ASSESSMENT OF THE DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF THE PARAMETERS OF IMMUNOPHENOTYPING OF BLOOD LEUKOCYTES IN ACUTE MYOCARDIAL INFARCTION

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Abstract:

The diagnostic and prognostic significance of immune status indicators obtained by laser flow cytometry in acute myocardial infarction, depending on the severity of the pathological process, has been analyzed with the help of systemic multivariate analysis using mathematical modeling.

Key words:

Myocardial infarction, pathological process, immune status, laser flow cytofluorometry, systemic multivariate analysis.

Myocardial infarction remains one of the leading diseases in terms of mortality and prevalence among the population of economically developed countries. The amount of information about the features of the clinic, pathogenesis, the possibility of early diagnosis of myocardial infarction has increased significantly, however, one of the important tasks remains the development of a set of clinical and laboratory methods that make it possible to timely assess the nature of the course of the disease, predict complications in its development. At the same time, the identification of a group of patients at increased risk became especially important after a real opportunity to improve their prognosis appeared [1, 2]. The rapid development of immunology in recent years has significantly changed the understanding of the processes of formation of the body's resistance, made it possible to reconsider in many respects the participation of these processes in the development of myocardial infarction. Understanding the multicomponent nature of the general biological system of the body's defense required an assessment of the interrelationships of its individual components in myocardial infarction and revealed a largely insufficiently studied and inadequate interpretation [3, 4, 5]. At the same time, a deeper study of the pathogenetic mechanisms of the onset and healing of myocardial infarction, and even more the role of the immune system in the implementation of this pathological process, will make it possible to develop new approaches to its diagnosis and treatment. The development and active implementation of flow cytofluorometry methods in the work of immunological laboratories made it possible to significantly increase the sensitivity and specificity of immunophenotyping, and the simultaneous use of two or more labels made it possible to more correctly identify subpopulations of lymphocytes, as well as identify minor populations and determine the stages of cell activation of specific populations, the intensity of expression of differentiation antigens cells [6].

The standard set of methods for assessing the immune status using laser flow cytofluorometry includes the determination of the content of the main populations and subpopulations of lymphocytes (mature T cells - CD3 +, T-helpers - CD3 + CD4 +, T-cytotoxic - CD3 + CD8 +, B-lymphocytes - CD3− CD19 + and natural killers - CD3− CD16 + CD56 +). At the same time, according to the WHO recommendations, monoclonal antibodies to CD45 and CD14 antigens are used for the correct isolation of the lymphocytic gate. As a result of expensive studies, the immune status is assessed by 8 parameters (16 taking into account absolute values). However, the instrumentation and software of modern laser flow cytometers allows one to obtain a much larger number of parameters, which, with appropriate mathematical processing, can have diagnostic and prognostic value in many pathological conditions, including myocardial infarction.

The aim of this study was to develop a complex of immunological methods based on a systemic multivariate analysis that characterize the functional state of the immune system in acute myocardial infarction and have diagnostic and prognostic value in this disease.

At the first stage of the study, we assessed the possibility of using the basic and additional quantitative characteristics obtained by immunophenotyping of blood leukocytes as part of assessing the immune status in patients with acute myocardial infarction of varying severity. First of all, this concerned CD14 + and CD45 + antigens, which are usually used only for the isolation of the lymphocyte gate. Analysis of the distribution of leukocytes by the expression of these antigens, along with taking into account the parameters of light scattering, also makes it possible to fairly cleanly isolate the gates of monocytes and granulocytes, therefore, to further evaluate their phenotype, since a number of antigens from the panel used are also presented on these cells (resting or activated). CD45 + antigen is panleukocyte, and CD14 + is present mainly on monocytes and to a lesser extent on blood granulocytes. In this regard, there was no point in determining the number of CD14 + and CD45 + leukocytes. Evaluation of changes in the fluorescence intensity of cells labeled with the corresponding antibodies was carried out, which showed that the luminescence characteristics of leukocytes expressing CD45 + antigen, regardless of the fluorochrome used, are stable in any combination of anti-CD45 with other antibodies of the panel used. At the same time, analysis of histograms revealed a different pattern of distribution of labeled CD45 + leukocytes in terms of fluorescence intensity in healthy donors and in patients with acute myocardial infarction, depending on the timing and severity of the disease. Thus, in healthy donors, the distribution of CD45 + leukocytes in terms of fluorescence intensity corresponded to normal, which made it possible to further use the statistical characteristics of the histogram (arithmetic mean, standard deviation, coefficient of variation and median) for comparing groups using parametric statistics and further systemic multivariate analysis.

In patients with acute myocardial infarction, simultaneously with a tendency to increase the total fluorescence intensity of labeled CD45 + leukocytes, a group of cells with less intense fluorescence appeared among lymphocytes, and among monocytes and granulocytes - with a brighter fluorescence. As a result, the distribution of cells in terms of luminescence intensity ceased to be normal, and for further mathematical processing of the results it was necessary to select the second peak on the histogram. The nature of the fluorescence intensity of labeled CD45 + leukocytes changed depending on the time elapsed from the onset of myocardial infarction and on the severity of the disease.

As for the CD14 antigen, in blood samples from healthy donors, the pattern of distribution of labeled monocytes and granulocytes in terms of fluorescence intensity corresponded to normal. In acute myocardial infarction, the normal distribution of cells in terms of luminescence intensity (which for CD14 + monocytes was statistically significantly higher than that of healthy donors) remained. The fluorescence parameters of CD14 + monocytes at different periods of observation in acute myocardial infarction with Q wave and without Q wave were different. The fluorescence intensity of CD14 + granulocytes did not undergo statistically significant changes.

Thus, only as a result of the analysis of the expression of CD14 + and CD45 + antigens on lymphocytes, monocytes and granulocytes of the blood of patients with acute myocardial infarction, additional 11 parameters were obtained, which were subsequently used for systemic multivariate analysis.

The next stage of the study was the assessment of the expression of CD3 antigen by lymphocytes, which makes it possible to identify mature resting T cells. The relative content of CD3 + lymphocytes did not undergo statistically significant changes in the dynamics of acute myocardial infarction in comparison with those in healthy donors and depending on the severity of the heart muscle damage. On average, 68.5 ± 7.2% of CD3 + lymphocytes were detected in the blood. In the first three days after the onset of myocardial infarction, there was a decrease (to 0.95 ± 0.06 cells × 109 / l, while the average values in the group of healthy donors were 1.85 ± 0.22 cells × 109 / l) in the absolute number of CD3 + lymphocytes, which was associated with a characteristic decrease in the absolute content of the total pool of lymphocytes due to an increase in the proportion of neutrophilic granulocytes in the blood formula (up to 71.9 ± 4.1% versus and 53.8 ± 6.4% in healthy donors). The distribution of lymphocytes in terms of the fluorescence intensity of CD3 + lymphocytes did not meet the criteria for a normal distribution, therefore, the parameters of the luminescence intensity were not included in further mathematical processing.

Analysis of mathematical models of the functional state of the immune system revealed oscillatory changes in its activity in the dynamics of the development of acute infarction with a maximum deviation from the norm 3 days after the onset of foci of necrosis in the heart muscle (see figure). In acute myocardial

infarction with a Q wave, the integral indicators of the system, in contrast to myocardial infarction without a Q wave, did not return to the normative, but deviated towards the activation of the immune system.

Thus, the use of systemic multivariate analysis made it possible to objectively assess the nature and direction of changes in the immune system depending on the stage of development of myocardial infarction, expand the range of indicators analyzed in a standard assessment of the immune system, identify the most significant prognostic indicators and substantiate the possibility of a repeated wave of system activation after 21 days. after the onset of myocardial infarction with a Q wave.

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