



Serum Autoantibodies Profile and Increased Levels of Circulating Intercellular Adhesion Molecule-1: a Reflection of the Immunologically Mediated Systemic Vasculopathy in Rheumatic Diseases?

ANNA KURLISZYN-MOSKAL*, PIOTR ADRIAN KLIMIUK and STANISŁAW SIERAKOWSKI

Department of Rheumatology and Internal Diseases, Medical University of Białystok, Skłodowskiej-Curie 24a, 15-276 Białystok, Poland

Abstract. The clinical manifestation of systemic vasculitis may be postulated as a consequence of immune response abnormalities in the course of connective tissue diseases (CTD). The aim of this study was to elucidate the significance of the different autoantibodies and soluble intercellular adhesion molecule 1 (sICAM-1) being shed into the circulation in the diagnosis of vasculitis in rheumatic diseases. Sera of 86 patients with rheumatic diseases (54 with rheumatoid arthritis (RA) and 32 with CTD) were analyzed for the concentrations of sICAM-1 levels by the enzyme-linked immunosorbent assay (ELISA). Control sera were obtained from 30 healthy individuals. Anti-nuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA) antibodies and anti-proteinase 3 (PR-3) antibodies (cytoplasmic specific anti-neutrophil cytoplasmic autoantibodies, cANCA) were assessed by the ELISA method. Fifty out of the 86 patients had systemic lesions. A pathological picture of the vascular loop under nailfold capillary microscopy was found in 84 patients. In 19 patients the microvascular changes were advanced, in 35 moderate and in 30 mild. All patients with articular manifestations had pathological changes under capillary microscopy. Patients with advanced changes under capillary microscopy had longer disease durations than patients with a mild intensity of vasculitis. The serum concentrations of sICAM-1 were significantly increased in RA and CTD patients compared with 30 controls (in both cases $p < 0.001$). Moreover, RA and CTD patients with systemic vasculitis showed significantly higher levels of sICAM-1 than those without vascular involvement ($p < 0.001$ and $p < 0.005$ respectively). ANA were observed in significantly elevated concentration among RA and CTD patients with the systemic damage compared with patients without organ injury ($p < 0.001$ and $p < 0.05$ respectively). Also, cANCA levels were two-fold higher, but only among CTD patients with systemic damage ($p < 0.05$). Serum concentrations of sICAM-1 were elevated in the patients showing the presence of ANA antibodies ($p < 0.05$). Significant correlations between ANA level and disease duration and hemoglobin concentration were observed. The concentrations of cANCA correlated with those of rheumatoid factor and of dsDNA with patient age. We conclude that systemic lesions in the course of RA and CTD are accompanied by the microvascular injury observed under nailfold capillary microscopy. Our data suggest that sICAM-1, ANA and cANCA serum levels may reflect the extent of the vascular involvement in RA and CTD patients.

Key words: autoantibodies; sICAM-1; rheumatoid arthritis; connective tissue diseases; vasculitis; capillary microscopy.

* Correspondence to: Anna Kuryliszyn-Moskal, M.D., Ph.D., Department of Rheumatology and Internal Diseases, Medical University of Białystok, M. Skłodowskiej-Curie 24a, 15-276 Białystok, Poland, tel.: +48 85 746 84 82, fax: +48 85 746 86 06, e-mail: klimp@amb.ac.bialystok.pl

Introduction

Vascular involvement in connective tissue diseases (CTD) is associated with a wide range of extra-articular complications and has been implicated in increased mortality among rheumatoid arthritis (RA) and CTD patients^{24, 31}. Damage of the internal organs occurs through a widespread disorder of the microvasculature and, therefore, early detection of the vascular involvement plays a crucial role in the diagnosis of vasculitis²⁷. Nailfold capillary microscopy has been used extensively as a non-invasive method for investigating microvascular involvement in rheumatic diseases^{1, 8, 19}. Furthermore, microvascular capillaroscopic abnormalities have been shown to be associated with histopathological changes in skin biopsy specimens of RA patients¹⁸.

Cytokines, adhesion molecules and autoantibodies are known to be involved in the pathogenic immune and inflammatory responses in vasculitis syndromes^{29, 30}. Cell adhesion molecules have been detected as soluble circulating forms (sCAM) in human serum, synovial fluid and synovial membrane in RA and other inflammatory arthropathies²¹. Intercellular adhesion molecule-1 (ICAM-1) is a membrane-bound molecule that plays an important role in the pathogenic inflammatory responses observed in vasculitis^{6, 15, 28}. In a previous study we demonstrated significantly elevated serum levels of soluble ICAM-1 (sICAM-1) in RA patients with systemic vasculitis¹⁷.

Growing evidence points to the pathophysiologic and diagnostic value of various anti-nuclear antibodies (ANA) and anti-neutrophil cytoplasmic autoantibodies (ANCAs) in rheumatic diseases^{7, 26, 34}. Over the past decade, ANCAs have been analyzed as markers for the underlying immunopathogenic disturbances in systemic vascular disorders^{4, 33}. To date, there is mounting evidence that ANCAs can induce the expression of ICAM-1 on endothelial cells¹² and the adhesion of neutrophils to cultured endothelial cells, and that endothelial cells stimulated by cytokines express ANCA antigens¹⁴. Data regarding the diagnostic value of ANCAs and their pathophysiological role in rheumatic diseases are controversial. In RA, associations between ANCAs and disease activity and extra-articular manifestations, including rheumatoid vasculitis, have been observed^{4, 22, 33}, but not confirmed by others¹³.

The aim of this study was to determine whether the levels of soluble ICAM-1 (sICAM-1) being shed into the circulation reflect the vascular injury found in the nailfold capillaroscopy as well as systemic vasculitis in patients with RA and CTD. Furthermore, to determine the presence of autoantibodies in patients with systemic

vasculitis, we investigated the incidence of ANA, anti-double-stranded DNA (anti-dsDNA) and cANCA in RA and CTD patients and their relationships to the clinical severity of the disease and extra-articular involvement.

Materials and Methods

Patients and controls. The study was carried out on 54 patients with RA and 32 with CTD (7 patients with systemic lupus erythematosus (SLE), 15 with scleroderma, 1 with dermatomyositis/polymyositis (DM/PM), 7 with overlap syndrome (SLE/RA) and 2 with mixed connective tissue disease (MCTD)), who met the diagnostic criteria of the American Rheumatism Association^{2, 11}. There were 2 males and 84 females with ages ranging from 18 to 70 years (mean 50.0 ± 12.0 years). The duration of the disease ranged from 0.5 to 30 years (mean 8.4 ± 7.2 years). Thirty healthy volunteers matched for sex and age were included in the study as a control group.

Clinical evaluation. Clinical disease manifestations, namely skin involvement (cutaneous vasculitis), nodules, joint involvement, heart, lung and renal involvement determined by pulmonary or renal function tests, as well as treatment status were recorded at the time of sampling. In addition, a chest X-ray, renal and heart sonography and bronchoscopy were used for the assessment of the extent and severity of the visceral manifestations.

All patients were classified into 2 groups according to the presence of extra-articular manifestations: those with the clinical and laboratory signs of systemic vasculitis (RA: $n=23$, CTD: $n=27$) and those without data suggestive of systemic vascular involvement (RA: $n=31$, CTD: $n=5$). Five of the 50 RA and CTD patients with the systemic manifestations showed renal involvement indicated by the presence of hematuria or proteinuria (3 patients with mesangial glomerulonephritis, 2 with amyloidosis, proven histologically). Seven patients suffered from dysphagia with esophagus involvement, 8 had cardiopulmonary involvement, 5 had hypertensive cardiovascular disease, 3 had fibrosing alveolitis, 4 had peripheral neuropathy, 1 had gastrointestinal manifestations and 4 RA patients had nodules. Five patients demonstrated constitutional features of fever, weight loss, fatigue, arthralgia and myalgia. Vasculitis of the skin was found in 8 patients in the form of palpable purpura, reticular livedo or digital ulcerations. All 50 patients had clinical, laboratory and/or morphological evidence of systemic involvement.

All patients were receiving treatment at the time of blood collection, including nonsteroidal anti-inflammatory drugs with or without other anti-rheumatic medication: methotrexate (33), sulphasalazine (9), IM gold (10 mg/week) (1) cyclosporine (2), chloroquine (6), cyclophosphamide (4) and prednisolone (5–10 mg/day in 8 cases and 15–45 mg/day in 8 others).

Nailfold capillary microscopy. Nailfold capillaroscopy examination was performed using a stereomicroscope SZ 4045 (Olympus, Germany). All fingers except the thumbs were examined.

The intensity of the morphological changes was evaluated on the basis of a semiquantitative method which included the parameters: loop density, percent of loops with architectural derangement, and the presence of extravasations into perivascular tissue. The intensity of capillaroscopic changes was expressed according to a scale from 0 to 3, where: 0 – indicated no vascular changes, 1 – mild vasculitis (30–50% morphologically changed capillaries with diminished loop density and without perivascular changes), 2 – moderate vasculitis (more than 50% morphologically changed loops with low capillary density without extravasations and clearly visible subpapillar venous plexus), and 3 – severe vasculitis (more than 75% of the loops with extravasations into perivascular tissue and extensive visible subpapillar venous plexus).

Laboratory studies. Comprehensive laboratory studies were performed on all patients. These included: complete blood count, Westergren erythrocyte sedimentation rate (ESR), serum levels of C-reactive protein (CRP), hemoglobin, urea, creatinine, urine analysis and urinary protein excretion, all according to standard techniques. Presence of the rheumatoid factor was assessed by the latex fixation test.

Serum levels of sICAM-1 were measured with a sandwich enzyme-linked immunosorbent assay (ELISA) (Biomedica GmbH, Austria).

Screening for the presence of ANCA was performed by indirect immunofluorescence microscopy on cytocentrifuged slides of ethanol and formalin-fixed human neutrophils according to the recommendations of the First International ANCA Workshop³⁵. Serial dilutions of the sera were tested (starting at 1:20) and a cANCA titer greater than or equal to 1:80 was considered to be positive (The Binding Site LTD, England). Fluorescence patterns were classified as classic cANCA, perinuclear ANCA (pANCA), or atypical (neither cANCA nor pANCA).

Levels of autoantibodies to nuclear antigens (ANA), dsDNA and proteinase 3 (PR-3) (cANCA) were assessed using commercial ELISA kits (Cogent Diagnostics Autostattm for ANA and dsDNA, and Shield Dia-

gnostics LTD Diastattm for anti PR-3, Scotland, UK).

Statistical analysis. The significance of the differences between abnormally distributed clinical variables and immunological parameters were assessed by the Mann-Whitney U test. The frequencies were compared by the Fisher exact test. The correlations between the variables were analyzed using the Spearman rank test. P values of <0.05 were considered statistically significant.

Results

Clinical features of the RA and CTD patients

The characteristics of both the RA and CTD groups with and without clinical signs of systemic vasculitis are shown in Table 1. There were no significant differences in age, sex and the number of tender or swollen joints between the two groups with different clinical features. The duration of disease was significantly longer in the group of RA patients with systemic involvement ($p < 0.05$). No significant differences in disease activity between patients with and without extra-articular manifestations were observed (Table 2).

Capillaroscopy findings in RA and CTD patients and in controls

Morphological abnormalities of nailfold capillary loops in RA and CTD patients are shown in Table 3. Capillary abnormalities were observed in 83 out of the 86 RA and CTD patients (96.5%). Architectural changes, such as tortuous or meandering patterns were observed. Mild capillaroscopic changes were found in 33.7% (29 out of 86), moderate in 40.7% (35 out of 86) and severe in 22.1% (19 out of 86) of the RA and CTD patients (data not shown). Patients in the group with severe capillaroscopic changes had a longer disease duration (9.7 ± 6.9 years) than did those in the group with mild vasculitis (6.0 ± 6.0 years $p < 0.05$ data not shown). On the other hand, no significant differences in age, sex, morning stiffness, the number of tender or swollen joints, ESR and steroid use between the three groups could be demonstrated. Only the CRP value in the sera of RA patients with severe vasculitis was significant higher (16.4 ± 35.3 mg/dl) than in patients with mild vascular capillaroscopic changes (7.5 ± 16.4 mg/dl) (data not shown). All patients with extra-articular manifestations showed pathological changes under capillary microscopy. Interestingly, 38 out of the 50 patients with

Table 1. Characteristics of RA and CTD patients and controls (mean $\pm$ SD)

	Number	Age (years)	Sex (male/female)	Disease duration (years)
Controls	30	48.4 $\pm$ 14.3	6/24	–
All RA	54	50.0 $\pm$ 12.0	1/53	8.4 $\pm$ 6.9
with systemic vasculitis (a)	23	49.9 $\pm$ 13.6	0/23	10.4 $\pm$ 6.4*
without systemic vasculitis (b)	31	50.1 $\pm$ 11.1	1/30	6.8 $\pm$ 7.0*
All CTD	32	49.8 $\pm$ 12.1	1/31	8.4 $\pm$ 7.4
with systemic vasculitis	27	49.5 $\pm$ 13.0	1/26	8.6 $\pm$ 7.9
without systemic vasculitis	5	51.6 $\pm$ 4.4	0/5	6.8 $\pm$ 3.1

* p (a–b) < 0.05 Mann-Whitney U test.

Table 2. Comparison of the immunological parameters in the sera of RA and CTD patients with different clinical features and in controls (mean $\pm$ SD)

	Parameters					
	ESR (mm/h)	CRP (mg/dl)	sICAM-1 (ng/ml)	ANA (IU/ml)	dsDNA (IU/ml)	ANCA (IU/ml)
Controls	12.0 $\pm$ 3.8	–	257.9 $\pm$ 43.4	–	–	–
All RA	73.3 $\pm$ 42.1	14.5 $\pm$ 29.5	378.9 $\pm$ 74.7	49.1 $\pm$ 93.5	55.9 $\pm$ 54.5	69.1 $\pm$ 67.3
with vasculitis (a)	86.2 $\pm$ 45.5	22.4 $\pm$ 39.7	417.9 $\pm$ 80.8	100.0 $\pm$ 108.1*	67.1 $\pm$ 77.4	87.5 $\pm$ 77.2
without vasculitis (b)	63.8 $\pm$ 37.3	9.6 $\pm$ 21.0	295.1 $\pm$ 57.0	25.7 $\pm$ 12.8*	50.4 $\pm$ 28.4	58.6 $\pm$ 63.0
All CTD	49.2 $\pm$ 39.3	9.3 $\pm$ 5.7	381.8 $\pm$ 95.9	43.3 $\pm$ 87.7	57.6 $\pm$ 39.0	105.0 $\pm$ 73.2
with vasculitis (c)	49.9 $\pm$ 42.6	12.1 $\pm$ 5.2	437.1 $\pm$ 97.2**	71.1 $\pm$ 48.7**	60.8 $\pm$ 41.8	137.8 $\pm$ 87.0***
without vasculitis (d)	45.8 $\pm$ 12.8	6.5 $\pm$ 4.2	291.4 $\pm$ 93.5**	20.0 $\pm$ 12.5**	40.6 $\pm$ 6.7	46.7 $\pm$ 31.5***

* p (a–b) < 0.001, ** p (c–d) < 0.005, *** p (c–d) < 0.05 Mann-Whitney U test.

Table 3. Morphological abnormalities of nailfold capillary loops in patients with RA and CTD

Vascular abnormality	RA (n=54)	Scleroderma (n=15)	SLE (n=7)	Other CTD (n=10)
Tortuous loops (in >1 digit)	39	12	6	9
Meandering vessels	9	5	6	9
Large dilated capillaries	0	12	0	4
Disorganized vascular pattern	4	3	4	8
Prominent subpapillary plexus	46	7	4	6
Avascularisation	0	15	5	6
Capillary hemorrhages	9	2	5	3

extra-articular manifestations (76%) showed moderate or severe vascular changes under nailfold capillaroscopy. In the group without extra-articular involvement severe vascular capillaroscopic abnormalities were found in only 3 patients (data not shown).

The capillaroscopic feature in the control group showed hairpin capillaries in a parallel arrangement. Isolated tortuous capillary loops or meandering vessels were found in 17% of the controls (data not shown).

Comparison of immunological parameters in the sera of RA and CTD patients with different clinical features and capillaroscopy findings

Serum levels of sICAM-1 and autoantibodies were determined in the 54 RA patients, 32 CTD patients and the 30 healthy subjects (Figs 1–4, Table 2). The RA and CTD patients had significantly elevated concentrations of sICAM-1 compared with age-matched healthy

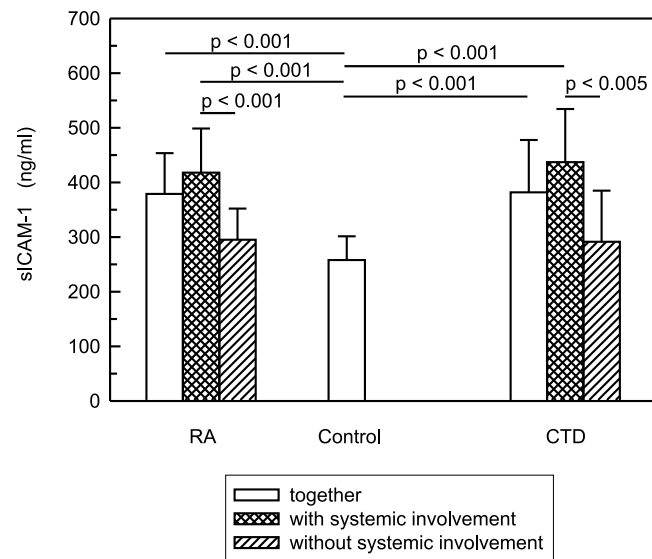


Fig. 1. The serum levels of sICAM-1 in RA and CTD patients depending on systemic involvement

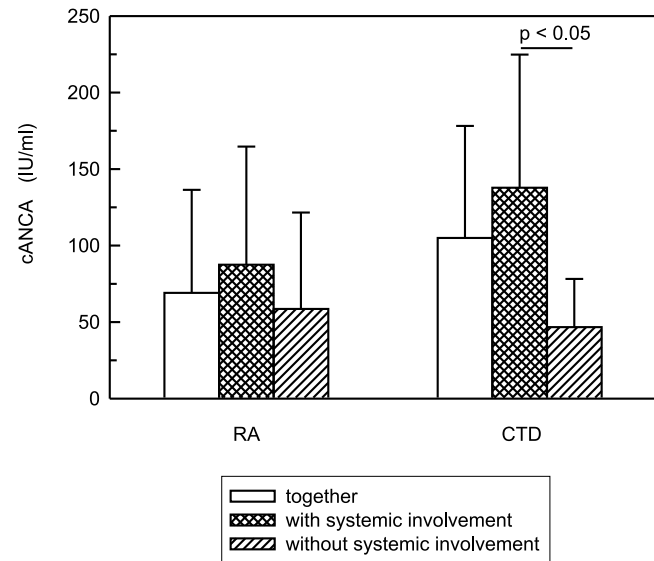


Fig. 3. The serum levels of cANCA in RA and CTD patients depending on systemic involvement

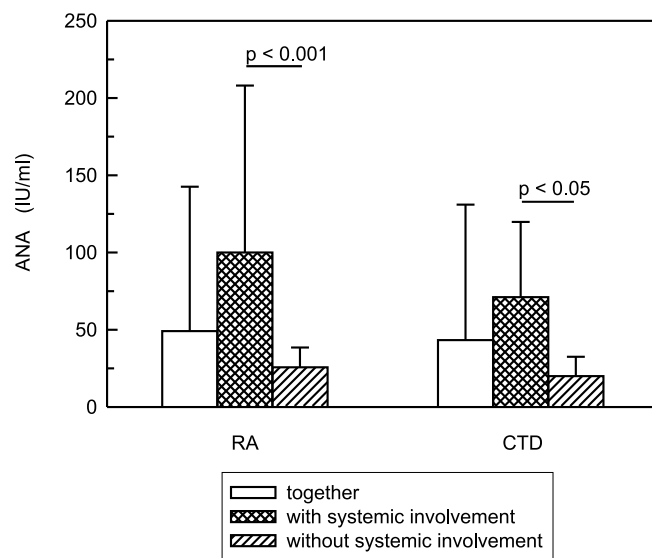


Fig. 2. The concentration of ANA in serum of RA and CTD patients with and without extraarticular manifestations

controls (in both cases $p < 0.001$) (Fig. 1). Also, the mean serum levels of sICAM-1 were significantly higher in the RA and CTD patients with systemic involvement than in the groups without any evidence of extra-articular manifestations ($p < 0.001$ and $p < 0.005$ respectively).

Comparison between both RA groups, with and without systemic vasculitis, showed a significantly higher ANA concentration in the sera of patients with systemic involvement ($p < 0.001$) (Fig. 2). Within the group of CTD patients, the ANA level was also significantly higher in the group with systemic vasculitis compared with non-vasculitis patients ($p < 0.05$) (Fig. 2).

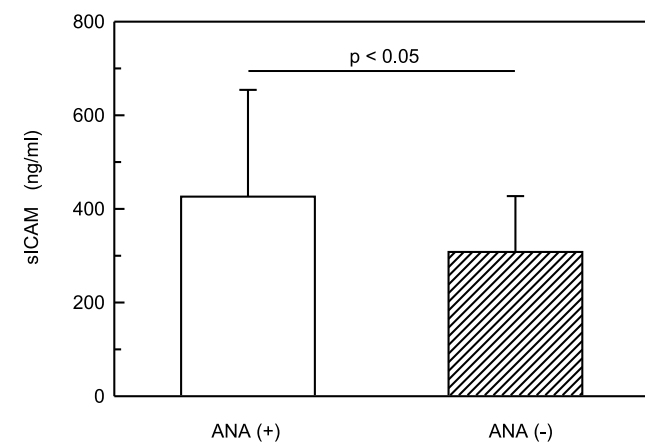


Fig. 4. The serum concentrations of sICAM-1 in serum of RA and CTD patients with and without presence of ANA antibodies

In the RA group, 8 out of the 54 patients (14.8%) were cANCA (anti-PR-3 antibodies)-positive (only in one RA patient was a high titer (1:640) of the cANCA pattern observed). Among the 32 CTD patients, cANCA antibodies were found in 9 samples of serum (28.1%) (data not shown).

The serum cANCA concentrations did not differ between RA patients with and without extra-articular manifestation (Fig. 3). However, it was higher in CTD patients with systemic vasculitis than those without this complication ($p < 0.05$).

The concentration of sICAM-1 in serum was higher in all patients (RA and CTD) with the presence of ANA antibodies ($p < 0.05$) (Fig. 4).

Tabel 4. Correlations among immunological parameters in the sera of CTD patients

		r	p
ANA	disease duration	0.169	0.002
	hemoglobin	−0.445	0.008
cANCA	rheumatoid factor	0.47	0.03
dsDNA	age	−0.21	0.05
sICAM	α_1 -globulins	0.42	0.01

Data expressed as r value (correlation coefficient) according to Spearman's rank correlation.

Furthermore, in cANCA-positive patients, the sICAM-1 level was significantly higher (449.7 ± 176.6 ng/ml) than that of the cANCA-negative group (293.4 ± 92.4 ng/ml, $p < 0.05$) (data not showed).

Cytoplasmic specific ANCA were not found in the serum of RA patients without extra-articular manifestation of the disease. All cANCA-positive patients suffering from CTD showed systemic involvement. Vasculopathy was observed in 87.5% of the ANA-positive patients and was rare (12.5%) among ANA-negative patients (data not showed).

Serum levels of sICAM-1, ANA, dsDNA and cANCA did not differ significantly between the RA and CTD groups with various capillaroscopic scores (data not shown).

Relationships between autoantibody levels and clinical or laboratory parameters in the patients studied

The correlations among autoantibody concentrations and other parameters in the RA and CTD patients are shown in Table 4. A significant correlation between ANA level and duration of the disease (positive) and hemoglobin concentration (negative) were found. Double-stranded DNA concentration correlated significantly with patient age, and cANCA with the rheumatoid factor in RA patients (Table 4). In CTD patients, negative correlations between cANCA and hemoglobin concentration ($r = -0.66$, $p < 0.02$), and erythrocyte blood count ($r = -0.78$, $p < 0.002$) and blood plates count ($r = -0.67$, $p < 0.02$) were demonstrated. Moreover, in CTD patients, a correlation between the level of antibodies against dsDNA and the duration of the disease ($r = 0.44$, $p < 0.02$) was found (data not showed).

No other correlations between serum autoantibodies and clinical parameters or laboratory indices of the disease activity were found.

Discussion

Systemic vasculitis is a rare but potentially serious complication of RA and CTD and is associated with

increased mortality^{24, 31}. Therefore, early detection of vascular involvement is very important. Nailfold capillary microscopy is widely used for the investigation of microvascular involvement in rheumatic diseases^{1, 8, 19}. But there are still no satisfactory clinical and laboratory parameters that would provide a quantitative assessment of the vascular damage in patients with vasculitis. Despite the heterogeneity of the clinical manifestations of particular connective tissue diseases, the immunopathogenesis of vasculitis demonstrates several common elements. Among these are cytokines, adhesion molecules and autoantibodies which are known to be involved in the immunopathology of vasculitis^{5, 12, 16, 25, 29, 30}. The aim of this study was to determine whether the levels of sICAM-1 and autoantibody profiles may reflect the vascular injury found in nailfold capillaroscopy as well as the systemic vasculopathy in patients with RA and CTD.

In the present study all patients with extra-articular manifestations showed the pathological changes in nailfold capillary microscopy. Among them, 76% of the patients with systemic involvement showed moderate or even severe vascular changes under capillaroscopy. In the group without the extra-articular manifestations severe vascular capillaroscopic abnormalities were found in only 3 patients (8%). Therefore it may be suggested that microvascular injury seen in nailfold capillary microscopy may be predictive of systemic vasculopathy in patients with the extra-articular manifestations of rheumatic diseases.

ICAM-1 is a membrane-bound molecule that plays an important role in the pathogenic inflammatory responses observed in vasculitis^{3, 6, 15, 28, 32}. The interactions of adhesion proteins with other cells culminate in three important cellular functions: homing to the lymphoid tissues, migration to the inflammatory sites and costimulation of the cellular activation²¹. We already showed that RA patients had significantly elevated levels of serum sICAM-1 compared with healthy controls¹⁷. In the present study we demonstrated the same phenomenon also in the case of CTD patients. Furthermore, we found that the concentration of sICAM-1 was significantly higher in the serum of RA and CTD pa-

tients with systemic involvement than in the groups without any evidence of extra-articular manifestation. Since sICAM-1 production by mononuclear cells reflects the state of the cells' activation, its increased serum level might also reflect the degree of vascular damage^{3, 5, 17}. It can be supposed that by involving larger vessels, the endothelial cells may, in the course of systemic vasculitis, release more ICAM-1 antigen into the circulation. Therefore, the increased levels of sICAM-1 might reflect a general vascular involvement rather than a local vascular injury in the course of RA or CTD.

Anti-nuclear antibodies and antibodies against PR-3 (cANCA) are also involved in the pathogenesis of vasculitis^{12, 20, 22, 33, 36}. It was shown that ANCAs may serve as a serological marker for systemic vascular disorders and the disease activity in CTD²⁰ and RA patients^{22, 23, 36}. FONT et al.⁹ suggest that ANCA positivity is associated with the presence of clinical manifestations attributable to vascular involvement, such as cutaneous vasculitis or peripheral neuropathy, in patients with primary Sjögren's syndrome. But the data regarding the diagnostic value of ANCAs and their pathophysiological role in rheumatic diseases are controversial. In RA, an association of ANCAs with the disease activity and the extra-articular manifestations, including rheumatoid vasculitis, was observed by some researchers^{4, 22, 23, 33, 36}. Other investigators were not able to confirm any association between ANCAs and RA disease activity^{10, 13}. In some studies, the presence of ANCAs was not able to predict the vasculopathy¹⁰. It was even suggested that ANCAs have the potential to mislead a physician towards a misdiagnosis of vasculitis¹⁰.

In our study we showed significantly higher ANA concentrations in the sera of RA and CTD patients with systemic involvement compared with to the patients without vasculopathy. ANA concentration correlated with disease duration and hemoglobin level. Vasculitis under nailfold capillaroscopy was present in most (78.6%) of the ANA-positive patients, but was very rare (21.4%) in the ANA-negative group (data not showed). Also, the serum cANCA level was increased in CTD patients with systemic vasculitis compared with those without. It did not differ between RA patients with and without extra-articular manifestation. It should be underlined that systemic vasculitis was observed in the all the cANCA-positive patients. Therefore, we suggest that both ANA and cANCA serum concentration may reflect systemic vascular involvement in the rheumatic patients.

We also observed higher serum concentrations of sICAM-1 in all patients (RA and CTD) with the

presence of ANA antibodies. This may be explained by the capacity of ANA and cANCA to up-regulate the expressions of the adhesion molecules on the endothelial cells¹² and the adhesion of neutrophils to the cultured endothelial cells¹⁷. Furthermore, it is known that endothelial cells, stimulated by cytokines, express ANCA antigens¹⁴. Taken together, our data show the associations of sICAM-1, ANA and cANCA with vasculopathy involvement and suggest that ANA and ANCA testing may alert the clinician to the possibility of underlying vasculitis and should be included in a rational diagnostic scheme.

This study confirms the concept that a widespread vascular involvement in RA and CTD affects not only the arteries and veins, but the capillaries as well. It was also shown that the microvascular capillaroscopic abnormalities were related to extra-articular manifestation in the course of RA and CTD. Increased serum levels of sICAM-1, ANA and cANCA in patients with systemic vasculopathy suggest an important role of immune response abnormalities in the pathogenesis of microvascular damage. The concentrations of these serological markers may also reflect the extent of the vascular involvement in RA and CTD patients.

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