UDC 616.72+616.711-002:616.13/.137-007-094:616.15-073.178

NATURE OF ENDOTHELIAL DYSFUNCTION IN ANKYLOSING SPONDYLITIS
ХАРАКТЕР ЭНДОТЕЛИАЛЬНОЙ ДИСФУНКЦИИ ПРИ АНКИЛОЗИРУЮЩЕМ СПОНДИЛИТЕ

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Abstract. In this research we evaluated the nature of endothelial dysfunction and vascular adsorption-rheological properties of serum in patients with ankylosing spondylitis, determined its characteristics in the presence of angiopathy and established communication with the clinical and instrumental parameters of vascular disease and the state of immunity. The study included 79 patients aged 15 to 66 years, among whom there were 95% of men and 5% of women. Endothelial vessels dysfunction occurs in each second patient with ankylosing spondylitis, which in the presence of clinical and instrumental vascular pathology accompanied by an increase in serum concentration of cyclic guanosine monophosphate and even greater reduction in prostacyclinemia parameters. The severity of disorders of vascular endothelial function in these patients is associated with disease duration, activity of the pathological process, the lesion of the peripheral nervous system, the severity of spondylopathies and sacroiliitis.
Keywords: ankylosing spondylitis, serum, vessels, endothelium, rheology.

Аннотация. В работе проведена оценка характера эндотелиальной дисфункции сосудов и адсорбционно-реологических свойств крови у больных анкилозирующим спондилитом, определены ее особенности при наличии ангиопатии, установлена связь с клинико-инструментальными показателями сосудистой патологии и состоянием иммунитета. Под наблюдением находились 79 больных в возрасте от 15 до 66 лет, среди которых были 95% мужчин и 5% женщин. Эндотелиальная дисфункция сосудов развивается у каждого второго больного анкилозирующим спондилитом, которая при наличии клинико-инструментальной сосудистой патологии сопровождается нарастанием в крови концентрации циклического гуанозинмонофосфата и еще большим уменьшением параметров простациклинемии. Степень выраженности нарушений эндотелиальной функции сосудов у таких больных связана с длительностью заболевания, активностью патологического процесса, поражением периферической нервной системы, выраженностью спондилопатии и сакроилеита.
Ключевые слова: спондилит анкилозирующий, сосуды, кровь, эндотелий, реология.

Introduction. Ankylosing spondylitis (AS) is a chronic progressive systemic autoimmune rheumatic disease of spine, peripheral joints, enthesis, ligamentous apparatus and internal organs [9, 11]. AS relates to the risk factor for severe vascular pathology [4, 10], and due to inflammation of small vessels, blood circulation of heart and brain is disrupted, and such cardiovascular and cerebrovascular changes correlate with the duration of the pathological process [13], as well as with the severity of changes in the locomotor system apparatus [7]. At present, the nature of the clinical-instrumental course of angiopathy in AS has not been studied sufficiently and pathogenetic constructs of vascular changes have not been elucidated [1, 2].

AS refers to diseases characterized by impaired vascular endothelial function [3], which is confirmed by experiments in mice with proteoglycan induced spondylitis [12]. Endothelial dysfunction in AS is accompanied by the higher indices of inflammatory proteins, pro-inflammatory cytokines, matrix metalloproteinases, cell adhesion

molecules, vascular endothelial growth factor (VEGF) and endothelin-1 (ET1) in blood [4, 8]. In most cases, vessels of brain, lungs and kidneys react with changes in the endothelium function and the rheological state of blood in these patients [5, 6]. It should be noted that the character of endothelial vascular dysfunction (EVD) in patients with AS requires further study, its features are not determined in the presence of angiopathy. The connection with clinical and instrumental indices of vascular pathology and the state of immunity and the adsorption-rheological properties of blood have not been studied properly [7, 13]. This was the purpose and objectives of this study.

Material and methods. 79 patients with AS at the age from 15 to 66 years old (on the average, 38.3 ± 1.28 years old) were observed, 94.9% of men and 5.1% of women among them. The duration of the disease from its first manifestations was 11.4 ± 0.83 years. The first degree of AS activity observed in 31.7% of observations, the second – in 39.2% and the third – in 29.1%, and the ratio of slowly to the rapidly progressive pathological process was 4: 1. Peripheral mono-oligo arthritis was found in 46.8% of the patients, polyartritis – in 53.2%.

The patients underwent X-ray examination of peripheral joints, sacroiliac and vertebra articulations (Multix-Compact-Siemens apparatus, Germany), joints' sonography (Envisor-Philips, The Netherlands), dual-energy X-ray osteodensitometry of the proximal femur (QDR-4500-Delphi –Hologic, USA), echocardiography (Acuson-Aspen-Siemens, Germany, Envisor-C-Philips, the Netherlands), ultrasonic vascular dopplerography (angioplasty Aplia-XG-Toshiba, Japan), conjunctival biomicroscopy (slit lamp "Haag-Streit-Bern-900", Switzerland).

The Lansbury index was 37.3 ± 3.20 points, the index of arthritis progression was 0.6 ± 0.18 r.u. Spondylopathy was diagnosed in 93.7% of patients, both-side sacroileiitis – in 73.4%, tendovaginitis – in 12.7%, enthesopathies – in 10,1%, the lesion of shoulder joints was detected in 43.0%, knee – in 40, 5%, metacarpophalngeal – in 19.0%, hip – in 17.7%, radiocarpal – in 16.5%, elbow – in 11.4%, ankle – in 3.8%, changes in heart (excitability and conductivity disorders, valves lesion, increase chambers in size) identified in 68.4% of cases, the kidneys (glomerulonephritis, secondary AA amyloidosis) – in 32.9%, lung (interstitial pneumonitis, fibrosing alveolitis) – in 13.9%. According to X-ray study, epiphyseal joints osteoporosis is set at 36.7% of patients, systemic – 29.1%, osteocystosis – 27.9%, subchondral sclerosis – 11.4%, osteousuras – 8.9%, Goff's intraarticular bodies – 6.3%. Studied clinical course angiopathy index (Ω) by the formula: $\Omega=(\Sigma:N)\sqrt{Z}$, where Σ – the scores sum of all AS clinical vascular signs, N – number of features, Z – the degree of disease activity, and the instrumental vascular index (Ψ) was determined, with each medium indicator in a patient (M), its standard deviation (SD) was evaluated by 1 point in case <M + SD, in M+SD↔M+2SD – 2 points, in M+2SD↔M+3SD – 3 points, in > M + 3SD – in 4 points. Counted Ψ on one patient by the formula: Ψ=(a+2b+3c+4d):n, where «a, b, c, d» – amount of patients with 1, 2, 3 and 4 points respectively, and "n" is the number of indicators .

The levels of VEGF, ET1, thromboxane-A2 (TxA2), homocysteine (HCys), prostacyclin (PgI2), cyclic guanosine monophosphate (cGMP), E-selectin (ESel), P-selectin (PSel) and antibodies to cyclic citrullinated peptide (aCCP) were studied in serum by the enzyme immunoassay (PR2100 Sanofi diagnostic pasteur, France), and the concentrations of rheumatoid factor (RF), C-reactive protein (CRP), fibrinogen (FG), immunoglobulins (Ig) A, G, M, circulating immune complexes (CIC) – with the Olympus-AU-640 analyzer (Japan). Surface parameters of viscosity (SV), elasticity (SE), tension (ST), serum relaxation (SR) and viscoelasticity modulus (VE) were evaluated using a computer tensiometer "PAT2-Sinterface" (Germany). Seropositivity by RF (> 14 IU/ml) was found in 6.3% of cases, by aCCP (> 17 U / ml) – in 83.6%. To study the integral index of EVD (Θ), the change degree (Δ) of vasoconstrictors parameters – VEGF, ET1 and TxA2, as well as PgI2 vasodilator, was calculated according to the formula: M = [(W-V): SD] 2, where W is indices in patients, V – in healthy, SD – standard deviation in healthy. Θ determined by the formula: $\Theta=\sqrt{(U+V+W):Y}$, where "U" is VEGF, "V" is ET1, "W" is TxA2, "Y" is PgI2 (EVD was diagnosed at Θ > 5 r.u.). As a control, laboratory parameters of blood-vessel rheological properties (BVRP) were studied in 30 practically healthy people (17 men and 13 women aged 18-65 years).

Statistical analysis of the research results was carried out by computer variational, nonparametric, correlation, regression, ANOVA and multivariate ANOVA / MANOVA variance analysis (Microsoft Excel and Statistica-Stat-Soft, USA). The mean values (M), their standard deviations (SD) and errors (m), the Pearson parametric correlation coefficients (r) and the nonparametric Kendall (τ), Brown-Forsythe dispersion (BF), the multiple regression (R), Student, Wilcoxon –Rao (WR), McNamara-Fisher (χ2) and the reliability of statistical indicators (p) were assess.

Results. In patients with AS, blood VEGF values were 103.9 ± 3.47 pg / ml, ET1 – 6.1 ± 0.18 pg / ml, TxA2 – 17.6 ± 0.93 ng / ml, HCys – 15.6 ± 0.43 μmol / l, PgI2 – 38.2 ± 0.88 ng / ml, cGMP – 13.4 ± 0.23 pkmol / ml, ESel – 44.1 ± 0.63 ng / ml, PSel – 217 , 4 ± 2.00 ng / ml, SV – 16.8 ± 0.24 mN / m, SE – 46.6 ± 0.64 mN / m, ST – 72.7 ± 4.45 mN / m, SR – 85,1 ± 4,22 s, VE – 26,1 ± 0,71 mN / m. Compared to the similar ones in healthy people, in case AS, there were significantly higher (5.3 times) PSel values (t = 52.35, p <0.001), 2.2 times TxA2 (t = 5.35, p <0.001), by 71% ST (t = 4.82, p <0.001), by 68% HCys (t = 8.41, p <0.001), by 53% ET1 (t = 7.16, p <0.001), by 20% cGMP (t = 5.52, p <0.001), by 16% VEGF (t = 2.14, p = 0.034), by 10% VE (t = 2.11, p = 0.037), by 9% SV and SE (respectively, t = 3.72, p <0.001 and t = 3.67, p <0.001), but lower by 24% SR (t = 4.15, p <0.001), by 47% PgI2 (t = 6.08, p <0.001) and 82% ESel (t = 27.31, p <0.001). In general, EVD was established in 53.2% of the number of the examined patients.

Among vessels pathology, discirculatory encephalopathy was diagnosed in 53.2% of patients, pulmonary hypertension (mean pressure more than 20 mmHg) – in 51.9%, peripheral vasoneuropathy – in 32.9%, and left common carotid artery (CCA) changes – in 27, 9%, uveitis – in 26,6%, changes in aorta and right CCA – in 20,3%, scleritis – in 17,7%, arterial hypertension (mean pressure more than 115 mmHg) – in 15,2%, Raynaud's syndrome – in 13,9%, changes in the left internal carotid artery (ICA) – in 11,4%, skin vasculitis – in 10,1%, lesion of the right ICA – in 7,6%, antiphospholipid syndrome – in 5,1% . 67 (84.8%) AS patients with angiopathy were included in the main group of surveyed, and the remaining 12 (15.2%) patients made up a control group. In the patients of the main group, values of cGMP (t = 2.22, p = 0.030) were established reliably higher by 12%, and values of PgI2 (t = 2.94, p = 0.004) – 15% lower.

There are relationships between separate immune parameters and parameters of BVRP, which is demonstrated by Pearson's analysis. Thus, the level of RF correlates with the content of PgI2 in blood (r = +0.266, p = 0.023), cGMP (r = –0.532, p <0.001) and ESel (r = –0.342, p = 0.002), aCCP – with HCys concentration (r = + 0.248, p = 0.028), CRP with PSel (r = + 0.473, p <0.001), FG – with ET1 (r = + 0.287, p = 0.010), SV (r = –0.390, p < 0.001), SE (r = –0.252, p = 0.025), ST (r = + 0.260, p = 0.021) and SR (r = –0.0249), IgA – with TxA2 –0.432, p <0.001), IgM – with VE (r = + 0.242, p = 0.032), CIC – with VEGF (r = + 0.304, p = 0.007).

As evidenced by the multifactorial variance analysis of Wilcoxon-Rao, the prevalence of peripheral articular syndrome (WR = 1.27, p = 0.035), the presence of discirculatory encephalopathy (WR = 5.74, p <0.001), peripheral neuropathy (WR = 3.97, p <0.001), pulmonary hypertension (WR = 2.21, p = 0.018), changes in the left and right CCA (respectively WR = 6.54, p <0.001 and WR = 3, 26, p = 0.002) are affect integral properties of BVRP.

Discussion. We selected those factors that had significant variance-correlation relationships between the separate parameters of BVRP and the clinical course of the disease, which simultaneously met the criteria of Brown-Forsythe and Kendall. Thus, the ESel level in blood depended on the duration of the disease (BF = 2.01, p = 0.017; τ= + 0.198, p = 0.010), cGMP – on the degree of the pathological process activity (BF=2,84, p=0,009; τ=-0,191, p=0,013), SV – from the severity of the spondylopathy (BF = 2.12, p = 0.035; = +0,183, p = 0,017), VE – from the severity of sacroiliitis (BF = 2.10, p = 0.012; = + 0.167, p = 0.029), PSel – from the degree of arthritis progression (BF = 7.51, p <0.001; τ = +0,160, p = 0,037), ET1 – from peripheral neuropathy (BF = 2,28, p = 0,038; τ= + 0,401, p <0,001), TxA2 – from the presence of uveitis (BF = 2.09, p = 0.016; = +0.296, p <0.001).

ANOVA / MANOVA showed no effect of EVD on integral indices of joint syndrome (WR = 0.75, p = 0.634) and extraarticular signs of AS (WR = 1.18, p = 0.314). The single-factor analysis of variance indicates the effect of VEGF (BF = 2.47, p = 0.005) and ET1 (BF = 3.27, p <0.001) on the development of enthesopathy, and HCys (BF = 2.07, p = 0.017) and PgI2 (BF = 2.71, p = 0.002) – on the development of tendovaginitis, SR (BF = 11.81, p = 0.001) – on the rate of arthritis progression, ESel (BF = 7.34, p = 0.008 and BF = 4.46, p = 0.038) – on the formation of uveitis and scleritis, PSel (BF = 4.55, p = 0.036) – on the formation of pulmonary hypertension, cGMP (BF = 4.75, p = 0.032) – on the development of the peripheral vasoneuropathy .

The index Θ affects the lesions of the central and peripheral nervous system (respectively, BF = 3.94, p = 0.048 and BF = 9.29, p = 0.003), and the presence of peripheral neuropathy directly correlates with this

integral severity criterion of EVD in patients (τ= + 0.296, p <0.001). In addition, the parameters of pulmonary vascular resistance (BF = 2.02, p = 0.026), CCA damage (BF = 10.94, p = 0.001 and BF = 10.31, p = 0.002), vessel conjunctival index (BF = 2.13, p = 0.049) and the sonographic index of vascular tension (BF = 2.65, p = 0.040) depends on Θ. Correlations relate to mean arterial pressure (τ= + 0.186, p = 0.015), vascular wall tension (τ= + 0,639, p <0,001) and the degree of changes in right CCA and ICA (respectively τ= + 0.256, p = 0.001 and τ= + 0.185, p = 0.016). Taking into account the statistical processing of the obtained research data, the following conclusion has been made, which has a practical focus: the index Θ> 10 r.u. (> M + SD AS patients with EVD) is a risk factor for the development of peripheral neuropathy, changes in carotid arteries and increased degree of vascular arterial wall tension.

The content of cGMP in blood of patients with AS is directly correlated with the levels of Ω and Ψ, as evidenced by Brown-Forsyth dispersion analysis (respectively, BF = 4.64, p <0.001 and BF = 4.25, p <0.001) and Pearson correlation (r = + 0.300 , p = 0.007 and r = + 0.371, p = 0.001). In addition, serum concentrations of ET1 (BF = 2.21, p = 0.043), TxA2 (BF = 2.70, p = 0.002) and ESel (BF = 3.66, p <0.001) are affected Ψ, and the correlation bounds of Ψ are related to values of VEGF (r = + 0.444, p <0.001), ESel (r = + 0.461, p <0.001), PSel (r = –0.242, p = 0.031) and SR (r = + 0.231, p = 0.044). In our opinion, the blood count of cGMP> 18 pmoles/ml (> M + SD patients in the main group) is a negative integral prognosis of vascular pathology in AS.

Conclusions.

1. EVD develops in 53% of the patients' number with AS, which in the presence of clinical and instrumental vascular pathology is accompanied by an increase in the concentration of cGMP in blood and greater reduction in the content of prostacyclinemia.
2. The degree of EVD intensity in patients with AS is associated with the duration of the disease, the degree of the pathological process activity, the damage of the peripheral nervous system, the severity of spondylopathy and sacroiliitis, and there are correlations between separate immune parameters and BVRP parameters.
3. BVRP in AS is involved in the pathogenetic constructions of enthesopathy, tendovaginitis, uveitis and scleritis, determine the rate of the joint syndrome progression and the integrated severity of angiopathy, and the cGMP content in the blood has prognostic significance.

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