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# THE DIAGNOSTIC AND PROGNOSTIC VALUE OF BIOMARKERS IN WOMEN WITH CORONARY ARTERY DISEASE AND OSTEOPOROSIS

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## Abstract

**Introduction.** Since the onset of menopause, the incidence of cardiovascular pathology and changes in bone mineral density (BMD) in the female population significantly increase.
**The objective of the study.** To determine the diagnostic and prognostic value of bone and cardiovascular remodeling biomarkers in terms of determining their interrelationship with coronary artery disease (CAD) progression on the background of postmenopausal osteoporosis (PMOS).
**Material and methods.** The study involved 115 women in the postmenopausal period, with a diagnosis of CAD. Depending on the BMD state, patients were divided into three groups: (1) – with normal BMD, (2) – with osteopenia, (3)- with osteoporosis. The selected control group consisted of 12 relatively healthy women of the corresponding age. Daily monitoring of electrocardiogram by Holter, two-dimensional echocardiography, intima-media thickness (IMT) measurement, the

## Résumé

**Valeur diagnostique et pronostique des biomarqueurs chez les femmes atteintes de maladie coronarienne et d'ostéoporose**

**Introduction.** Depuis le début de la ménopause chez la population féminine, l'incidence des pathologies cardiovasculaires et les modifications de la densité minérale osseuse (DMO) ont considérablement augmenté
**Le but de l'étude.** Étudier la valeur diagnostique et pronostique des biomarqueurs du remodelage osseux et vasculaire en déterminant leur relation avec les facteurs de progression de la maladie coronarienne en combinaison avec l'ostéoporose post-ménopause (PMOS).
**Matériels et méthodes.** L'étude comprenait 115 femmes ménopausées chez lesquelles on avait diagnostiqué une maladie coronarienne. En fonction de l'état de la DMO, les patientes sont divisées en trois groupes: (1) avec une DMO normale, (2) avec une ostéopénie, (3) avec une ostéoporose. Le groupe de contrôle était

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ultrasound densitometry, and FRAX algorithm were performed. The levels of cardiovascular and bone remodeling biomarkers were assessed.

**Results.** In women with CAD and PMOS, there was a significant increase in the level of bone (osteoprotegerin, osteocalcin) and cardiovascular remodeling biomarkers (VEGF-A, homocysteine). By means of the ROC analysis, the cut-off values of biomarkers for osteopenia and osteoporosis were determined. The results of the relative risk (RR) assessment showed a probable interrelationship between the course of CAD and PMOS.

**Conclusions.** The levels of biomarkers, determined by the ROC analysis, can be used for early diagnosis of bone structure disorders, and also for predicting the course of CAD on the background of PMOS.

**Keywords:** coronary artery disease, postmenopausal osteoporosis, osteopenia, serum biomarkers, relative risk.

**Abbreviations:**
AUC – area under curve
BMD – bone mineral density
CAD – coronary artery disease
CCA – common carotid arteries
CI – confidence interval
FC – functional class
HDL – high density cholesterol
HF – high frequency
HR – heart rate
HRV – heart rate variability
IMC – intima-media complex
LA – left atrium
LDL – low density cholesterol
LF – low frequency
LV – left ventricle
LV DD – left ventricle diastolic dysfunction
PMOS – postmenopausal osteoporosis
RR – relative risk
TG – triglycerides
TC – total cholesterol
VEGF-A – vasculo-endothelial growth factor-A
VNS – vegetative nervous system
WHO – World Health Organizatio

composé de 12 femmes relativement en bonne santé et d'un âge approprié. Les enregistrements ECG Holter, une échocardioscopie bidimensionnelle et une imagerie Doppler à onde pulsée, la mesure du complexe intima-média, une ostéodensitométrie à ultrasons et un algorithme FRAX ont été réalisés. Le niveau de biomarqueurs du remodelage cardiovasculaire et osseux a été évalué.

**Résultats.** Chez les femmes atteintes de la maladie coronarienne et d'ostéoporose post-ménopausique, il y avait une augmentation significative du niveau des marqueurs du remodelage osseux (ostéoprotégérine, ostécalcine) et vasculaire (WEFR-A, homocystéine). Les points critiques de distribution des biomarqueurs pour le diagnostic de l'ostéopénie et de l'ostéoporose ont été identifiés par l'analyse ROC. Sur la base du calcul du risque relatif, une relation probable entre l'évolution de la maladie coronarienne et le PMOS est prouvée.

**Conclusions.** Les niveaux de biomarqueurs déterminés par l'analyse ROC peuvent être utilisés pour le diagnostic précoce de troubles de la structure osseuse, ainsi que pour la prévision de l'évolution de la maladie coronarienne chez les femmes avec PMOS.

**Mots-clés:** maladie coronarienne, ostéoporose post-ménopausique, ostéopénie, marqueurs sériques, risque relatif.

## Introduction

According to World Health Organization (WHO), women predominate among the elderly people all over the world and the proportion of elderly women is constantly increasing; this requires studying the women' health state in the postmenopausal period[1].

Since the onset of menopause, which accelerates the development of dyslipidemia, insulin resistance and obesity, endothelial dysfunction, cardiovascular remodeling, the incidence of cardiovascular pathology in the female population increases significantly, especially coronary artery disease[2,3]. Menopause is not only a proven factor in the progression of cardiovascular disease, but also a risk factor for bone

mineral density (BMD) loss[4,5]. One of the most common forms of this pathology is postmenopausal osteoporosis (PMOS), which occurs on account of the development of pathological bone remodeling associated with increased resorption of mature bone tissue, non-traumatic spinal and tubular bone fractures, leading to increased disability and mortality of patients[6].

The interrelationship between PMOS and CAD in women has been proven due to the presence of common risk factors, as well as similar morphological and molecular properties of bone and vascular tissue[7,8]. Bone metabolism proteins, as well as markers of systemic inflammation and endothelial dysfunction, have been found to be involved in both the pathogenesis of atherosclerosis and the development of osteoporosis[9]. In particular, osteoprotegerin disrupts the process of osteoclastogenesis and reduces the activity of bone resorption[9], at the same time it acts as an inhibitor of ectopic calcification in patients with coronary artery disease[10]; osteocalcin is an activator for osteoblasts, besides, its elevated level is found in atherosclerotic plaques[11]; vasculoendothelial growth factor-A (VEGF-A) is a marker for neoangiogenesis[12], but numerous studies confirm its role in the activation of osteoclastogenesis and subsequent bone resorption[13,14]. Increased levels of homocysteine exert negative effects on the mechanisms involved in the regulation of vascular tone, lipid metabolism, and the coagulation cascade. Homocysteine is an atherogenic factor that plays an important role in the early stages of atherogenesis: it inhibits the growth of endothelial cells, has a prooxidant, mitogenic effect on smooth muscle cells and collagen biosynthesis, stimulates the accumulation of proteins in atheroma[15]. These processes result in the development of endothelial dysfunction, structural and geometric changes in the vascular wall, which contributes to the development of coronary artery disease[16]. In recent years, it has been established that hyperhomocysteinemia also affects adversely the structural and functional state of bone tissue, increases the risk of osteoporosis and osteoporotic fractures. The osteo-toxic effect of homocysteine is by and large associated with the activation of osteoclastogenesis and increased bone resorption processes, as well as oxidative and proatherogenic damage to peripheral vessels, impaired vascular production of nitric oxide[16].

However, the clinical and pathogenetic role of biomarkers taken with coronary artery disease with PMOS requires further clarification. Therefore, it is important to study the interrelationship between bone and cardiovascular remodeling biomarkers with the factors of CAD progression on the background of PMOS.

## The objective of the study

To determine the diagnostic and prognostic value of bone and cardiovascular remodeling biomarkers in terms of determining their interrelationship with progression of CAD on the background of PMOS.

## Materials and methods

A double open cross-sectional monocentric clinical study in parallel groups involved 115 women in the postmenopausal period with CAD: stable exertional angina of II-III functional class (FC) (mean age 67.07±0.92 years). Depending on the BMD state, all patients were divided into 3 groups: group 1 – 24 patients with normal BMD; group 2 – 34 patients with osteopenia; group 3 – 44 patients with osteoporosis. The control selected group consisted of 12 relatively healthy women of the corresponding age.

The inclusion criteria in the study were: presence of verified (documented) stable exertional angina of II-III FC; the duration of the postmenopausal period in women for more than 5 years, signed informed consent of the patient. The exclusion criteria: chronic heart failure of NYHA class III-IV; acute myocardial infarction or unstable angina; valvular heart defects; acute infectious diseases; severe somatic diseases during exacerbation and decompensation; endocrine pathology (hypogonadism, diabetes mellitus, hyperparathyroidism, thyrotoxicosis, acromegaly, hyperprolactinemia, hypercorticism) and diseases that induce the development of secondary osteoporosis; cancer and systemic diseases; mental disorders; alcohol abuse, drug addiction.

Angina pectoris and its functional class were diagnosed according to a comprehensive analysis of complaints, physical examination data, laboratory and instrumental findings in accordance with generally accepted standards.

The BMD state was assessed according to WHO criteria. For the screening assessment of the degree of BMD loss, the ultrasound osteodensitometry on the Omnisense 7000 apparatus (Israel) with sensors for the phalanx of the finger, the radial and tibia bones was used. X-ray densitometry on Medix DR (France) apparatus was used to confirm the diagnosis if necessary. The degree of BMD loss was evaluated according to the T-criterion (the standard deviations' value – SD from the mean values of the "peak bone mass"): the decrease in BMD to –1 SD was regarded as normal, from –1 to –2.5 SD – osteopenia, more than –2, 5 SD – osteoporosis. The FRAX algorithm was used to assess the 10-year risk of osteoporotic fractures development.

All patients underwent biochemical studies to determine total cholesterol (TC), triglycerides (TG), high (HDL) and low density (LDL) lipoproteins level using a PLIVA-Lachema BIOLATEST reagent, low-density lipoprotein levels were calculated under the Friedewald formula.

Holter electrocardiogram (ECG) daily monitoring was performed using a Cardiosens K instrument (KhAI MEDICA, Ukraine). Average, minimum and maximum heart rate (HR) per day, the frequency, duration and maximum depth of depression of the ST segment were evaluated. To determine the signs of electrical instability of the heart, we investigated the number of cardiac arrhythmias per day. While analyzing heart rate variability (HRV), the time-domain and frequency-domain methods recommended by the Committee of Experts of the North American Society of Stimulation and Electrophysiology, the European Society of Cardiologists, and the Ukrainian Association of Cardiologists were used.

Two-dimensional echocardiography was performed with the Esaote MyLab 50 X-vision ultrasound scanner, under the generally accepted practice according to ASE/EAE recommendations (2011). Quantitative and qualitative characteristics of the intima-media thickness (IMT) of the common carotid arteries (CCA) were evaluated under the ultrasound visualization in B-mode. An increase in the thickness of IMT greater than 0.9 mm was considered to be a marker of atherosclerotic vascular damage.

All patients underwent the study of osteocalcin (set of reagents by Immudiagnostic systems limited, England) osteoprotegerin (a set of reagents by Bender MedSystems GmbH, Austria), VEGF-A (eBioscience, An Affymetrix Company, USA), homocysteine (a set of reagents by Axis-Shield, Great Britain) using ELISA method on enzyme-linked immunosorbent analyzer "SIRIO S" (Italy) on the basis of the educational medical and laboratory center of the Zaporizhizhia State Medical University.

Statistical data processing was carried out by using the method of variation statistics with the help of software package "Statistica 13.0". (StatSoft Inc., N° JPZ8041382130ARCN10-J), under the generally accepted practice. The pattern of the distribution of the studied variables was assessed using the Shapiro-Wilk's criterion. Quantitative characteristics were represented as M ± m (arithmetic mean ± standard error of arithmetic mean) or Me (Q25; Q75) (median, 25 and 75 distribution quartiles) depending on the type of data distribution. Under the normal distribution, the validity of the differences was estimated using the Student's t-criterion; under the distribution different from normal, Mann-Whitney's non-parametric U-criterion was used. To assess the diagnostic significance of vascular and bone biomarkers, the ROC-analysis (Receiver Operating Characteristic) with a construction the characteristic curves for the dependence of the sensitivity and specificity of the investigated features and the calculation of the area under the working characteristic curve (AUC) was used. To quantify the interrelationship between the impact of a specific factor and the type of pathological changes, a relative risk (RR) analysis was performed, with a 95% confidence interval (CI) determined by constructing four-field tables.

When constructing the tables the following indicators were taken into account: presence/absence of atherogenic dyslipidemia, IMT thickening of the CCA over 0.9 mm, dilation of the right and left ventricles, systolic (left ventricular ejection fraction less than 45%) and diastolic dysfunction (E/A ratio less than 1), of the left ventricle (LV), cardiac arrhythmias, sympathetic-parasympathetic imbalance (LF/HF ratio greater than 2), and ischemic myocardial changes (ST segment depression episodes significant in depth and duration), BMD disorders (T-score less than –1 SD). At RR> 1, the probability of an adverse outcome occurrence in the group with exposure to the risk factor is higher, and at RR <1 is lower than in individuals without exposure to the risk factor. Differences were considered statistically significant at $p<0.05$.

## Results

The level of biomarkers of bone and vascular remodeling in women with CAD in the postmenopausal period depending on BMD state is presented in Table 1.

In women with CAD and BMD disorders, if compared to the control selected group and if compared to women with CAD and normal BMD state, an increase in osteocalcin level was observed: in the group of women with CAD and osteopenia – by 16.22% and 5.68% respectively, by 19.52% and 8.68% in the group of women with CAD and osteoporosis correspondingly ($p<0.05$). The level of osteoprotegerin also increased if compared to the control selected group: by 5.10% in the group of women with CAD and osteopenia, by 7.17% in the group of women with CAD and osteoporosis ($p<0.05$). In women with CAD and osteoporosis there was a significant increase in osteoprotegerin level if compared to women with CAD and normal BMD state – by 4.83% ($p<0.05$), meanwhile this biomarker trended to increase in women with CAD and osteopenia compared with women with CAD and normal BMD state.

It is known that VEGF-A is not only a regulator of neoangiogenesis and stimulates blood supply to bone and cartilage[12], but is also a stimulator of

**Table 1.** Levels of biomarkers in postmenopausal women with CAD, depending on the BMD state, Me (Q25; Q75)

| Indicator, units of measurement | Control selected group (n=12) | CAD (n=24) | CAD +osteopenia (n=34) | CAD +osteoporosis (n=44) |
|---|---|---|---|---|
| Osteocalcin, ng/mL | 14.24 (12.54; 17.12) | 15.66 (12.75; 18.04) | 16.55 (9.95; 25.51) #* | 17.02 (14.30; 30.26) #* |
| Osteoprotegerin, pg/mL | 216.85 (170.58; 231.35) | 221.69 (213.40; 232.74) | 227.90 (196.82; 241.02) # | 232.39 (209.60; 248.62) #* |
| VEGF-A, pg/mL | 105.96 (102.66; 119.74) | 119.84 (77.05; 225.21) | 190.19 (89.53; 253.86) # | 253,04 (103.32; 379.56) #* |
| Homocysteine, mmol/mL | 10.11 (9.16; 11.08) | 11.18 (10.03;12.83) | 12.01 (10.26;14.48) # | 14.38 (13.53; 20.09) #* |

Note: # – the probability of indexes difference if compared to the control selected group (p<0.05); * – the probability of indexes difference if compared to the patients with CAD and normal BMD state (p <0,05).
**Legend:** CAD – coronary artery disease; BMD – bone mineral density; VEGF-A – vasculo-endothelial growth factor-A

**Table 2.** The prognostic value of biomarkers in patients with CAD for the presence of osteopenia according to the results of ROC analysis

| Indicator, units of measurement | Cut-off value | Area under curve (AUC) | Sensitivity, % | Specificity, % | p-value |
|---|---|---|---|---|---|
| Osteocalcin, ng/mL | > 15.89 | 0.875 | 83.3 | 81.0 | <0.001 |
| Osteoprotegerin, pg/mL | >223.76 | 0.787 | 70.0 | 71.4 | <0.001 |
| VEGF-A, pg/mL | > 112.52 | 0.952 | 88.9 | 82.4 | <0.001 |
| Homocysteine, mmol/mL | >11.40 | 0.850 | 83.3 | 80.0 | <0.001 |

**Legend:** AUC – area under curve; CAD – coronary artery disease; VEGF-A – vasculo-endothelial growth factor-A

**Table 3.** The prognostic value of biomarkers in patients with CAD for the presence of osteopenia according to the results of ROC analysis

| Indicator, units of measurement | Cut-off value | Area under curve (AUC) | Sensitivity, % | Specificity, % | p-value |
|---|---|---|---|---|---|
| Osteocalcin, ng/mL | > 16.71 | 0.844 | 65.6 | 77.8 | <0.001 |
| Osteoprotegerin, pg/mL | >224.44 | 0.762 | 67.6 | 68.9 | <0.001 |
| VEGF-A, pg/mL | >123.31 | 0.964 | 85.7 | 94.3 | <0.001 |
| Homocysteine, mmol/mL | >12.83 | 0.800 | 90.0 | 70.0 | <0.001 |

**Legend:** AUC – area under curve; CAD – coronary artery disease; VEGF-A – vasculo-endothelial growth factor-A

osteoclastogenesis[13,14]. In our study an increase in the VEGF-A level was observed: 1.8 times higher in the group of women with CAD and osteopenia, and 2.4 times higher in the group of women with CAD and osteoporosis if compared to the control selected group and patients with CAD and normal BMD respectively (p<0.05). In patients with CAD and osteoporosis, the VEGF-A level was 2.1 times higher than in group with CAD and normal BMD state (p<0,05), whereas in group with osteopenia it tended to increase compared to CAD patients.

An increase in homocysteine level, which is a classic marker of endothelial dysfunction and an independent risk factor for atherosclerotic vascular damage in patients with CAD and also of a BMD loss, was observed[16]: by 18.79% in the group of women with CAD and osteopenia, by 42.2% in the group of women with CAD and osteoporosis if compared to the control selected group. There was a tendency to its increase in the group of patients with CAD and osteopenia and a significant increase by 28.62% in the group of women with CAD and osteoporosis if compared to the group of patients with CAD and normal BMD state (p<0,05).

While comparing the level of bone and vascular biomarkers in the group of women with CAD and

osteopenia to the group with CAD and osteoporosis, there was a tendency to an increase, but no significant difference was found.

In order to determine the optimal threshold values of biomarkers as indicators of BMD disorders at different stages, the ROC analysis was conducted with the calculation of sensitivity (%), specificity (%), area under the curve (AUC) and cut-off value. The results of the ROC analysis are presented in Tables 2 and 3.

As it can be seen from Tables 2 and 3, all indicators had an area under the curve close to 0.8 and higher, which indicates the value of the selected biomarkers (high and very high model quality). According to the results of the ROC analysis, patients with CAD are at increased risk of osteopenia at osteocalcin level >15.89 ng/mL, osteoprotegerin level >223.76 pg/mL, VEGF-A level >112.52 pg/mL, homocysteine level >11, 40 mmol/mL, and at risk of osteoporosis at osteocalcin level > 16.71 ng/mL, osteoprotegerin level >224.44 pg/mL, VEGF-A level >123.31 pg/mL, homocysteine level >12.83 mmol/mL. VEGF-A showed the highest sensitivity, the optimal ratio of sensitivity and specificity, the critical value of which according to the ROC analysis was equal to 112.52 pg/mL (Se = 88.9%, Sp = 82.4%; AUC = 0.952; 95 % CI 0.842-0.994) at osteopenia identification, 123.31 pg/mL (Se = 85.7%, Sp = 94.3%; AUC = 0.964; 95% CI 0.869-0.996) – at osteoporosis identification.

At the next stage, the relative risk of complicated course of CAD on the background of PMOS was determined, which made it possible to quantify the interrelationship between the influence of a certain factor and the type of pathological changes. It was found that in women with CAD combined with BMD disorders who had an osteocalcin level >15.89 ng/mL the relative risk of having sympathetic-parasympathetic imbalance increased by 2.1 times (RR=2.143; 95% CI 1.013-4.532; p<0.05), of atherogenic dyslipidemia – by 1.5 times (RR=1,540; 95% CI 1.013-2.343; p<0.05), of diastolic dysfunction of the LV (LV DD) – by 2 times (RR=2.027; 95% CI 1.064-3.863; p<0.05), of dilation of the left atrium (LA) – by 1.7 times (RR=1.679; 95% CI 1.026-2.749; p<0.05).

In women with CAD combined with BMD disorders who had osteoprotegerin level >223.76 pg/mL the relative risk of having sympathetic-parasympathetic imbalance increased by 1.9 times (RR=1.890; 95% CI 1.040-3.435; p<0.05), of atherogenic dyslipidemia – by 1.5 times (RR=1.486; 95% CI 1.045-2.112; p<0.05), of dilation of the LA – by 1.6 times (RR=1.607; 95% CI 1.004-2.574; p<0.05).

In patients with CAD and BMD disorders who had a VEGF-A level higher than 112.52 pg/mL, the relative risk of having an intima-media complex (IMC) thickened increased by 2.1 times (RR=2.141; 95% CI 1.040-4,408; p<0.05), of sympathetic-parasympathetic imbalance increased by 2 times (RR=2,040; 95% CI 1.025-4.061; p<0.05), of atherogenic dyslipidemia – by 1.5 times (RR=1,500; 95 % CI 1.043-2.157; p<0.05).

In patients with CAD and BMD disorders who had a homocysteine level higher than 11.40 mmol/mL, the relative risk of having an IMC thickened increased by 3.6 times (RR=3.556; 95% CI 1.047-12.071; p<0.05), of atherogenic dyslipidemia – by 2.1 times (RR=2.074; 95% CI 1.052-4.090; p<0.05), of LV diastolic dysfunction – by 2.3 times (RR=2.308; 95% DI 1.126-4.731; p<0.05), of dilation of the LA – by 2.5 times (RR=2.528; 95% CI 1.052-6.072; p<0.05), of arrhythmic complications – by 2.5 times (RR=2.566; 95% CI 1.050-6.267; p<0.05).

A general scheme of biomarkers' influence on the clinical course of CAD with concomitant BMD disorders is presented in Fig. 1.

## Discussion

Our study showed a significant increase in levels of bone remodeling biomarkers – osteoprotegerin and osteocalcin in groups of women with CAD and BMD disorders (osteopenia and osteoporosis), which can be due to the participation of osteoprotegerin in the process of osteoclastogenesis inhibition and bone tissue resorption, as well as its compensatory enhancement to perform a protective role in the development of vascular calcification[10]. An increase in osteocalcin level may indicate a violation of its inclusion in normal bone structure, compensatory activation of osteoblasts on the background of decreased BMD and its participation in the process of ectopic calcification of the heart and blood vessels[11,17]. The results obtained confirm the data of Sagalovsky et al (2016) on the participation of osteoprotegerin in the regulation of calcium deposition in the vessel wall, increasing its rigidity with the subsequent development of vascular calcification, as well as in the formation of arterial endothelial dysfunction and cardiac remodeling[9]. There is literary evidence that osteoprotegerin is a marker for the severity of the atherosclerotic process, increased level of which leads to destabilization of the atherosclerotic plaque and the development of atherothrombosis[18,19].

According to many authors, the increase of osteocalcin serum concentration can be considered as a possible prognostic indicator of disturbance of bone architecture[20,21]. Also, many studies have revealed its involvement in the processes of vascular calcification, which occur with the progression of atherosclerotic process[21,22].

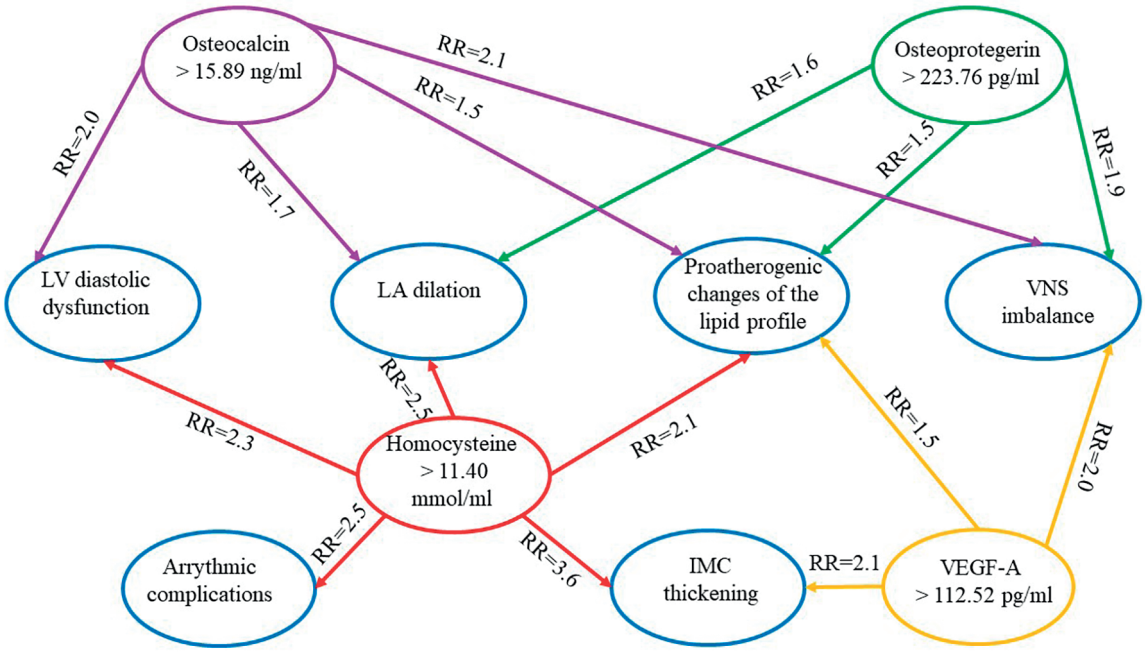


**Figure 1.** Interrelationship of biomarkers' level with clinical course of CAD on the background of BMD disorders.

**Legend:** IMC – intima-media complex; LA – left atrium; LV – left ventricle; RR – relative risk; VEGF-A – vasculo-endothelial growth factor-A; VNS – vegetative nervous system

Modern scientific evidence indicates that the content of free VEGF-A in the blood of healthy individuals is low, it is secreted by endothelial cells and pericytes in hypoxia and is also a promoter of collateral formation in ischemic myocardium, carries a positive influence on myocardial revascularization through a number of mechanisms (activation of NO synthase, increase of NO content and vasodilation)[12,22,23]. On the other hand, overexpression of VEGF-A may also cause excessive bone resorption through the activation of osteoblasts[25]. In our study, an increase in the level of VEGF-A was observed. The obtained data are related to the overexpression of VEGF-A level under the development of chronic tissue hypoxia in CAD[13], which may lead to the malfunction of the normal molecular mechanisms of the ossification development, activation of osteoclastogenesis with the subsequent stimulation of osteoclast-mediated bone resorption[14].

Nowadays, it is proven that hyperhomocysteinemia is one of the potential risk factors for the development of atherosclerosis and hypertension[15,25]. Increase in homocysteine level has a direct effect on the vascular wall with the formation of endothelium-dependent vasodilation and oxidative stress, the development of lipid peroxidation due to increased synthesis of superoxide dismutase, activation of thrombogenesis[16], coagulation[26]. In addition, hyperhomocysteinemia contributes to the reduction of bone blood supply and is able to influence its biomechanical properties, allowing homocysteine to be considered as a new risk marker for osteoporosis[16,27]. Therefore, the obtained data on the increase in homocysteine, which was the highest in women with CAD and PMOS, can be explained not only by the development and progression of endothelial dysfunction, but also by the progression of bone remodeling.

Thus, the obtained results confirm the existing scientific data on the presence of general pathogenetic bases of bone and vascular remodeling biomarkers' levels growth, impaired osteogenesis and the progression of cardiovascular disturbance in postmenopausal women with CAD[28-30].

## Conclusions

The presence of BMD disorders (osteopenia, osteoporosis) in postmenopausal women with CAD is accompanied with an increase in the level of bone and vascular remodeling biomarkers (osteoprotegerin, ostecalcin, VEGF-A, homocysteine).

The levels of osteoprotegerin and/or ostecalcine and/or VEGF-A and/or homocysteine determined by the ROC analysis can be used as early diagnostic markers of bone tissue disturbances and stratification of patients in the category of increased risk of osteoporosis fractures development.

The calculation of the relative risk proves the existing interrelationship of osteoprotegerin, osteocalcin, WEFR-A and homocysteine levels with the development of atherogenic dyslipidemia, diastolic dysfunction of the LV, dilatation of LA, the occurrence of arrhythmic complications, VNS imbalance, which indicates its influence on the course of CAD on the background of PMOS.

### Author Contributions:

*Conceptualization, N.M.; methodology, N.M. and I.S.; software, T.K.; validation, T.K.; formal analysis, I.S.; investigation, I.S.; resources, I.S.; data curation, T.K. and I.S.; writing–original draft preparation, I.S.; writing–review and editing, N.M, L.F.; visualization, I.S.; supervision, N.M. and L.F.; project administration, N.M. All the authors have read and agreed with the final version of the article.*

### Compliance with Ethics Requirements:

*„The authors declare no conflict of interest regarding this article"*

*„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law"*

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