SUMMARY

Title: „Evaluation of the kinurenin system in systemic scleroderma”

Systemic sclerosis (SSc) is a chronic systemic connective tissue disease characterized by immune dysregulation, vasculopathy and fibrosis of the skin and internal organs. Due to the variety of organ manifestations in SSc, patients require targeted organ-specific therapy. However, despite the implementation of novel therapies, the disease is still characterized by an aggressive course and a high mortality rate. Therefore, further scientific research is needed to search for new molecules or metabolic pathways that are potential points of action for new drugs and/or have prognostic significance. Methabolites synthesized from tryptophan in the so called kynurenine pathway are involved in the regulation of processes which are of key importance in SSc pathogenesis: immune response, vascular function and remodeling of connective tissue.

Study Objective: The aim of the present study was to evaluate the role of the kinurenin system in SSc by comparative analysis of kinurenin system parameters (tryptophan (TRP), kinurenin (KYN), 3OH-kinurenin (3-HKYN) concentrations and tryptophan/kinurenin-T/K ratio) in SSc patients and healthy subjects and correlation of their concentrations with clinical picture .

Material and methods: Seventy-two patients with SSc diagnosed on the basis of the current 2013 ACR/EULAR classification criteria for SSc and/or the earlier classification criteria developed by the ACR in 1980 were included in the study. The control group included 31 healthy subjects. The clinical evaluation of SSc patients included a number of clinical and laboratory parameters performed as part of the routine clinical evaluation of patients. The current project evaluated serum concentrations of the following metabolites of the kinurenine pathway: tryptophan (TRP), kinurenine (KYN) and 3OH-kinurenine (3-HKYN). In addition, the ratio of tryptophan to kinurenine concentration was calculated: tryptophan/kinurenine ratio (T/K). Determinations of the above-mentioned metabolites of the kinurenine system were performed at the MUB Pharmacodynamics Department by high-performance liquid chromatography (HPLC) using Agilent Technologies LC system 1260 series chromatographic equipment. TRP and KYN concentrations were measured by the Holmes method, while 3-HKYN was performed as described by Heyes. Statistical analysis was performed using STATISTICA version 12.

Results: Seventy-two patients with SSc were included in the analysis, including 64 women (accounting for 88.9% of all patients) and 8 men (11.1% of the study group), ranging in age from 25 to 78 years (mean; +/- SD: 54 years; +/- 12 years). Twenty-two (30.55%) patients had the diffuse systemic sclerosis (dSSc), and the remaining 50 (69.44%) patients had the limited systemic sclerosis (lSSc). The duration of the disease, defined by the first non-Raynaud symptom, ranged from 0.3 years to 30 years and averaged (+/- SD): 5.39 (+/- 5.6) years. Forty (56%) patients had early onset SSc, while the remaining 32 (44%) patients had late onset SSc. Organ involvements in the form of interstitial lung disease (ILD), pulmonary hypertension (PH) and/or digital ulcers were found in: 38, 10 and 13 patients, which accounted for (respectively): 56%, 14.93% and 20.65% of patients with available data. Antinuclear antibodies (ANA) were found in 70 (97.22%) patients, while individual specific antibodies were detected: anticentromere antibodies (ACA) in 26 patients, against topoisomerase I (anti-topo I, anti-Scl70) in 24 patients, and against RNA polymerase type III (anti-RNAPolIII) in the remaining

4 patients, accounting for, respectively: 37.14%, 34.28% and 5.71% of patients with the presence of autoantibodies.

The group of patients with SSc had significantly higher levels of KYN (2.80 +/- 1.11 μM/L) and 3-HKYN (139.79 +/- 124.90 nM/L) compared to the control group (2.12±0.58 μM/L, p=0.001 and 70.49±41.01 nM/L, p=0.003, respectively) and a significantly lower T/K ratio (13.04±7.05) compared to the control (17.46±6.11) (p=0.003). It was shown that the T/K ratio was significantly lower in male patients (8.90+/- 2.95) compared to female patients (13.56±7.26, p=0.027); in patients with early onset disease (11.30+/- 4.74) compared to patients with late onset systemic sclerosis (15.23+/-8.77, p=0.026); in patients with ILD (10.99 +/- 4.68) compared to patients without ILD (16.27 +/- 8.62, p=0.001). In addition, the T/K ratio showed the highest number of significant correlations with the clinical or laboratory parameters studied: the extent of skin involvement measured by Rodnan Scale (mRSS) (rS=-0.531), DLCO value (rS=0.350), and inflammatory parameters ESR (rS=-0.496) and CRP (rS=-0.312).

In contrast, KYN levels showed a significant correlation with the presence of ILD (2.99 +/- 1.02 μM/L in patients with ILD compared to 2.56 +/- 1.27 μM/L without ILD, p=0.022), and correlated with mRSS (rS=0.461) and ESR values (rS=0.387).

We have also shown that 3-HKYN levels were significantly higher (214.06 +/- 95.39 nM/L) in patients with PH compared to those without PH (119.10 +/- 114.19 nM/L) (p=0.0016), and correlated with PASP values (rS=0.415).