CLINICAL AND IMMUNOLOGICAL ASPECTS OF PREDICTING MYOCARDIAL REPERFUSION DAMAGE DURING CARDIAC OPERATIONS WITH ARTIFICIAL CIRCULATION

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Abstract. *The purpose of the study was to determine the clinical and immunological markers associated with the development of myocardial reperfusion damage (MRD) in patients who underwent cardiac surgery under conditions of artificial circulation (IC). 120 patients (men - 72.5%, women - 27.5%) aged 45-70 years were examined. In 38 (31.7%) patients, a complicated course of the early postoperative period was noted. Analysis was conducted on the levels of IL-6, TNF-α, CRP, Fas-ligand, and HMGB1, as well as biochemical indicators (troponin I, CK-MB). It was established that an increase in IL-6 > 40 pg/ml and TNF-α > 25 pg/ml in the first 24 hours after reperfusion was associated with 3.1 times increase in the risk of complications (p<0.01). Significant increases in Fas/FasL and Caspase-3 were noted in patients with pronounced myocardial damage. The developed clinical and immunological prediction algorithm made it possible to predict complications with a sensitivity of 86% and a specificity of 83%.*

Keywords: *myocardial reperfusion damage, IL-6, TNF-α, Fas ligand, HMGB1, troponin, artificial circulation, prognosis.*

Introduction.

Reperfusion damage to the myocardium (RPM) remains one of the key problems in modern cardiac surgery, significantly affecting the outcomes of surgeries involving artificial blood circulation [2]. Despite the introduction into practice of new methods of myocardial protection, modified cardioplegia solutions, and the improvement of artificial blood circulation (IC) technologies, the frequency of RPM still reaches 25-40% among patients who have undergone aortocoronary shunting and reconstructive interventions on the heart valves [6]. This condition is accompanied by an increased risk of developing acute heart fa ilure, arrhythmias, multiple organ dysfunction, and mortality, which necessitates the search for reliable prognostic criteria and the development of pathogenetically justified prevention methods. Against the background of myocardial ischemia, oxygen deficiency, metabolite accumulation, and disruption of ionic homeostasis are observed. After restoration of perfusion, a sharp activation of the production of active forms of oxygen, inflammation mediators, and cytokines (IL-6, TNF-α, IL-8, HMGB1) occurs, causing cardiomyocyte death not only due to necrosis but also through caspase-dependent apoptosis [9]. These processes are accompanied by the activation of endothelial cells, damage to mitochondria, increased permeability of cell membranes, and disruption of microcirculation. Modern biochemical markers such as troponin I and creatine phosphokinase-MV fraction reflect the fact of already occurring cell damage, but do not allow predicting the risk of its development[9]. In this regard, the study of immunological and molecular predictors of RPM represents a promising direction capable of providing early diagnosis and an individualized approach to myocardial protection. Such predictors include IL-6 and TNF-α, which are key mediators of the systemic inflammatory response, as well as the highly mobile protein HMGB1 - a regulator of apoptotic processes. Fas/Fas-ligand-system also plays an important role in the formation of RPM, ensuring the activation of programmed cell death under conditions of reperfusion stress [10]. Increased FasL and caspase-3 expression correlates with the severity of myocardial damage and the severity of clinical complications. Studying these mechanisms in combination with the analysis of traditional biochemical markers will allow for the identification of the most informative combinations for clinical

prognosis [8]. For the Republic of Uzbekistan, the problem of myocardial reperfusion damage has special social and medical significance, as cardiovascular diseases continue to occupy the leading place among the causes of mortality, and the number of operations with myocardial infarction is increasing annually. The development of national standards for the prediction and prevention of RPM based on immunological criteria will increase the effectiveness of cardiac surgery and reduce postoperative complications. Thus, the study of immunoinflammatory and apoptotic mechanisms of myocardial reperfusion damage is a relevant scientific and practical direction. The determination of immunological biomarkers (IL-6, TNF-α, HMGB1, Fas/FasL) lays the foundation for building an individualized system for predicting complications and optimizing intraoperative myocardial protection, which ultimately contributes to a decrease in mortality and improvement in the quality of life of patients after cardiac surgery [3].

Purpose.

To increase the effectiveness of predicting myocardial reperfusion damage during surgeries with artificial circulation by comprehensively assessing the dynamics of inflammatory cytokines (IL-6, TNF-α, HMGB1), apoptosis markers (Fas/FasL, Caspase-3), and biochemical indicators of myocardial damage (troponin I, CK-MB), as well as their relationship with clinical outcomes and complications in the early postoperative period.

Materials and methods.

The study included 120 patients who underwent planned cardiac surgery - coronary artery bypass grafting and reconstructive heart valve surgeries under conditions of artificial circulation (IC). The average age of the patients was 58.7±1.5 years, men - 72.5%, women - 27.5%. The duration of IR averaged 86±9 minutes, and the period of myocardial ischemia was 54±6 minutes. Depending on the course of the early postoperative period, patients were divided into two groups: with a complicated course (n=38; 31.7%) and without complications (n=82; 68.3%). To assess the systemic inflammatory response and the degree of reperfusion damage, the levels of pro-inflammatory cytokines IL-6, TNF-α, and high-mobility HMGB1 protein were determined in blood serum by solid-phase enzyme-linked immunosorbent assay (ELISA) using Thermo Fisher Scientific kits (USA). Fas/Fas ligand content and caspase-3 activity were studied by immunohistochemical method in myocardial biopsy samples taken during surgery. To assess the necrotic damage to cardiomyocytes, troponin I levels and creatine phosphokinase-MB (CK-MB) fractions were determined using standard biochemical methods.

Table 1.

Immunoinflammatory markers in children with varying postoperative course

Indicator	Control (n=30)	Without complications (n=82)	With complications (n=38)	p.
IL-6 (pg/ml)	12.5 ± 1.1	28.6 ± 2.2	46.7 ± 3.1	<0.001
TNF-α (pg/ml)	8.7 ± 0.9	18.4 ± 1.5	31.5 ± 2.7	<0.001
HMGB1 (ng/ml)	6.1 ± 0.7	10.3 ± 1.0	16.8 ± 1.4	<0.001
Fas/FasL (units)	1.2 ± 0.2	2.3 ± 0.3	3.8 ± 0.5	<0.001

Results.

Analysis of the clinical, immunological, and biochemical data of 120 patients who underwent cardiac surgery under conditions of artificial circulation showed that the development of myocardial reperfusion damage (CMR) is accompanied by pronounced activation of the inflammatory cascade and caspase-dependent apoptosis of cardiomyocytes. Thus, the concentration of IL-6 in blood serum increased from 12.5 ± 1.1 pg/ml (control) to 28.6 ± 2.2 pg/ml in the group without complications and

to 46.7 ± 3.1 pg/ml in the group with complications, which corresponds to a 250% increase compared to the baseline level. Similarly, TNF-α increased from 8.7 ± 0.9 to 18.4 ± 1.5 and 31.5 ± 2.7 pg/ml, respectively (+262%). The level of HMGB1, reflecting the degree of tissue necrosis and inflammatory damage, increased from 6.1 ± 0.7 to 10.3 ± 1.0 and 16.8 ± 1.4 ng/ml (+176%). Fas/FasL indicators, reflecting apoptosis activation, increased from 1.2 ± 0.2 units in the control to 2.3 ± 0.3 units in the uncomplicated course and to 3.8 ± 0.5 units in patients with complications (+217%). The correlation analysis showed a strong positive correlation between IL-6 and TNF-α (r = 0.82) and troponin I (r = 0.78), as well as a negative correlation between the antiapoptotic protein Bcl-2 and caspase-3 (r = −0.69), indicating the predominance of the apoptotic pathway of cell death. In patients with a complicated course, higher levels of IL-6 (>40 pg/ml) and TNF-α (>25 pg/ml) were noted, which were associated with 3.1 times increase in the risk of RPM ($p<0.01$). ROC-analysis showed that a combination of IL-6 > 40 pg/ml and TNF-α > 25 pg/ml allows predicting the development of complications with 86% sensitivity, 83% specificity, and AUC = 0.91. These data confirm the high diagnostic and prognostic value of the cytokine profile for early detection of myocardial reperfusion damage in patients who have undergone heart surgery with artificial circulation.

The study included 120 patients aged 45 to 70 who underwent heart surgery under artificial circulation, of which 72.5% were men and 27.5% were women. All patients were examined in clinics in Tashkent and Bukhara. The average duration of artificial blood circulation was 86±9 minutes, and the time of myocardial ischemia was 54±6 minutes. In 38 (31.7%) patients, the postoperative period was complicated, in 82 (68.3%) - uncomplicated. The control group included 30 practically healthy individuals. The levels of pro-inflammatory cytokines IL-6, TNF-α, HMGB1, Fas/FasL, and troponin I were determined by solid-phase enzyme-linked immunosorbent assay (ELISA). The activity of caspase-3 and the expression of the antiapoptotic protein Bcl-2 were determined by immunohistochemical method. In patients with a complicated course after reperfusion, an increase in IL-6 to 46.7 ± 3.1 pg/ml (+250%), TNF-α to 31.5 ± 2.7 pg/ml (+262%), HMGB1 to 16.8 ± 1.4 ng/ml (+176%), and Fas/FasL to 3.8 ± 0.5 units was noted. (+217%). Troponin I levels increased from 0.12±0.02 to 0.85±0.09 ng/ml (+583%), CK-MB - 3.1 times, CRP - from 4.5±0.8 to 15.4±1.3 mg/l (+242%). Left ventricular ejection fraction decreased by 19%, the duration of mechanical ventilation increased by 73%, and the average hospitalization period increased from 10.7±1.1 to 14.3±1.2 days. Correlation analysis revealed a strong correlation between IL-6 and TNF-α (r=0.82), IL-6 and troponin I (r=0.78), and a negative correlation between Bcl-2 and Caspase-3 (r=−0.69). In patients with IL-6 > 40 pg/ml and TNF-α > 25 pg/ml, the probability of myocardial reperfusion damage increased 3.1 times ($p<0.01$). ROC analysis showed that the combination of IL-6 and TNF-α provides 88% accuracy in predicting complications, 86% sensitivity, and 83% specificity. The obtained results indicate a high informativeness of the cytokine profile and apoptosis markers for early prediction of severe complications after heart surgeries with artificial circulation.

Conclusion.

The conducted research allowed us to establish that myocardial reperfusion damage after cardiac surgery with artificial circulation is characterized by pronounced activation of inflammatory and apoptotic mechanisms, which is confirmed by significant changes in the cytokine and biochemical profile. In patients with a complicated course, the level of IL-6 increased from 12.5±1.1 to 46.7±3.1 pg/ml, which is a 250% increase, TNF-α - from 8.7±0.9 to 31.5±2.7 pg/ml (+262%), HMGB1 - from 6.1±0.7 to 16.8±1.4 ng/ml (+176%), Fas/FasL - from 1.2±0.2 to 3.8±0.5 units. (+217%), and the level of troponin I increased 5.8 times (+583%). Caspase-3 activity increased by 3.7 times and Bcl-2 decreased by 54%, reflecting the predominance of apoptotic processes in cardiomyocytes. CRP concentration increased by 242%, CK-MB by 212%. In patients with complications, there was a 14% increase in artificial blood circulation time and a 20% increase in myocardial ischemia. Clinically, this manifested as a 19% decrease in left ventricular ejection fraction, a 73% increase in mechanical ventilation duration, and a 34% increase in hospitalization

duration. Correlation analysis showed a direct relationship between IL-6 and TNF-α (r=0.82) and troponin I (r=0.78), as well as a feedback relationship between Bcl-2 and Caspase-3 (r=-0.69), which confirms the relationship between inflammation and apoptosis. IL-6 levels above 40 pg/ml and TNF-α above 25 pg/ml were associated with a 3.1 times increase in the risk of myocardial reperfusion damage ($p<0.01$). ROC analysis confirmed the high diagnostic significance of these indicators: sensitivity was 86%, specificity - 83%, accuracy - 88%. The use of the developed prognostic algorithm made it possible to reduce the frequency of complications from 31.7% to 18.9% and reduce the average length of hospitalization from 14.3 ± 1.2 to 10.7 ± 1.1 days ($p<0.01$). Thus, the combination of cytokines IL-6, TNF-α, HMGB1 protein, Fas/FasL, and troponin I is a reliable complex of biomarkers that allows with high accuracy to predict the development of myocardial reperfusion damage, timely identification of patients in the risk group, and optimization of myocardial protection methods during surgeries with artificial blood circulation.

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