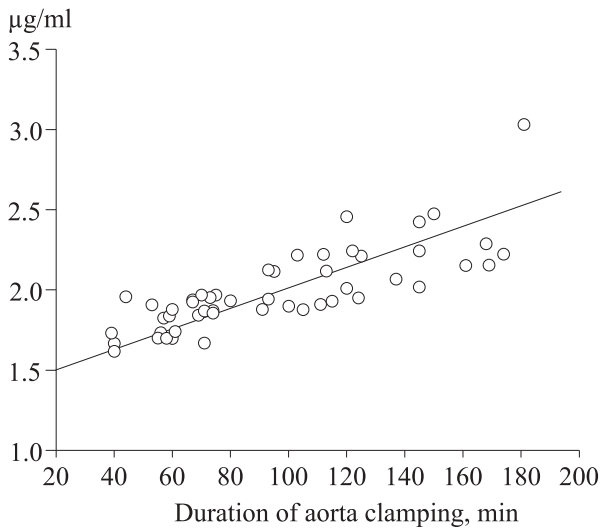

Fig. 2. Correlation between the duration of aorta clamping and blood concentration of autoantibodies to MLC 6 h after resumption of coronary bloodflow.

150% of the initial value) after 6 h. In group 3, the mean concentrations of autoantibodies to MLC were significantly higher than in other groups and in group 2 were higher than in group 1. In group 3, high concentrations of troponin B were detected, which however peaked only 18 h after resumption of coronary bloodflow and then decreased.

The treshold connections of autoantibodies to MLC were determined from the results of group 2. Higher values attested to intraoperative injury to cardiomyocytes leading to irreversible changes in the myocardium (Fig. 1).

The duration of aorta clamping is the main factor determining the severity of ischemic and reperfusion injury to the myocardium. A relationship between the duration of aorta clamping and changes in the concentrations of autoantibodies to MLC after resumption of coronary bloodflow was observed. In order to describe the relationship between these parameters, we evaluated the degree of their proportionality to each other using Pearson's linear correlation test (Fig. 2). It was found that the longer was aorta clamping, the higher was blood concentration of autoantibodies to MLC (r=0.774; p<0.001). Intraoperative myocardial ischemia results in metabolic and then ultrastructural disorders, eventuating in impairment of membrane permeability and integrity. This leads to the release of MLC from damaged cells, and subsequent reoxygenation just augments cardiomyocyte injury.

Autoantibodies to MLC were detected before surgery; their concentration during this period varied from 1.062 to 1.971 μg/ml (standard deviation 0.0167 μg/ml). The factors essential for the blood concentration of autoantibodies to MLC were identified by multivariant regression analysis. Thorough

TABLE 3. Blood Concentrations of Autoantibodies to MLC

Time of measurements	Group 1	Group 2	Group 3
Before surgery	1.388±0.044	1.415±0.083	1.561±0.175
After coronary bloodflow recovery			
3 h	1.528±0.041	1.619±0.050	1.775±0.134
6 h	1.839±0.036	2.084±0.077	2.436±0.215
9 h	1.988±0.037	2.28±0.07	3.047±0.512
12 h	2.088±0.037	2.413±0.068	3.461±0.536
18 h	2.168±0.040	2.585±0.093	3.854±0.707
24 h	2.204±0.041	2.746±0.137	4.230±0.663
36 h	2.235±0.044	2.873±0.179	4.530±0.811

Note. Significant differences between groups 1 and 3 before surgery: p<0.05. Significant differences between all groups after surgery: p<0.05 after 3 h, p<0.01 during other periods.

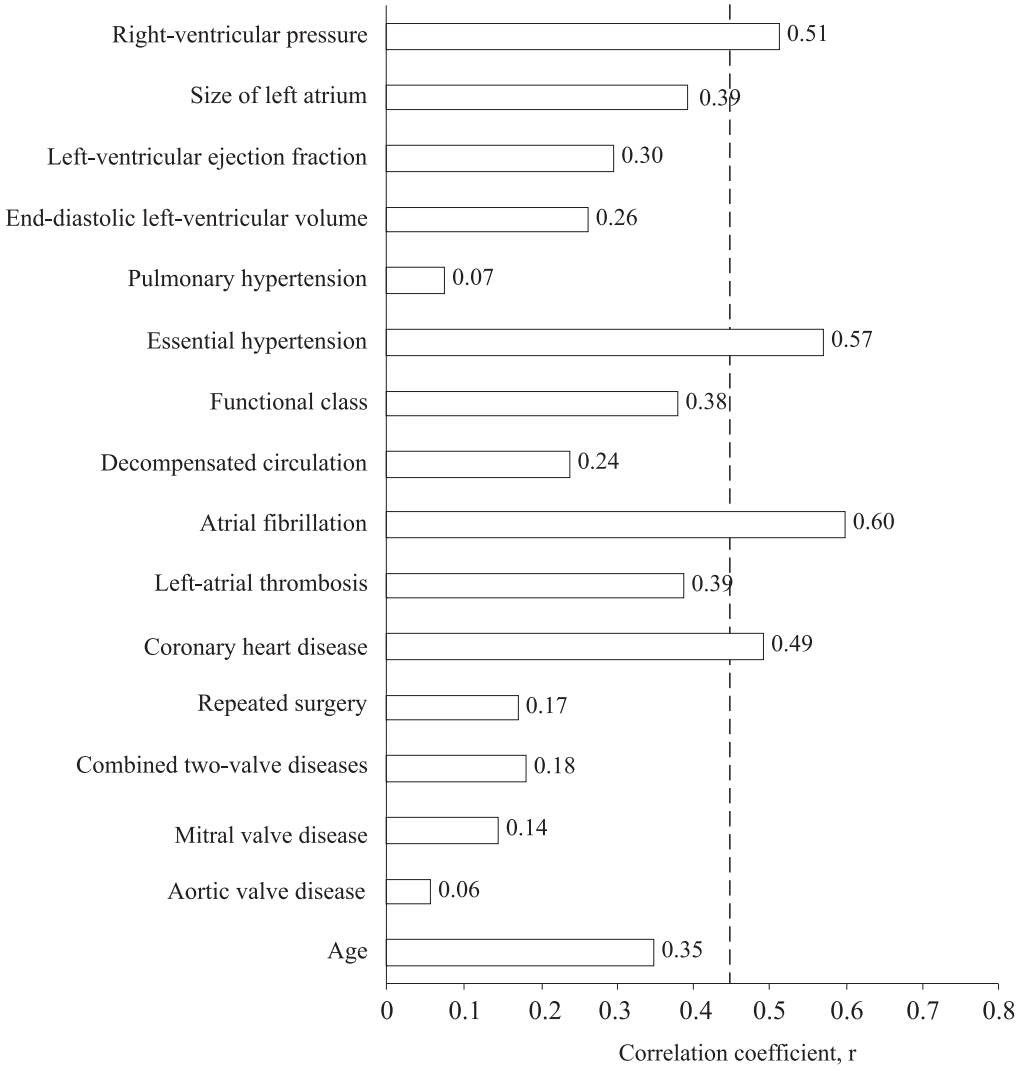

Fig. 3. Risk factors for high concentration of autoantibodies to MLC in the blood. Significant risk factors cross the vertical dashed line.

analysis of case histories and preoperative clinical hemodynamic status was carried out and the parameters presumably essential for the blood concentration of autoantibodies to MLC were determined. The regression equation derived in this analysis was evaluated by Pearson's correlation test. The effect of individual factors in the resultant regression equation was significant at r=0.423 and p=0.05. Of 16 independent factors analyzed, blood concentration of autoantibodies to MLC was reliably influenced by right-ventricular pressure, essential hypertension, atrial fibrillation, and coronary disease (Fig. 3).

Hence, blood concentration of autoantibodies to MLC increases virtually in all cardiosurgical patients during the postischemic period and depends strongly on the time of aorta clamping: the longer was the aorta clamping, the higher was the concentration of autoantibodies and coefficient of correlation (r=0.774; p<0.001) between these parameters. The maximum concentrations of autoantibodies to MLC were determined. High specificity, early appearance, and long presence of autoantibodies to MLC in the blood were shown, as well as their sufficiently high concentrations for the detection in the blood, which indicates high efficiency of this marker. Early detection of autoantibodies to MLC in the blood of patients before surgery helps to evaluate the severity of myocardial injury. Measurement of the concentration of autoantibodies to myocardial MLC by ELISA can become an effective method for evaluation of the adequacy of myocardial protection in heart surgery.

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