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Original article

Characteristics of pathogenetic links in vascular remodeling and bone tissue destruction in postmenopausal women with arterial hypertension

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Abstract: *Background* — Much attention is drawn to the role of the nonspecific immune inflammatory vascular response as a link of general pathogenetic mechanisms with changes in the elastic properties of arteries and the phenomena of destructive changes in bones.
Objective — We aimed to study the role of nonspecific immune inflammatory markers, parathyroid hormone and female sex hormones as predictors of cardiovascular and degenerative bone changes in postmenopausal women with arterial hypertension (AH) and osteoporosis (OP).
Methods — We examined 104 patients (mean age: 54.03±9.56 years) distributed among three groups: healthy women, females with AH and osteopenia, and women with AH and OP. The markers of immune inflammatory response, endothelial dysfunction, hormonal, mineral, and vitamin statuses were analyzed simultaneously with 24-hour ambulatory monitoring of blood pressure, and parameters of vascular wall stiffness and densitometry to elucidate the predictors of cardiovascular and degenerative bone changes in postmenopausal women.
Results — For patients with AH and osteopenia, a significant parameter associated with the risk of OP was pulse wave velocity (PWV); its increase exceeding 12.05 m/s was associated with a 3.8-fold increase in the risk of OP. Levels of proinflammatory indicators, interleukins IL-6 and IL-8, tumor necrosis factor-α, high-sensitivity C-reactive protein, and parathyroid hormone were elevated, while levels of progesterone and IL-10 were decreased.
Conclusion — Timely specialized multidirectional research of biochemical and instrumental parameters (PWV and densitometry) could become the basis for the development of a personalized strategy for the prevention and treatment of women in order to avoid dangerous cardiovascular and bone complications.

Keywords: atherosclerosis, osteoporosis, immune inflammatory response, hormonal status, vitamin status, mineral status, T-score peak

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Introduction

Currently, atherosclerosis (AS) and osteoporosis (OP) are among the most common chronic noninfectious diseases. They are age-related and also are associated with increase in life expectancy and etiopathogenetic relationships. AS and OP have several common features and, especially, asymptomatic course at the onset as well as a high risk of delayed complications such as heart attack and stroke for AS, and low-trauma fractures with a rate of 30-40% for OP [1].

Contemporary medicine finds it extremely important to identify certain relationships between various diseases and their common pathogenetic mechanisms in order to develop an integrated and individualized approach to the prevention and treatment of diseases. The results of experimental and clinical studies conducted during the last decade confirmed that AS and OP with asymptomatic onset had common pathogenetic links resulting in demonstrated complications. A relationship was shown between the development of AS and decrease in bone mineral density (BMD), regardless of the patient age and increased risk of morbidity and mortality due to AS complications in patients with OP [2].

Various factors affecting bone metabolism are involved in the mechanisms of vascular diseases. In order to assess the relationship between OP and cardiovascular diseases (CVD) caused by AS, surrogate markers of these diseases are commonly used, e.g., parameters of vascular wall stiffness or vascular calcification and BMD. It was revealed that vascular and bone tissues have a number of common properties, and vascular calcification consists of the same elements as bone tissue: calcium salts, type I collagen, phosphates, bone morphogenetic protein, etc. It was suggested that low BMD may be a direct risk factor for the development of coronary artery AS [3].

The connection between AS and AP is most obvious in postmenopausal women. Estrogen deficiency reduces the ability of endothelial cells to produce nitric oxide, which maintains arterial elasticity and has a stimulating effect on osteoblasts, leading to endothelial dysfunction and disorders of bone metabolism [2, 3].

Along with the deficiency of sex steroids, a negative calcium balance is of great importance, caused by a deficiency of vitamin D

and a decrease in calcium absorption in the intestine, which ultimately leads to secondary hyperparathyroidism and increased bone resorption [4, 5]. Disorders leading to both OP and CVD include increased activity of the sympathetic autonomic nervous system, which, together with endothelial dysfunction, causes disorders in the microcirculatory system. The most important mechanism for reducing BMD is the deterioration of bone tissue perfusion associated with disorders of the microcirculation system. Microcirculation which determines the value of peripheral vascular resistance, due to the subclavian steal syndrome, significantly affects the state of perfusion of internal organs, including bone tissue [6].

The role of angiotensin II in the development of CVD is well known. In addition to its vasoconstrictor effect, it has significant proinflammatory activity in the vascular wall via stimulating the production of reactive oxidized species, inflammatory cytokines and adhesion molecules) and promotes the formation and progression of AS. Angiotensin II receptors were identified in cultured bone cells (osteoblasts and osteoclasts). Angiotensin II promotes the production of receptor activator of nuclear factor kappa B ligand (RANK-L) by osteoblasts, which leads to additional activation of osteoclasts and increased bone resorption, as well as inhibition of bone mineralization [7, 8].

The results of clinical studies of the relationship between BMD and the level of arterial hypertension (AH) and blood pressure (BP) were contradictory. Some of them showed a negative association between BP and BMD, while others showed no association between BP and BMD whatsoever [9, 10]. Data have also been published showing that arterial stiffness was higher in women with moderate cardiovascular risk and postmenopausal OP and was closely associated with BMD and markers of bone turnover. It was shown that a decrease in femoral neck BMD was an independent factor in increasing arterial stiffness. Published results implied that disorders of mineral metabolism in bone tissue could have been an additional risk factor for damage to the vascular wall, which must be taken into account when determining the overall cardiovascular risk in patients [11-14].

With the steady aging of the population in the 21st century, data on the connection between the processes of cardiovascular remodeling and bone resorption during the postmenopausal period continue to be of interest. Recently, more and more attention has been paid to the role of the nonspecific immunoinflammatory response of blood vessels as a link between the general pathogenetic mechanisms of atherosclerotic lesions of the vascular bed with changes in the elastic properties of arteries and the phenomena of degenerative changes in bones. These are of great importance at the subclinical level in order to provide comprehensive measures to prevent complications of these comorbidities in general.

The goal of our study was to examine the role of nonspecific immune inflammatory markers, parathyroid hormone, and female sex hormones as predictors of cardiovascular and degenerative bone changes in postmenopausal women with AH and OP.

Material and Methods

Data collection

The study involved 104 patients (mean age: 54.03±9.56 years) who were distributed among three groups. Group 1 included 39 healthy women, Group 2 comprised 30 patients with AH and osteopenia, and Group 3 encompassed 35 women with AH and OP. The study protocol was approved by the Ethics Committee of Tyumen Cardiology Research Center, Tomsk National Medical Research Center of the Russian Academy of Sciences, Tomsk, Russia. Prior to the enrollment, each study participant gave a written informed consent to use the study results for scientific purposes.

Exclusion criteria were as follows: presence of acute cerebrovascular accident less than 6 months ago, coronary artery disease, type 2 diabetes mellitus, chronic heart failure of functional class (FC) III-IV sensu the New York Heart Association (NYHA) classification, cancer, and mental illness. AH was diagnosed in accordance with the current recommendations of the European Society of Cardiology and Russian Society of Cardiology. The scope of diagnostic measures included clinical examination, laboratory and instrumental tests to evaluate the cardiovascular and skeletal systems. The parameters of 24-hour ambulatory BP monitoring (ABPM) were measured in all examined patients according to the standard scheme by oscillometric method on the equipment of BPLAB LLC “Pyotr Telegin” (the Russian Federation).

Instrumentation

Examination of elastic properties of the vascular walls was performed with a sphygmograph Vasera VS-1000 Series (Fukuda Denishi, Japan), and the following parameters were assessed: pulse wave velocity in elastic arteries on the right or left (PWV-R/L) and ankle-brachial index (ABI-R, ABI-L) as a parameter of peripheral vascular blood flow and a screening parameter for the presence of AS of the vessels in the

lower limbs. Osteodensitometry to identify BMD was performed with Siemens Somatom Emotion spiral computed tomography scanner. Calcium content expressed in terms of hydroxyapatite (CA-HA) and the standard deviation of the T-score peak were assessed as well (normal values range from 2.0 to -1.0; in osteopenia, values vary from -1.0 to -2.5; OP is diagnosed at values from lower than -2.5).

We also carried out ultrasound scanning of brachiocephalic arteries was performed; the parameters of intima-media thickness (IMT) of the carotid artery, state of the vascular wall, and the presence of atherosclerotic plaques were taken into account. IMT was determined at a distance of 2 cm from the bifurcation of the common carotid artery on the posterior wall (a value smaller than 0.8 mm was considered normal, 0.9 mm represented the upper limit of normal, more than 0.9 mm indicated the thickening). Atherosclerotic plaque was a local thickening of the arterial wall exceeding 50% or more of the thickness of the adjacent unchanged IMT, protruding into the lumen of the vessel and having different structure vs. unchanged arterial wall and/or thickening of the IMT of above 1.3 mm [13]. Fasting venous blood was collected into disposable VACUETTE tubes (Japan). The blood was centrifuged for 15 min at 2,500 rpm on a Sigma centrifuge (Germany). Blood serum of patients was divided in aliquots for further freezing (at -70 °C) and analyses.

Biochemical markers

The parameters of lipid metabolism were examined using the Cobas Integra 400 plus automated biochemical analyzer (Switzerland). Total cholesterol and triglycerides (TG) in blood serum were determined using the enzymatic colorimetric method; high-density lipoproteins (HDL) and low-density lipoproteins (LDL)

were measured by the direct enzymatic colorimetric method; concentrations of apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo-B), and lipoprotein a (Lp(a)) were identified via immunoturbidimetry with the analytical kits and control materials by Roche Diagnostics Gmbh (Germany).

The following biochemical markers of inflammation were investigated: high-sensitivity C-reactive protein (hs-CRP) by immunoturbidimetric method using the hs-CRP analytical kit (BioSystem, Spain) on Clima MC-15 semi-automatic open-type analyzer (Spain); interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor α (TNF-α) and homocysteine (HYC) via sandwich-ELISA and solid-phase competitive chemiluminescence enzyme immunoassay with the following analytical kits: IL-1β, IL-6, IL-8, TNF-α, and HYC, respectively. The levels of sex hormones (estradiol, progesterone, testosterone) and parathyroid hormone were determined using a solid-phase competitive chemiluminescent ELISA method with the Siemens Diagnostics reagents. Myeloperoxidase was measured by the sandwich ELISA method using the eBioscience reagents.

Carbohydrate metabolism was assessed based on glucose and glycated hemoglobin (HbA1c) concentrations. Blood glucose was determined by the hexokinase method using the Cobas Integra 400 plus biochemical analyzer. Glycated hemoglobin was determined via chromatography with the Bio-Rad D10 analyzer, USA. The atherogenic coefficient (AC) was calculated as:

$$AC = Apo\ B/Apo\ A\text{-}I.$$

The parameters of vascular endothelium functional activity in blood serum were determined as follows: nitrite levels with the Human HUMALYZER 2000 biochemical analyzer (Germany) and endothelin 1-21 with the Dynatech semi-automated immunoassay analyzer (Germany).

Statistical data processing

Statistical data processing was carried out using the STATISTICA software package (SPSS, Inc., version 11.5). Parameter distribution was tested using the Kolmogorov-Smirnov test. To determine the statistical significance of the differences in continuous values depending on the distribution parameters, we employed one-way analysis of variance with the Holm-Sidak correction for multiple comparisons or the Kruskal-Wallis test with the Bonferroni correction for multiple comparisons. Continuous variables are presented as M±SD (mean ± standard deviation) or Me [Q25; Q75] (median and interquartile range). To assess the differences in qualitative variables, the chi-squared test and the Fisher's exact test were used. Spearman and Pearson linear correlation coefficients, logistic regression, and discriminant analysis were used to identify the relationship between variables

Results

Characteristics of clinical and anamnestic data of the examined patients are presented in *Table* 1. It is clear that the age of the patients in Groups 2 and 3 significantly differed from Group 1 ($p<0.005$; $p<0.001$, respectively). There were no significant differences in smoking and body mass index (BMI) in all groups, or duration of AH in the groups with AH. The percentage of family history of AH in the groups with AH did not differ, but was significantly lower in the control group of healthy patients. Regarding the grade of AH, the maximum share of patients with grade 1 AH was in Group 2 and the maximum percentage of patients with grade 3 AH was in Group 3. Besides, patients in the group with AH and OP had significantly longer postmenopausal period vs. Group 1 patients ($p<0.001$).

Table 1. Clinical and anamnestic characteristics in groups of examined patients

Parameter		*Group 1* *Healthy patients (n=39)*	*Group 2* *Patients with AH and osteopenia (n=30)*	*Group 3* *Patients with AH and osteoporosis (n=35)*	*p* (groups 1 and 3)
Age (years)		42.92±13.41	58.91±8.28***	62.68±7.16	<0.001
Smoking		0%	1(3.4%)	3(8.6%)	0.181
Non-smoking		39(100%)	29(96.6%)	32(91.4%)	
AH grade	1		11(36.7%)	5(14.3%)	0.029
	2		11(36.7%)	17(48.6%)	0.386
	3		8(26.6%)	13(37.1%)	0.259
BMI (kg/m²)		25±0.8	26.14±2.48	25.48±2.61	0.460
Waist volume (WV) (cm)		71.01±6.08	83.83±8.31	81.65±12.38	0.165
Hips volume (HV) (cm)		93.01±1.41	96.22±7.28	97.51±9.93	0.739
Family history of AH	Yes	17(43.5%)	20(66.7%)	29(82.9%)	0.082
	No	22(56.6%)	10(33.3%)	6(17.1%)	
Postmenopausal period (years)		1.0 [1.0;1.75]	7.0 [4.0;10.0]***	10.0 [5.5;21.5]##	<0.001

*** p<0.001 – comparison between groups 1 and 2; ## p< 0.01 – comparison between groups 2 and 3; p – comparison between groups 1 and 3.

Table 2. Structural and functional characteristics of the vascular wall and bone tissue in the groups of examined patients (M±SD)

Parameter	*Healthy patients* (n=39)	*Patients with AH and osteopenia* (n=30)	*Patients with AH and osteoporosis* (n=35)	*p* (groups 1 and 3)
PWV-R, m/s	11.29±0.84	12.99±1.52**	14.82±2.81#	<0.001
PWV-L, m/s	9.60±0.77	13.32±1.44*	15.09±2.97#	0.001
IMT CCA d, mm	0.70 [0.55; 0.80]	0.80 [0.75; 1.0]	0.90 [0.80; 0.90]	0.046
IMT CCA s, mm	0.70[0.55; 0.75]	0.80 [0.70; 0.85]	0.90 [0.9; 1.00]	0.007
T Score	-0.40±0.22	-1.47±0.93	-3.08±0.64	<0.001
AC	-	110.56±16.27	60.37±26.79	<0.001

* p<0.05; ** p<0.01; # p< 0.05 – comparison between groups 2 and 3; p – comparison between groups 1 and 3.

Table 3. Characteristics of biochemical parameters in the examined groups of patients (M±SD)

Parameter	*Healthy patients* (n=39)	*Patients with AH and osteopenia* (n=30)	*Patients with AH and osteoporosis* (n=35)	*p* (groups 1 and 3)
Lipid profile				
TCh (mmol/L)	5.09±1.01	5.54±1.15	5.57±1.18	0.124
HDL (mmol/L)	1.59±0.39	1.54±0.4	1.73±0.52	0.230
LDL (mmol/L)	2.83±0.76	3.32±1.05	3.25±1.17	0.081
TG (mmol/L)	1.21±0.75	1.44±0.85	1.18±0.43	0.246
Apo-A (mg/dL)	187.26±35.7	177.13±27.64	178.7±29.0	0.358
Apo-B (mg/dL)	116.02±139.8	105.75±29.78	105.97±29.42	0.261
Apo-A1/Apo-B (mg/dL)	0.53±0.14	0.6±0.19	0.61±0.16	0.112
Myeloperoxidase (mg/dL)	7.69 [2.12; 11.87]	9.75 [7.13; 13.02]	10.07 [8.09; 13.7]	0.05
Inflammatory markers				
High-sensitivity C-reactive protein (hs-CRP) (mg/L)	1.05 [0.45; 3.13]	2.10 [1.01; 4.05]	3.11 [1.76; 5.51]*	0.002
TNF-α (pg/ml)	4.47±0.06	4.78±1.35	5.04±1.26	0.219
Homocysteine (μmol/L)	10.88±2.28	12.42±5.47	13.21±5.21	0.076
IL-1β (pg/ml)	2.49±0.46	2.54±0.53	2.67±1.04	0.539
IL-6 (pg/ml)	1.65±0.41	2.05±0.80	1.92±0.67	0.285
IL-8 (pg/ml)	10.40±4.27	10.42±4.64	12.91±4.75*	0.034
IL-10 (pg/ml)	3.71±0.88	3.33±0.81	3.17±0.76	0.020
Endothelial dysfunction				
Endothelin-1 (fmol/L)	0.47 [0.13; 1.13]	0.32 [0.05; 0.99]	0.51 [0.24; 1.166]	0.269
Nitrites (μmol/L)	56.21±31.13	68.36±33.63	78.25±40.23*	0.036
Nitrates (μmol/L)	67.53±38.22	77.61±30.80	90.9±40.33*	0.037
Nitrites / Nitrates	34.11±30.31	29.12±22.53	31.5±26.49	0.782
Parameters of hormonal and calcium metabolism, vitamin D				
Estrogen (nmol/L)	35.54±22.34	26.76±9.54	26.7±9.68	0.449
Progesterone (nmol/L)	1.99 [0.99; 4.9]	0.64 [0.64; 0.81]***	0.64 [0.64; 0.82]*	<0.001
Testosterone (nmol/L)	0.70 [0.69; 1.01]	0.69 [0.69; 0.72]	0.69 [0.68; 0.69]	0.010
Parathyroid hormone (pg/mL)	22.5 [16.7; 39.5]	29.3 [21.1; 45.9]	37.7 [22.7; 59.0]*	0.029
Calcitonin (pg/mL)	1.39 [1.31; 1.61]	1.26 [1.14; 1.56]	1.18 [1.13; 1.49]	0.152
Vitamin D (ng/mL)	46.24±14.82	41.51±21.96**	39.1±15.14#	0.001
Total calcium (mmol/L)	2.39±0.11	2.44±0.09	2.35±0.18	0.226
Ionized calcium (mmol/L)	1.14±0.02	1.17±0.03	1.17±0.43	0.728

* p<0.05; ** p<0.01; *** p<0.001 – comparison between groups 1 and 2; # p< 0.05 – comparison between groups 2 and 3; p – comparison between groups 1 and 3.

According to ABPM, statistically significant differences in the parameter values were observed for the levels of 24-hour systolic BP (SBP 24) and diastolic BP (DBP 24) between apparently healthy subjects in Group 1 and patients in Group 2, as well as for the levels of variability of SBP 24 and nocturnal DBP between patients of Groups 2 and 3 (p<0.01). The absence of other statistically significant changes in ABPM parameters can be explained by sufficient adherence of patients with AH to antihypertensive therapy. The characteristics of structural and functional parameters of vascular wall and bone tissue in the groups of examined patients are presented in *Table 2*.

According to the results presented in *Table 2*, PWV-R/L was significantly higher in the groups of patients with AH vs. the control group. The maximum values were observed in Group 3 (much higher than in Groups 1 and 2), which agrees with the data of other researchers who noted an increase in the rigidity of the vascular wall in postmenopausal women [2, 3].

Maximum values of the common carotid artery IMT (IMT CCA) d/s were observed in Group 3 of patients with AH and OP: they substantially exceeded the values in Groups 1 and 2. T-score and AC were naturally significantly smaller in Group 3 of patients with AH and OP vs. Groups 1 and 2. Correlation analysis of parameters presented in the table demonstrated moderate associations in Group 2 (PWV-R with IMT CCA d, r=0.415, p<0.06) and Group 3 (AC with PWV-R, r=0.871, p<0.06; and AC with IMT CCA d, r=-0.673, p<0.002).

We intended to examine common relationships between the studied parameters by determining their associations with the biochemical parameters of the lipid profile, inflammatory response and endothelial dysfunction of the vascular wall, as well as parameters of hormonal, mineral and vitamin metabolisms.

Laboratory biochemical parameters in the examined groups of patients are presented in *Table 3*.

According to the table, there is a persistent trend of the increase in the level of total cholesterol and its atherogenic fractions in groups with AH compared with the control group of patients. There is a clear tendency towards an increase in myeloperoxidase as an indicator reflecting an increase in the process of peroxidation in Group 3 of patients with AH and OP vs. Groups 1 and 2.

According to the results of vascular inflammatory response markers, the levels of hs-CRP, HYC and IL-8 were significantly higher in Group 3. The trend of elevated levels of endothelin 1 and significantly increased nitrites in Group 3 imply significant endothelial dysfunction in patients with AH and OP. The results of the study of indicators of hormonal, mineral and vitamin metabolisms showed maximum levels of parathyroid hormone (p<0.029) and a decrease in the levels of estrogen, progesterone and testosterone, along with a trend towards a reduction in calcitonin, total calcium, and ionized calcium; and also, a significantly low level of vitamin D (p<0.001) in patients of Group 3. Our results were consistent with published data and reflected the severity of changes in biochemical markers in different groups of patients [2, 3].

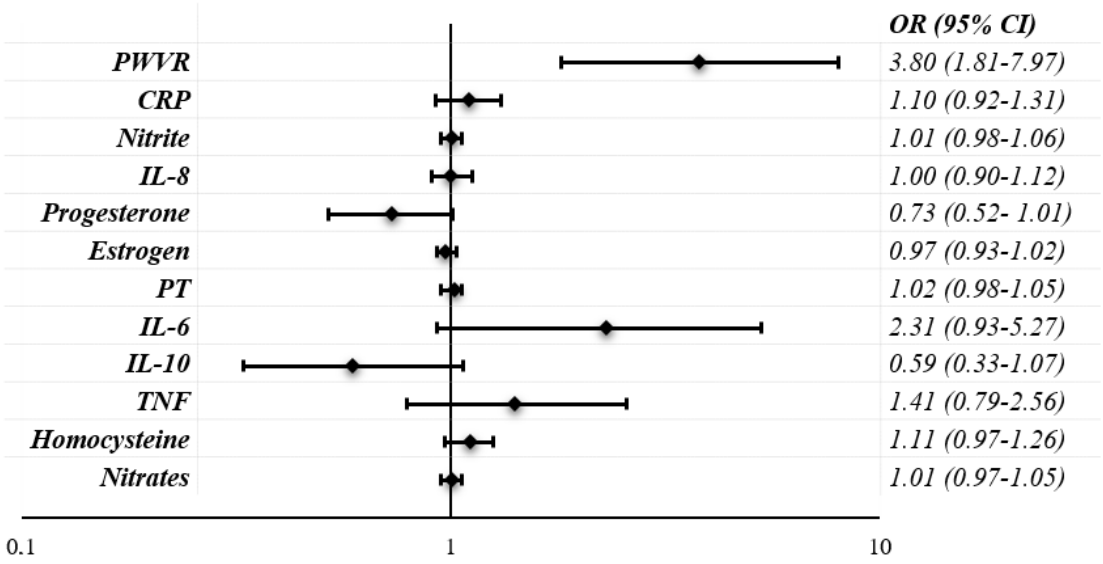

Figure 1. Odds ratios maximizing the risk of developing osteoporosis in hypertensive patients with osteopenia. PWVR, pulse wave velocity in elastic arteries on the right; CRP, C-reactive protein; PT, parathyroid hormone; IL, interleukin; TNF, tumor necrosis factor.

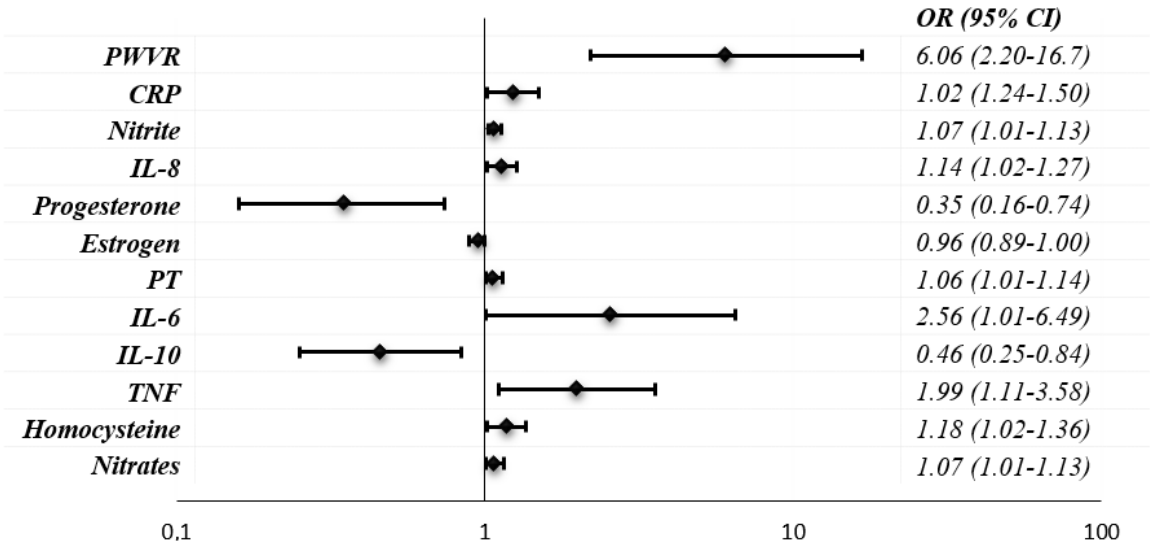

Figure 2. Odds ratios maximizing the risk of osteoporosis progression in hypertensive patients with preexisting osteoporosis. PWVR, pulse wave velocity in elastic arteries on the right; CRP, C-reactive protein; PT, parathyroid hormone; IL, interleukin; TNF, tumor necrosis factor.

The study showed multiple multidirectional moderate correlations (r=0.452, p<0.05) between the structural, functional and biochemical parameters. In Group 3, inverse correlations were observed between the peak of T-score and age, PWV-L/R, 24-hour and night SBP and DBP, duration of menopause, IL-6, hs-CRP and HYC, as well as between PWV-L and estradiol; whereas direct correlations were established between T-score and progesterone, and between PWV-R/L and IL-6, LDL, hs-CRP, TNF-α, endothelin 1, mean 24-hour SBP, along with daily variability of SBP and DBP.

Back in 1935, Allen et al. showed that estrogens dilated blood vessels, improve blood circulation and normalize cardiac function. In 1957, Popovici et al. stated that a decrease in estrogen levels yielded a reduction in acetylcholine, which in turn resulted in coronary and arterial ischemic syndrome. Modern data convincingly prove the existence of the relationships of the levels of sex hormones with CVD and bone destructive processes, and long-term vascular inflammatory response is considered a pathogenetically associated link in this relationship.

The risks of the development and progression of destructive changes were calculated using logistic regression for the group of patients with AH, osteopenia and OP in the postmenopausal period. E.g., for the patients with AH and osteopenia, statistically significant parameter associated with the risk of OP was PWV-R: its increase by 1 point was associated with 3.8-fold increase in the risk of OP (odds ratio (OR) 3.8, 95% confidence interval (CI) 1.81-7.97). We found no statistically significant relationships between biochemical parameters and the risk of OP in this group of patients at this stage of the study.

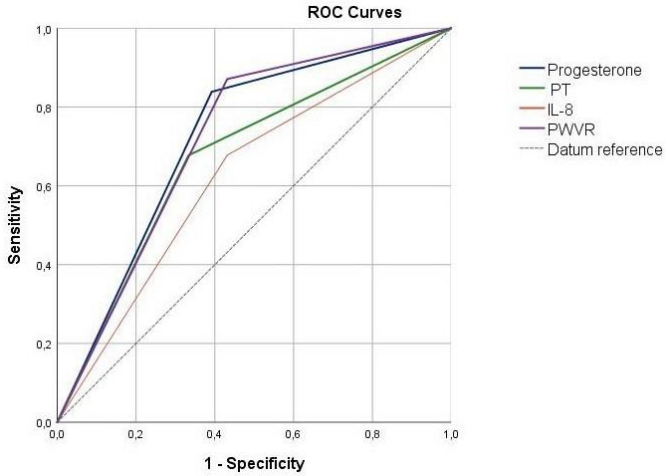

Figure 3. ROC curves for risk of OP progression in patients with AH and OP.

PT, parathyroid hormone; IL, interleukin; PWVR, pulse wave velocity in elastic arteries on the right.

In the group with AH and OP, risks of the bone destruction process progression were observed with changes in certain biochemical markers. For instance, the risk of OP increased 2.5-fold with an increase in IL-6 by 1 pg/mL (OR 1.037 CI 1.01; 1.065, p=0.048). Also, this risk increased twofold with increase in TNF-α by 1 pg/mL (OR 1.99 CI 1.107; 0.58, p=0.022), by 6.5% with decrease in estrogen by 1 nmol/L (OR 0.967 CI 0.935; 1.00, p=0.052), by 18% with increase in HYC by 1 µmol/L (OR 1.18 CI 1.023; 1.361, p=0.023), by 65% with decrease in progesterone by 1 nmol/L (OR 0.348 95% CI 0.164; 0.739, p=0.006), by 3.7% with increase in parathyroid hormone by 1 pg/mL (OR 1.037 95% CI 1.01; 1.065, p=0.009), by 13.6% with increase in IL-8 by 1 pg/ml (OR 1.136 95% CI 1.016; 61.27, p=0.025), and by 54% with decrease in IL-10 by 1 pg/mL (OR 0.459 95% CI 0.252; 0.837, p=0.011). As for functional parameters, the risk of OP increased sixfold with an increase in PWV by 1 m/s (OR 6.06 95% CI 2.203; 16.69, p=0.00048).

The characteristics of OR that maximally determined the risk of OP in the study groups with AH, and with osteopenia and OP are presented in *Figures* 1 and 2. According to the data presented in *Figure* 1, in the OP Group, the most reliable parameter that determined the risk of OP was PWV-R value. According to the data presented in *Figure* 2, increased levels of parathyroid hormone and inflammatory markers IL-6 and 8, TNF-α, hs-CRP, as well as reduced levels of progesterone and anti-inflammatory IL-10, were most actively involved in the aggravation of pre-existing destruction of bone tissue.

In addition, during the receiver operating characteristic (ROC) analysis in the group of patients with AH and OP, cut-off points for augmented risk of OP progression were determined: for example, with decrease in progesterone levels below 0.93 nmol/L, the risk of OP increased ninefold (sensitivity 76.9%, specificity 85.7); with increase in parathyroid hormone levels over 28.14 pg/mL, the risk of OP increased by 3.7% (sensitivity 68.6%, specificity 69.2%); with increase in IL-8 over 10.25 pg/mL, the risk of OP rose by 13.6% (sensitivity 71.4%, specificity 64.1); with decrease in IL-10 levels below 3.465 pg/mL, the risk of OP augmented by 54.1% (sensitivity 66.7%, specificity 62.9%). As for functional parameters, with increase in PWV-R over 12.05 m/s, the risk of OP increased sixfold (sensitivity 87.1%, specificity 89.3) (*Figure* 3).

The results of the discriminant analysis clarified that the most significant parameters for the progression of OP in Group 3 of patients were progesterone, parathyroid hormone, IL-8 and PWV-R. The model obtained as a result of the discriminant function calculations was statistically significant (the Wilks lambda = 0.201, p<0.001) with a canonical correlation coefficient of 0.894.

At the same time, the greatest diagnostic contribution to the progression of OP was made by the level of progesterone (standardized coefficient 0.843). Standardized coefficients of parathyroid hormone (0.523), IL-8 (0.367) and PWV-R (0.413) demonstrated similar diagnostic significance of these variables. The equation of the resulting discriminant function is as follows:

$$F = -2.618 + 42.951\times\text{progesterone} + 1.293\times\text{parathyroid hormone} + 0.749\times\text{IL-8} + 1.025\times\text{PWV-R}.$$

The specificity of this model was 100%, the sensitivity was 92%; 96% of original observations were classified correctly. To enable classification, centroids were calculated for each group and a cut-off point to more accurately identify group membership. The mean value of the function was -1.915 for Group 1 and -1.992 for Group 2; the cut-off point (or threshold value) was equal to the function value (0.039).

Discussion

The demographic situation worldwide is characterized by a steady increase in the proportion of older people. In the context of a steadily aging population, the problem of much higher number of socially significant diseases in women is becoming increasingly important. The most common causes of disability and mortality in older postmenopausal women include the clinical consequences of AS and OP, such as cardiovascular accidents and bone fractures. It is known that many factors affecting bone metabolism are involved in the mechanisms of vascular diseases. There are similarities in the course of these diseases, since they can be asymptomatic for many years and often have clinical manifestations after menopause.

Recently, much attention has been drawn to the role of the nonspecific immunoinflammatory vascular response as a link between the general pathogenetic mechanisms of atherosclerotic damage to the vascular bed and the phenomenon of destructive changes in bones.

A multimarker approach to studying the general links in the pathogenesis of socially significant diseases made it possible to clarify the main risk factors, laboratory levels of markers of a nonspecific immune inflammatory response and parameters of hormonal and vitamin statuses that determine the degree of elastic function impairment. The properties of the vascular wall and the risk of OP progression may be predictors of cardiovascular and degenerative bone complications in postmenopausal women with AH.

In our study, the following markers of vascular inflammation were increased: hs-CRP, HYC, IL-8, endothelin 1, parathyroid hormone, total cholesterol and atherogenic lipid fractions, with a simultaneous decrease in the levels of estrogen, progesterone, calcium and vitamin D. Multiple statistically significant relationships were observed between indicators of inflammation and those of lipid metabolism, hormonal status, and vitamin status.

To some extent, this means that less sun exposure leads to vitamin D deficiency and decreased calcium absorption in the body, along with anxiety and depression, leading to unhealthy psychological states.

Results of the analyses showed that postmenopausal OP affects both the physical and psychological health of patients. Fractures resulting from postmenopausal OP were negatively associated with overall quality of life and physical functioning [15]. Published systematic reviews and meta-analyses demonstrated that postmenopausal women with osteoporotic fractures had lower quality of life than women with controlled postmenopausal OP.

However, we would like to emphasize that the exact mechanism of OP development is not entirely clear. There is already a large body of evidence indicating a close relationship between the gut microbiome, bone metabolism, and mineral metabolism both in healthy condition and in the presence of a disease. The human intestine contains a huge number of microorganisms that maintain close symbiotic relationships with the host's body and its state of health. With the development of modern sequencing platforms, there is increasing evidence that the gut microbiome plays an important role in the development of CVD associated with AS and the development of OP, which combines the socially significant conditions presented in the article. Experimental evidence using mice lacking gut microbiota has shown that the gut microbiome was more likely to regulate bone metabolism by influencing osteoclast activity. The influence of the gut microbiome on calcium absorption has been studied, and the process has been shown to be regulated by short-chain fatty acids (SCFAs), which increase calcium absorption into the intestinal wall by lowering intestinal pH. In addition, a connection has been established between microbiota and vitamin D, a decrease in the level of which can regulate the development of OP by directly controlling the intestinal microbiome, in particular, by reducing the proportion of Firmicutes.

A number of scientists showed that deficiency of sex steroids in mice increases the production of inflammatory cytokines TFN-a, IL-17, and affects the response of neurotransmitters determining the development of depressive states in OP. Our study demonstrated gender- and age-related changes in the metabolome of the intestinal microbiota in patients with AH and abdominal obesity. In our earlier study, we observed a significant increase in the levels of TMA and TMAO, IL-1.6, hs-CRP and GDF15 and a decrease in the levels of SCFA, adiponectin, sex hormones, intestinal hormones and calcium in the presence of multidirectional correlations of biomarkers with PWV and left ventricular hypertrophy [16].

In this regard, the problem under discussion remains very relevant.

Conclusion

The results of the study imply the necessity of early examination of women with AH to identify increased rigidity of the vascular wall and decreased BMD, which create conditions for an increased risk of development and progression of subclinical AS and OP prior to postmenopause.

Timely in-depth examination of premenopausal women with AH should become the main strategy for developing personalized prevention and treatment for women in order to prevent socially significant cardiovascular and bone complications, such as coronary artery disease, stroke, and low-trauma fractures.

Limitations of the study

This is a pilot project that requires a larger patient sample to clarify the mechanisms of the relationships between the processes under study. The planned comparative characteristics of the studied indicators between groups of women and men will require expanding the range of statistical methods for processing the collected data.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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