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Diagnostic significance of apoptosis of peripheral blood lymphocytes in patients with chronic heart failure

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Abstract
The aim of our research has been the study of changes in the apoptosis of peripheral blood lymphocytes in patients with chronic heart failure depending on the stage of the disease.
Materials and Methods: The study involved 86 patients with hypertension complicated by heart failure. There was conducted a general clinical, ultrasound and immunological examination of patients. Determination of apoptosis-mediated factor CD95 (Fas, APO-1) expression on the surface of lymphocytes was performed using an indirect immunofluorescence method with monoclonal antibodies. The state of cellular immunity was assessed by the immunofluorescence method using monoclonal antibodies. The tumor necrosis factor alpha (TNFα) content in the patients blood was determined by the immunoassay method.
Results: In patients suffering from AH with CHF there was revealed of apoptosis activation of peripheral blood lymphocytes in 49 (76.56%) patients with CHF NYHA class III and in 21 (95.46%) patients with CHF NYHA class IV. The apoptosis activation of peripheral blood lymphocytes in CHF patients accompanied by the secondary immunodeficiency. A direct correlation between the increase of pro-inflammatory cytokine TNF-α content in the blood and the increase of expression level of CD95 (Fas, APO-1) on peripheral blood lymphocytes was established ($r=0.61$; $p<0.05$).
Conclusion: In patients with CHF the activation of peripheral blood lymphocytes apoptosis was identified, which increases with the CHF stage increase and accompanied by secondary immunodeficiency and increase of TNF-α content in the blood.

Keywords: Chronic heart failure, apoptosis of peripheral blood lymphocytes, secondary immunodeficiency, tumor necrosis factor alpha.

1. Introduction
The problem of chronic heart failure remains topical in modern medicine, and has both medical and social significance. The rate of chronic heart failure (CHF) in the structure of cardiovascular diseases is permanently increasing [1, 2]. Today, in spite of significant advances in the treatment of CHF, mortality remains high [3, 4].
Systemic-inflammatory processes, immune response involving pro-inflammatory cytokines, play an important role in the development and progression of CHF [5]. The role of cytokines – tumour-necrotizing factor alpha (TNF-α), interleukin-six (IL-6), and interleukin-eight (IL-8) – was proved. Special hopes to solve the problem of CHF, including the one of hypertensive origin, are associated with the subsequent work out of inflammatory concept of the development and progression of myocardial dysfunction, as well as with identification of the key factors of immunopathogenesis and development of imunomodulating, in particular anticytokine, therapy [6, 7, 8]. However, the role of apoptosis in peripheral blood lymphocytes in patients with CHF is poorly studied.
The aim of the work is to study the indicators of apoptosis of peripheral blood lymphocytes in patients with CHF, depending on the stage and changes in the immune system.

2. Materials and Methods of Investigation
There were examined 86 patients with arterial hypertension (AH) of the II-IIIrd stage of CHF, 45 men and 41 women, aged (65.7±5.9) years. Duration of the disease was (7.6±4.3) years. Among the patients there were 64 patients with CHF NYHA class III (I group) and 22 – with CHF NYHA class IV (II group). The control group consisted of 20 healthy volunteers.
A general clinical, immunological and ultrasonic examination of patients was performed. Determination of apoptosis-mediated factor CD95 (Fas, APO-1) expression on the surface of lymphocytes was performed using an indirect immunofluorescence method with monoclonal antibodies of Experimental Pathology, Oncology and Radiobiology Institute named after

R. Ye. Kavetsky NAS of Ukraine and "Sigma" (USA) according to the methods of firms-manufacturers. In order to detect apoptotic cells the staining with Hoechst 33342 colourant (Sigma, USA), and Annexin V-FITC (Clonteck, USA) was used. Evaluation of the results was performed using a fluorescence microscope Axiolab (Zeiss, Germany) with magnification of *900.

The immuno-phenotyping of subpopulations of T- and B-lymphocytes using monoclonal antibodies was performed. Lymphocytes were isolated from the peripheral blood according to the method of L.B. Heifets, V.F. Abalkin. State of cellular immunity was evaluated according to the content of serum subpopulations of CD3+-lymphocytes general, CD4+-lymphocytes-helpers (Th), CD8 +-lymphocytes-suppressors (Ts), IPO47 (HLA-DR) – activated T-lymphocytes, CD24 + - B-lymphocytes general, CD 150 (HPI) + - activated B-lymphocytes, CD56+ -natural killers and immunoregulatory index value of CD4+/CD8+ (Th/Ts).

Content of TNF-α was determined in blood serum with the help of immune-enzyme method (ELISA) using commercial kits of "Cytimmune" (USA).

Statistical processing of the results using the programme "Statistika-7.1" was performed.

3. The Received Results and their Discussion

Analysis of the study results allowed to reveal activation of apoptosis of peripheral blood lymphocytes in 49 (76.56%) patients of the Ist group and in 21 (95.46%) patients of the IInd group. Expression of Fas/APO-1 (CD95) on peripheral blood lymphocytes in the first group has increased to (12.63±1.04)% ($p<0.05$), in the second group – up to (20.64±1.72)% ($p<0.05$), compared to (3.65±0.29)% in the control group.

It was determined, that in hypertensive patients with CHF secondary immunodeficiency has developed, which was manifested by a decrease of the number of CD3+-T-lymphocytes, CD4+-T-lymphocytes, CD56+-natural killers, IPO47-activated T-lymphocytes in the blood with a decrease of immunoregulatory index CD4+/CD8 ($p<0.05$). Change of these parameters has increased with the increase of CHF stage (Table 1). Moderate reduction of CD3+-T-lymphocytes in the blood by 9.94% ($p<0.05$), CD4+-T-lymphocytes – by 10.27% ($p<0,05$), CD56+-natural killers – by 9.55% ($p<0.05$) and IPO47-activated T-lymphocytes – by 16.08% ($p<0.05$), accompanied by a decrease of the immunoregulatory index to 22.76% ($p<0. 05$) was revealed in patients of the Ist group. At the same time, in these patients, the number of CD24+-B-lymphocytes general has not changed significantly, and the number of CD150 (IPO3)+-B-lymphocytes activated has moderately increased – by 12.12% ($p<0.05$), along with the increase of CD8+-T-lymphocytes number, which have immune-regulating influence on the state of humoral immunity, – by 11.69% ($p<0.05$). There were determined changes which, in our opinion, indicate the development of an imbalance of cellular immunity in patients with CHF FC III NYHA.

In patients of the IInd group the dynamics of cellular immunity was more expressed. In particular, the number of CD3+-T-lymphocytes – by 15.24% ($p<0.05$), CD4+-T-lymphocytes – by 30.46% ($p<0.05$), CD56+-natural killers – by 21.58% ($p<0.05$) and IPO47-activated T-lymphocytes – by 21.04% ($p<0.05$) with the reduction of immunoregulatory index by 36.55% ($p<0.05$), has decreased more in blood. According to the received results of study, the pronounced secondary immunodeficiency develops in patients with NYHA FC IV. It was found that in patients of the IInd group in contrast to patients of the Ist group the number of CD24+-B-lymphocytes general has decreased by 16.56% ($p<0.05$); it may be due to the exhaustion of the immune system at this stage of the disease. However, the number of CD150 (IPO3)+-B-lymphocytes activated has increased by 16.29% ($p<0.05$), along with the increase of CD8+-T-lymphocytes number by 17.86% ($p<0.05$), indicating the deepening the imbalance of cellular immunity in patients with FC IV NYHA.

The inverse correlation links between the increase of apoptotic lymphocytes number and the decrease of CD-4-lymphocytes amount ($r=-0.59$; $p<0.05$) and the indicator of immunoregulatory index ($r=-0.51$; $p<0.05$) were established, that, in our opinion, indicates the role of lymphocytes' apoptosis activation in the development of the secondary immunodeficiency in patients with CHF.

The study has revealed an increase of TNF-α in 91% of patients, that grew with the increase of the disease stage. In particular, in patients of the Ist group the content of TNF-α in the blood was (32.65±2.29) pg/ml ($p<0.05$), of the IInd group – (54.71±3.86) pg/ml ($p<0.05$) compared to (20.31±1.05) pg/ml in healthy persons.

A direct correlation between the increase of pro-inflammatory cytokine TNF-α content in the blood and the increase of expression level of CD95 (Fas, APO-1) on peripheral blood lymphocytes was established ($r=0.61$; $p<0.05$).

4. Conclusion

In patients with CHF the activation of peripheral blood lymphocytes apoptosis was identified, which increases with the CHF stage increase. 2. The development of secondary immunodeficiency in patients with chronic heart failure, accompanied by increase of apoptosis of peripheral blood lymphocytes was revealed. 3. The relation between the increase of TNF-α in patients with chronic heart failure and activation of apoptosis of peripheral blood lymphocytes was determined.

Table 1: Comparative characteristic of cellular immunity parameters in patients with chronic heart failure depending on its stage (M±m)

Indexes	Control, n=10	NYHA class III n=64	NYHA class IV n=22
CD3+(%)	70.51±4.66	62.91±4.05*	55.27±3.60*•
CD4+(%)	45.76±2.80	37.72±2.83*	31.82±2.75*•
CD8+(%)	32.09±1.92	35.90±2.56*	37.81±1.84*
CD24+(%)	15.82±1.04	14.35±1.20*	13.09±1.26*
CD 95(%)	3.65±0.29	12.63±1.04	20.64±1.72
CD56+(%)	17.38±1.26	114.92±1.45*	13.50±1.21*•
CD150(%)	5.28±0.37	5.98±0.51*	6.125±0.60*
IPO47(%)	13.12±0.98	11.23±0.89*	10.29±1.01*
Th/Ts (CD4+/CD8+)	1.45±0.08	1.09±0.09*	0.89±0.08*•

Notes: *– probability of difference of the parameters from those in the control group, $p<0.05$; •– probability of difference of the parameters in CHF IIA group from those in CHF IIB group, $p<0.05$.

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