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Стаття надійшла 30.08.2024 р.

DOI 10.26724/2079-8334-2025-3-93-13-17
UDC 616.12-008.46:616.153:577.1:615.07

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CLINICAL SIGNIFICANCE OF NOVEL BIOMOLECULES SUCH AS GALECTIN-3, APOPTOSIS-INDUCING FACTOR, ENDOTHELIN, AND VEGF-A IN THE DIAGNOSIS OF CHRONIC HEART FAILURE

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The use of molecular biomarkers, alongside classical clinical and instrumental methods, is becoming increasingly relevant for the early diagnosis and prognosis of chronic heart failure. The study aimed to assess the diagnostic value of novel biomarkers (galectin-3, apoptosis-inducing factor, endothelin, and VEGF-A) by comparing their levels across various functional stages (I–II, III, IV) of chronic heart failure. The study involved 219 patients aged between 34 and 89 years who were diagnosed with CHF and examined and treated at the Clinical Medical Center between 2018 and 2024. The concentrations of the biomarkers galectin-3, AIF, endothelin, and VEGF-A in the blood serum were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. More pronounced changes in the levels of the studied biomarkers were observed in patients with NYHA class IV chronic heart failure. In this group, compared to patients in NYHA class I, the concentration of AIF increased by 80 % ($p<0.001$), endothelin-1 by 51 % ($p=0.037$), and galectin-3 by 70 % ($p<0.001$), all of which were statistically significant. In contrast, the concentration of VEGF-A showed a tendency to decrease ($p=0.251$). Compared to NYHA class II, a statistically significant 2.4 fold decrease in VEGF-A concentration ($p<0.001$) was observed in the NYHA class IV. The analyses revealed that during chronic heart failure, the levels of galectin-3, AIF, endothelin-1, and VEGF-A change significantly depending on the functional stage of the disease, with a marked increase observed as the disease progresses.

Key words: chronic heart failure, galectin-3, AIF, endothelin-1, VEGF-A.

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КЛІНІЧНЕ ЗНАЧЕННЯ НОВИХ БІОМОЛЕКУЛ, ТАКИХ ЯК ГАЛЕКТИН-3, АПОПТОЗ-ІНДУКУЮЧИЙ ФАКТОР, ЕНДОТЕЛІН І VEGF-A, У ДІАГНОСТИЦІ ХРОНІЧНОЇ СЕРЦЕВОЇ НЕДОСТАТНОСТІ

Застосування молекулярних біомаркерів поряд з класичними клінічними та інструментальними методами набуває все більшої актуальності для ранньої діагностики та прогнозу хронічної серцевої недостатності. Метою дослідження була оцінка діагностичної значущості нових біомаркерів (галектин-3, апоптоз-індукувальний фактор, ендотелін та VEGF-A) шляхом порівняння їх рівнів на різних функціональних стадіях хронічної серцевої недостатності (I-II, III, IV). До дослідження було включено 219 пацієнтів віком від 34 до 89 років, яким було поставлено діагноз хронічна серцева недостатність та які проходили обстеження та лікування у Клініко-медичному центрі у період з 2018 по 2024 рік. Концентрації біомаркерів галектину-3, апоптоз-індукувального фактору, ендотеліну та VEGF-A у сироватці крові визначалися методом імуноферментного аналізу (ELISA). Більше виражені зміни рівнів досліджуваних біомаркерів спостерігалися у пацієнтів із хронічною серцевою недостатністю IV функціонального класу за класифікацією NYHA. У цій групі порівняно з пацієнтами I класу концентрація апоптоз-індукувального фактору збільшилася на 80% ($p<0,001$), ендотеліну-1 – на 51% ($p=0,037$), галектину-3 – на 70% ($p<0,001$); всі зміни були статистично значущими. Навпаки, концентрація VEGF-A мала тенденцію до зниження ($p=0,251$). У пацієнтів із хронічною серцевою недостатністю IV функціонального класу за класифікацією NYHA, порівняно з пацієнтами II класу, спостерігалося статистично значуще 2,4-кратне зниження концентрації VEGF-A ($p<0,001$). Проведений аналіз показав, що при прогресуванні хронічної серцевої недостатності рівні галектину-3, апоптоз-індукувального фактору, ендотеліну-1 та VEGF-A змінюються залежно від функціонального класу захворювання, причому підвищення концентрацій більшості з них було більш вираженим на пізніх стадіях.

Ключові слова: хронічна серцева недостатність, галектин-3, апоптоз-індукувальний фактор, ендотелін-1, VEGF-A.

Chronic heart failure (CHF) remains a serious social and medical problem worldwide, accompanied by high rates of disability and mortality. Affecting over 56 million individuals globally, with a prevalence of 1 %–2 %, HF has emerged as a significant public health challenge [8]. The main contributing factors to the development of chronic heart failure include arterial hypertension,

atherosclerotic lesions of the coronary arteries, valvular heart diseases, cardiomyopathies, as well as atrial fibrillation and other cardiac arrhythmias [1]. It is characterized by impaired pump function of the heart due to the exhaustion of compensatory mechanisms of the myocardium, leading to insufficient delivery of oxygen and nutrients to tissues [10].

Alongside classical clinical and instrumental methods, the use of molecular biomarkers is becoming increasingly relevant for the early diagnosis and prognosis of CHF. Investigating various biomarkers involved in the pathogenesis of CHF may aid in the early detection and prognostication of the disease in clinical practice. In recent years, biomarkers such as galectin-3, apoptosis-inducing factor (AIF), endothelin-1, and vascular endothelial growth factor A (VEGF-A) have been identified as playing a significant role in the pathogenesis and clinical course of CHF [5, 11, 12, 15].

Galectin-3 plays a key role in the development of fibrosis in cardiac tissue, the enhancement of inflammatory responses, and myocardial remodeling. Its elevated levels are associated with disease severity and a poor prognosis in heart failure patients [12, 13]. Apoptosis-inducing factor (AIF) is a mitochondrial protein located in the membranes and is responsible for regulating cell apoptosis. In heart failure, mitochondrial dysfunction and oxidative stress cause AIF to translocate into the cytoplasm, where it induces the apoptotic process. This can lead to the destruction of cardiomyocytes, deterioration of cardiac function, and further progression of chronic heart failure [15].

Endothelin-1 (ET-1), as a potent vasoconstrictor, plays a significant role in the disruption of vascular tone, myocardial hypertrophy, and fibrosis processes; its elevated levels indicate the progression of the disease. ET-1 is primarily secreted by endothelial cells and vascular smooth muscle cells and functions through ET_A and ET_B receptors. It plays a key role in maintaining basal vascular tone and contributes to systemic and pulmonary arterial resistance. In addition to its potent vasoconstrictive properties, ET-1 also exhibits pro-inflammatory and mitogenic activity, promoting vascular smooth muscle proliferation, cardiac hypertrophy, and myocardial fibrosis [5].

VEGF-A stimulates angiogenesis in response to hypoxia in the heart and participates in vascular regeneration; however, its long-term elevation may have adverse effects [14].

Each of these biomarkers reflects different pathophysiological mechanisms of the disease, and their combined assessment may enable a more accurate diagnosis of CHF and the development of treatment strategies. The stage-dependent variations in biomarker levels suggest their potential as diagnostic indicators and as biomarkers for determining the stage of the disease.

The purpose of the study was to evaluate the comparative diagnostic significance of new biomarkers (galectin-3, AIF, endothelin, and VEGF-1) at different functional stages (I-II, III, IV) of chronic heart failure.

Materials and methods. The study analyzed the concentrations of galectin-3, AIF, endothelin, and VEGF-A biomarkers in the blood serum of 219 patients aged between 34 and 89 years, diagnosed with CHF and examined and treated at the Clinical Medical Center during the period from 2018 to 2024. Patients with CHF were divided into three groups based on the New York Heart Association (NYHA) functional classification. Among them, 123 patients were classified as having CHF of NYHA functional classes I–II (Group I), 84 patients as class III (Group II), and 12 patients as class IV NYHA (Group III).

The control group consisted of 51 practically healthy individuals aged between 20 and 77 years.

Venous blood samples collected from patients and healthy individuals were centrifuged at 4000 rpm, and the obtained serum was stored at –25°C until analysis. The concentration of galectin-3 in the blood serum was measured using the Human Galectin-3 Quantikine ELISA Kit (R&D Systems, USA); AIF using the Human AIF ELISA Kit (Elabscience, China); endothelin-1 using the Human Endothelin-1 ELISA Kit (Abcam, UK); and VEGF-A using the Human VEGF-A Quantikine ELISA Kit (R&D Systems, USA). All measurements were performed by the enzyme-linked immunosorbent assay (ELISA) method.

The obtained results were statistically analyzed by the Kruskal–Wallis and Mann–Whitney U tests in the SPSS 26.0 software. A p-value of less than 0.05 was considered statistically significant.

Results of the study and their discussion. In Group I, the concentration of AIF increased by 3.6 fold ($p<0.001$), endothelin-1 by 64 % ($p=0.005$), galectin-3 by 94 % ($p=0.012$), and VEGF-A by 4.4 fold ($p<0.001$) compared to the control group, all showing statistically significant increases. A similar trend was observed in Group II, where the concentration of AIF increased 5.0 fold ($p<0.001$), endothelin-1 by 2.1 fold ($p=0.003$), galectin-3 by 2.5 fold ($p=0.001$), and VEGF-A by 9.3 fold ($p<0.001$) compared to the control group, all showing statistically significant increases. In Group III, a significant increase in the concentration of biomarkers was also observed compared to the control group. Thus, AIF increased by 6.6 fold ($p<0.001$), endothelin-1 by 2.5 fold ($p<0.001$), galectin-3 by 3.3 fold ($p=0.001$), and VEGF-A by 3.9 fold ($p=0.047$).

According to statistical analysis, in NYHA class III (Group II) of CHF, the concentration of AIF increased by 39 % (p=0.031), endothelin-1 by 25.7 % (p=0.046), galectin-3 by 26.8 % (p=0.048), and VEGF-A by 2.1 fold (p=0.001) compared to NYHA classes I–II (Group I), all showing statistically significant increases (Table 1).

Table 1

Changes in the concentration of certain biomarkers in the blood of patients with CHF (M±m, min-max)

Biomarkers	Groups			
	Control (n=51)	CHF I–II (Group I) (n=123)	CHF III (Group II) (n=84)	CHF IV (Group III) (n=12)
AIF (ng/mL)	1.08± 0.06 (0.42–1.8)	3.89±0.18 (2.12–6.23) p<0.001	5.42±0.18 (2.43-8.87) p=0.001 p_1=0.032	7.03±0.51 (4.78-10.37) p<0.001 p_1<0.001 p_2=0.061
Endothelin-1 (pg/mL)	5.60 ±0.21 (3.1–8.0)	9.16±0.37 (4.31–13.31) p=0.005	11.51±0.23 (6.47-17.28) p=0.003 p_1=0.046	13.79±0.96 (9.49-20.16) p<0.001 p_1=0.037 p_2=0.073
Galectin-3 (ng/mL)	7.31±0.36 (3.5–11.9)	14.18±0.25 (8.32–19.54) p=0.012	17.94±0.29 (11.54-23.89) p=0.001 p_1=0.048	24.05±2.58 (14.09-48.65) p<0.001 p_1<0.001 p_2=0.026
VEGF-A (pg/mL)	35.3±1.16 (19.8–48.1)	155.29±3.24 (96–216) p<0.001	323.78±4.44 (258.6-394.8) p<0.001 p_1=0.001	137.55±11.07 (74.3-191.9) p=0.047 p_1=0.251 p_1<0.001

Note: p – compared to the control group; p_1 – compared to Group I; p_2 – comparison between Group III and Group II.

More pronounced changes in the levels of the studied biomarkers were observed in NYHA class IV of CHF. In this group, compared to NYHA class I (Group I), the concentration of AIF increased by 80 % (p<0.001), endothelin-1 by 51 % (p=0.037), and galectin-3 by 70 % (p<0.001), all showing statistically significant increases. In contrast, the concentration of VEGF-A tended to decrease (p=0.251). In this group, compared to Group II, an increase was observed in the concentrations of AIF by 30 % (p=0.061), endothelin-1 by 20 % (p=0.073), and galectin-3 by 34 % (p=0.023). However, in contrast, the concentration of VEGF-A showed a statistically significant decrease by 2.4 fold (p<0.001).

Thus, the levels of all biomarkers were significantly higher in patients with CHF compared to the control group. The analyses showed that during CHF, the levels of galectin-3, AIF, and endothelin-1 increase sharply depending on the functional stage of the disease. In particular, the elevations observed in VEGF-A and AIF levels at stage III may indicate progressive structural changes and endothelial dysfunction.

Galectin-3 plays a key role in the processes of fibrosis and inflammation in the pathophysiology of CHF. By stimulating fibroblast proliferation and collagen synthesis, galectin-3 may contribute to myocardial remodeling, ultimately leading to deterioration in cardiac function. Recent studies have shown that elevated serum levels of galectin-3 are associated with a worse prognosis in patients with CHF. A meta-analysis conducted in 2022, which analyzed data from 6,440 CHF patients, revealed that increased levels of galectin-3 are associated with a higher risk of cardiovascular mortality [2, 3].

However, some studies have reported that galectin-3 levels are not associated with specific cardiological parameters in patients with heart failure. For example, one study showed that there was no correlation between galectin-3 levels in myocardial biopsy samples and plasma galectin-3 levels, and no association with cardiac fibrosis. They demonstrated that galectin-3 is synthesized not only in cardiac tissue but also in other organs and tissues and that its plasma level may therefore depend on various factors [3]. Based on the results of our study, we believe that galectin-3 may be a valuable prognostic biomarker in patients with CHF. An increase in its level may provide additional information for assessing both short- and long-term prognosis. Future larger and more specific studies will help to more accurately determine the clinical utility of galectin-3.

We found that the level of AIF was significantly increased in patients with CHF, particularly those in functional class III–IV, compared to the control group. This indicates that AIF may accelerate apoptotic processes in the progressive stages of CHF [15].

In CHF, elevated levels of ET-1 play a significant role in the development of endothelial dysfunction, myocardial hypertrophy, and fibrosis. The increase in ET-1 is associated with enhanced cardiac load, oxidative stress, and inflammatory processes. Clinical studies have shown that elevated ET-1 levels in patients with CHF are linked to a poor prognosis, while a decrease in its level may indicate treatment efficacy [4].

In our study, the level of ET-1 was found to be significantly increased in patients with CHF, especially those in functional class III–IV, compared to the control group. This suggests that ET-1 may play an important role in the pathophysiology and prognosis of CHF.

VEGF is a key regulator of the angiogenesis process and plays a crucial role in endothelial cell proliferation, migration, and vessel formation. In vitro, VEGF stimulates the division of endothelial cells of arteries, veins, and lymphatic vessels, prevents apoptosis, induces the expression of anti-apoptotic proteins Bcl2 and A1 in endothelial cells, and also promotes the recruitment of monocytes and the formation of macrophage colonies in certain vascular networks. VEGF plays an important role in regulating endothelial homeostasis observed during CHF. Recent studies have shown that it not only induces the proliferation of endothelial cells but is also involved in their repair [6, 7].

Ischemia-induced angiogenesis is a process in which vascular endothelial growth factor (VEGF) plays a crucial role. To conduct research on VEGF's involvement in cardiovascular diseases, it is essential to understand its role in both physiological and pathological processes in the heart [7].

The increase in VEGF-A levels during CHF may reflect compensatory angiogenesis mechanisms in response to hypoxia and impaired perfusion in cardiac tissue [3]. Changes in its levels may vary depending on the stages of CHF, which are mainly associated with the phases of compensation and decompensation. In functional classes I–II (NYHA), at the early stage, VEGF levels are lower compared to class III (NYHA). At this stage, oxygen deficiency begins to develop in the myocardium, which in turn activates the compensatory angiogenesis process. The elevation of VEGF is a physiological response aimed at improving oxygen supply to the tissues.

In our study, the level of VEGF-A was found to be significantly elevated in patients with CHF, particularly in those classified as NYHA functional class III, compared to the control group. This suggests that VEGF-A may serve as an indicator of the angiogenic response in the progressive stages of CHF. Some studies have shown that despite elevated VEGF levels, its functional effect may be diminished ("dysfunctional angiogenesis"). In patients classified as NYHA class IV, a significant decrease in VEGF-A concentration has been observed compared to those in class III. Prolonged hypoxia and endothelial dysfunction may lead to the exhaustion of the angiogenic response. Structural changes in the myocardium, such as fibrosis and apoptotic processes, can hinder VEGF expression. Our findings are consistent with the scientific evidence reported by other researchers. Kobusiak-Prokopowicz M. and colleagues also demonstrated in their study that VEGF levels increased significantly in stage III compared to stages I–II [8]. Pannella M. and co-authors, in their *in vitro* studies, confirmed that serum samples obtained from patients with CHF induced angiogenesis and transformed the Notch signaling pathway in HUVECs (Human Umbilical Vein Endothelial Cells) [11].

In addition, increased VEGF-A levels are also associated with enhanced vascular permeability and tissue edema, which may negatively contribute to the progression of the disease. The rise in VEGF-A levels across the functional stages of heart failure is closely related to the activation of compensatory mechanisms and the advancement of the disease.

Conclusion

Thus, the obtained results demonstrated that biomolecules such as galectin-3, AIF, endothelin-1, and VEGF-A undergo statistically significant changes at different stages of chronic heart failure (CHF). Each of these biomarkers reflects specific pathophysiological mechanisms of CHF, and their concentration levels are closely associated with the clinical stage of the disease. The concentrations of galectin-3, AIF, and endothelin-1 were significantly elevated in the blood of patients with CHF, with the most pronounced increases observed in those with NYHA class IV heart failure. In contrast to the upward trend observed in NYHA classes I–III, the concentration of VEGF-A decreased in NYHA class IV, which may indicate that prolonged hypoxia and endothelial dysfunction contribute to the exhaustion of the angiogenic response. These findings suggest that as CHF progresses, biomarker levels change in a differential manner depending on disease severity. The combined assessment of these biomarkers may

improve early diagnosis and allow for individualized prognostic evaluation according to the functional stage of chronic heart failure. Furthermore, these biomarkers can be used in clinical practice for both prognostic and diagnostic purposes.

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Стаття надійшла 24.06.2024 р.