Clinical Significance of Selected Endothelial Activation Markers in Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Systemic lupus erythematosus (SLE) is an autoimmune disease in which immunologically mediated vascular endothelial cell activation is regarded as a potential pathophysiological mechanism of systemic organ damage. We investigated selected endothelial cell activation markers in serum of patients with SLE and their relationships with systemic organ manifestations and disease activity.

Methods. Serum levels of endothelin-1 (ET-1), soluble E-selectin, and thrombomodulin (sTM) were determined by ELISA in 76 SLE patients and in 34 healthy controls.

Results. Higher serum concentrations of ET-1, sE-selectin ($p < 0.05$), and sTM ($p < 0.001$) were observed in SLE patients in comparison with controls. Significant differences of ET-1, ($p < 0.01$), sTM ($p < 0.001$), and sE-selectin serum concentrations ($p < 0.01$) were found between SLE patients with systemic involvement and controls. Patients with organ manifestations ($n = 34$) showed significantly higher serum levels of ET-1 than patients without systemic involvement ($n = 42$) ($p < 0.05$). Comparison between patients with active and inactive SLE according to SLE Disease Activity Index (SLEDAI) score showed significantly higher concentration of ET-1 in the sera of patients with active SLE compared with inactive patients and the controls ($p < 0.001$).

Conclusion. Our findings suggest that the elevated serum concentrations of ET-1, sTM, and sE-selectin reflect persisting endothelial cell activation in SLE, and point to an important role of ET-1 in the pathogenesis of internal organ involvement. Moreover, elevated ET-1 concentrations are related to disease activity, suggesting a key role of endothelial cell activation in systemic manifestations in SLE patients. (First Release May 15 2008; J Rheumatol 2008;35:1307–13)

Key Indexing Terms:
ENDOTHELIAL ACTIVATION MARKERS SYSTEMIC LUPUS ERYTHEMATOSUS
ENDOTHELIN-1 E-SELECTIN THROMBOMODULIN

Systemic lupus erythematosus (SLE) is a progressive autoimmune disease of unknown etiology, characterized by a chronic course with flares and remissions. Although progress in early diagnosis, understanding of the disease process, and better approaches to treatment have contributed to improved survival rates of SLE, comorbidity and longterm organ damage still remain high[1]. Internal organ involvement occurring in the late period of the disease is one of the main causes of increased mortality in SLE[2].

It is thought that pathological changes within the blood vessels accompanied by immunological disturbances are implicated in the pathogenesis of life-threatening systemic organ manifestations in the course of SLE[3]. Endothelium as a target and source of numerous multifunctional substances involved in the control of blood coagulation, vascular tone, and inflammatory conditions plays a crucial role in the regulation of vascular function[4]. In addition, endothelial cells can express a number of molecules and growth factors, such as endothelin-1 (ET-1), thrombomodulin (TM), vascular endothelial growth factor (VEGF), and E-selectin, that play an important role in immunological reactivity[5].

Angiogenesis is a complex process of formation of new blood vessels from preexisting ones. Pathological angiogenesis is the integral part of vascular changes in the course of systemic connective tissue disease. It occurs very early in rheumatoid arthritis (RA), often preceding clinical and histological manifestations of the inflammatory process[6]. Increased concentrations of VEGF, the main mediator of angiogenesis, were observed in synovial fluid and serum of patients with RA[7]. Moreover, we previously described significantly elevated serum levels of VEGF in patients with SLE[8] and RA[9], especially in cases with internal organ

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involvement. Increased concentrations of VEGF were also observed in patients with dermatomyositis/polymyositis, systemic sclerosis (SSc) complicated by interstitial lung disease[10], and SLE, particularly with kidney involvement[11].

Endothelin, which is involved in numerous processes such as mitogenesis, vascular hypertrophy, fibrosis, inflammation, extracellular matrix synthesis, and tissue remodeling, plays an essential role in the pathogenesis of multiple vascular abnormalities in rheumatic diseases[12]. Increased production of endothelin has been demonstrated in various systemic rheumatic diseases in which vascular pathology is related to endothelial cell activation, including SLE[13], RA[14], and SSc, especially in the diffuse form with pulmonary fibrosis[12]. Previously, we showed significantly elevated serum endothelin levels in patients with RA, especially in cases of extraarticular manifestations of the disease[9]. Further, it has been suggested that ET-1 may play an important role in the cardiovascular system and kidney damage in the course of SLE[13].

TM, an endothelial cell membrane glycoprotein, is released after endothelial injury as a soluble form (sTM) detectable in plasma, serum, and urine. sTM has been used as a marker for microvascular damage, and its elevation in serum has been reported in patients with systemic vasculitides[15]. Further, it has been assumed that elevated serum TM concentrations are associated with nephritis, vasculitis, and neurological involvement in the course of SLE[16].

E-selectin, mainly expressed on activated endothelial cells, is another marker reflecting endothelial cell damage[17]. It has been suggested that soluble forms of E-selectin (sE-selectin) play an important role in the development of the inflammatory process and angiogenesis[17]. Elevated serum concentration of E-selectin has been reported in various systemic rheumatic diseases such as Wegener's granulomatosis[15], giant cell arteritis, polyarteritis nodosa[18], vasculitis[19], local scleroderma[20], and SSc[21]. Moreover, increased concentrations of sE-selectin have been described in cutaneous[22] and systemic lupus erythematosus[23].

Based on these findings, suggesting a fundamental role of vascular damage in the pathogenesis of autoimmune rheumatic diseases, we evaluated the relationship between serum concentrations of the selected factors modulating endothelial function such as ET-1, sTM, and sE-selectin and disease activity and systemic organ manifestations in patients with SLE.

MATERIALS AND METHODS

Patients. Seventy-six patients (72 women, 4 men, mean age 39.6 ± 3.0 yrs) who fulfilled the updated 1982 American College of Rheumatology (ACR) revised criteria for SLE[24] were recruited into the study. The mean duration of disease was 7.9 ± 7.1 years. Overlap syndromes had been excluded.

All patients were evaluated by extensive clinical and laboratory studies. Physical examination was performed on the day of blood collection. In all patients the activity of the disease was determined according to the SLE Disease Activity Index (SLEDAI)[25]. The maximum score in this system is 105 points. In our group of patients, the score of points ranged from 2 to 26; we considered a score of 0–11 points as inactive disease (53 patients) and a score ≥ 12 points as active disease (23 patients).

All SLE patients were classified into 2 groups: those with internal organ involvement (34 cases) and those with no evidence of systemic organ manifestation in the course of SLE (42 patients). The group with internal organ manifestation included 20 patients with renal involvement (lupus nephritis, proven histologically), one with lung fibrosis [diagnosed on high-resolution computed tomography (HRCT)], 2 with cardiovascular manifestations, 12 with central nervous system manifestations (in the form of cognitive dysfunction, severe anxiety, and altered mental function with impaired orientation), and 8 patients with severe vasculitis (ulceration, infarction, gangrene). In 9 patients, systemic involvement in more than one organ was observed. Table 1 shows the characteristics of the patient groups.

Seventy-one patients were treated with prednisolone at low dosage (5–10 mg/day), 27 with hydrochloroquine, and 7 with immunosuppressive agents (azathioprine or cyclophosphamide) on a stable dose for at least 6 months. In 41 patients receiving high-dose steroids (pulsed intravenous methylprednisolone), blood samples had been drawn at least 8 weeks after the last course of pulse therapy.

The control group consisted of 34 healthy subjects, matched with patients for sex and age. All participants gave written informed consent for all procedures. The protocol was approved by the Ethical Committee of the Medical University of Bialystok, and adhered to the tenets of the Helsinki Declaration.

Clinical and laboratory analysis. Clinical and laboratory data recorded at the time of serum collection included pulmonary and renal function tests, chest radiography, and renal sonography as well as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) concentration measured by radial immunodiffusion (Nanorid, The Binding Site Ltd., Birmingham, UK), the concentration of hemoglobin, serum creatinine, peripheral blood cell counts for platelets, urinalysis, creatinine clearance, and urinary protein excretion. Organ system involvement was as follows; lung: bibasilar pulmonary fibrosis on chest radiograph; heart involvement: pericarditis or congestive heart failure on echocardiography; and kidney: renal function tests

Table 1. Clinical and laboratory measures of SLE patients (mean ± SD).

Characteristics	No. Patients (%)
Sex, M/F	4/72
Age, yrs, mean ± SD	39.5 ± 13.5 (range 19–72)
Disease duration, yrs, mean ± SD	8.2 ± 7.2 (range 0.25–27)
SLEDAI, mean ± SD	8.8 ± 5.9
Active/inactive	23/53 (30.3/69.7)
Fever	16 (21.1)
Cutaneous involvement	25 (32.9)
Arthritis	35 (46.1)
Renal involvement	20 (26.3)
Cardiac involvement	2 (2.6)
Neurological involvement	12 (15.8)
Pulmonary fibrosis	1 (1.3)
Vasculitis	8 (10.5)
Anemia, hemoglobin < 12 g/dl	18 (23.7)
Leukopenia (white blood cells < 3.5×10^9/l)	11 (14.5)
Thrombocytopenia (platelets < 150×10^9/l)	13 (17.1)
ESR, mm/h (> 20 mm/h)	42 (55.3)
ANA-positive	71 (93.4)
Anticardiolipin antibody	22 (28.9)
Treatment	
Low-dose steroids	30 (39.5)
High-dose steroids	41 (53.9)
Hydroxychloroquine	33 (43.4)
Immunosuppressive agents	17 (22.4)

and sonography. Renal biopsy was done if patients fulfilled the ACR criteria for renal involvement, i.e., persistent proteinuria > 0.5 g/24 h or cellular casts in the absence of infection[24].

Serum specimen preparation. Blood samples were clotted for 30 min and centrifuged for 10 min at 1000 × g. Serum aliquots were frozen at –80°C immediately after collection.

Laboratory studies. Screening for presence of autoantibodies to nuclear antigens (ANA) was by indirect immunofluorescence microscopy on commercial human Hep-2 cell substrate (Viro-Immun Labor-Diagnostica GmbH, Oberursel, Germany). Serial dilutions of the sera were tested (starting at 1:80) and ANA titer ≥ 1:80 was considered positive. Levels of IgG and IgM anticardiolipin antibodies (aCL) were measured using a commercial ELISA kit (Pharmacia Diagnostics AB GmbH, Freiburg, Germany).

Serum concentration of sE-selectin was assessed by ELISA (R&D Systems, Wiesbaden-Nordenstadt, Germany). Commercial kits were used for measuring TM (Imubind® thrombomodulin ELISA kit; American Diagnostica, Stamford, CT, USA) and ET-1 (Endotelin-1 Biotrak™ ELISA system; Amersham Biosciences, Freiburg, Germany). Experimental procedures were performed according to the manufacturer's instructions.

Statistical analysis. Data were analyzed by Mann-Whitney U test. The probability of differences in frequency distributions was determined by chi-square test or Fisher's exact test. Data were correlated by Spearman rank-order test. P values < 0.05 were considered statistically significant.

RESULTS

The characteristics of SLE patients with and without systemic involvement are shown in Table 2. No significant differences in age, sex, ESR levels, and ANA positivity between 2 groups of SLE patients with and without systemic involvement were seen. The duration of disease was longer in SLE patients with systemic organ manifestations. Moreover, disease activity measured by SLEDAI score was significantly greater in SLE patients with systemic involvement. The mean SLEDAI score for the active group was 16.39 ± 3.89 and for the inactive patients was 5.51 ± 2.73 ($p < 0.001$). Cutaneous involvement was observed more frequently in the group with systemic organ manifestations.

Serum levels of ET-1, sE-selectin, and sTM were determined in 76 SLE patients, including 42 patients with systemic involvement and 34 without internal organ manifestation and in 34 healthy subjects (Figure 1A). In comparison with the control group, higher serum concentrations of ET-1 ($p < 0.05$), sE-selectin ($p < 0.05$), and sTM ($p < 0.001$) were observed in SLE patients. Moreover, significant differences of ET-1 ($p < 0.01$), sTM ($p < 0.001$), and sE-selectin ($p < 0.01$) serum concentrations were found between SLE patients with systemic involvement and controls (Figures 1A, 3, and 5).

Comparison of the SLE groups with and without organ manifestations showed significantly higher ET-1 serum levels in patients with systemic involvement ($p < 0.05$). However, there were no significant differences in serum concentrations of sTM and sE-selectin in the 2 SLE groups (Figures 1A, 3, and 5).

The mean serum levels of ET-1 and sTM were significantly higher ($p < 0.001$, in both cases) in patients with active SLE than in the control group (Figures 2 and 4), but levels of sE-selectin were not (Figure 6). However, only the serum concentrations of ET-1 were significantly higher in patients with active SLE compared to those with inactive disease, according to SLEDAI score ($p < 0.001$; Figure 2). Between the SLE patients with and without systemic involvement, 2 subgroups were distinguished according to their disease activity. In both patient groups the mean ET-1 serum level remained significantly higher in patients with active SLE than in the cases with inactive disease (Figure 1B). The concentrations of other endothelial activation markers did not differ significantly between the patients with active and those with inactive disease (data not shown).

As for the effect of treatment on serum levels of ET-1, sTM, and sE-selectin in SLE patients, no significant differences were observed between the patients receiving and those not receiving corticosteroids and immunosuppressive drugs (data not shown).

DISCUSSION

SLE is a multisystem autoimmune disease characterized by a wide spectrum of clinical manifestations. According to recent studies, the vascular inflammatory process resulting

Table 2. Clinical and laboratory differences in SLE patients according to presence of systemic organ involvement.

Characteristics	SLE Together	SLE Patients without Systemic Involvement, n = 42	SLE Patients with Systemic Involvement, n = 34	p
Sex, M/F	4/72	2/40	2/32	NS
Age, yrs, mean ± SD	39.5 ± 13.5	39.9 ± 14.5	39.1 ± 12.4	NS
Disease duration, yrs, mean ± SD	8.2 ± 7.2	6.2 ± 6.0	10.6 ± 7.9	< 0.01
SLEDAI, mean ± SD	8.8 ± 5.9	7.1 ± 4.9	10.9 ± 6.4	< 0.01
ESR, mm/h, mean ± SD	28.1 ± 25.3	22.0 ± 17.6	35.8 ± 31.1	NS
Cutaneous involvement, n (%)	25 (32.9)	8 (19.0)	17 (50.0)	< 0.01
ANA positive, n (%)	71 (93.4)	38 (90.5)	32 (94.1)	NS
Treatment				
Hydroxychloroquine, n (%)	33 (43.4)	23 (54.8)	10 (29.4)	< 0.05
Immunosuppressive agents, n (%)	17 (22.4)	4 (9.5)	13 (38.2)	< 0.01

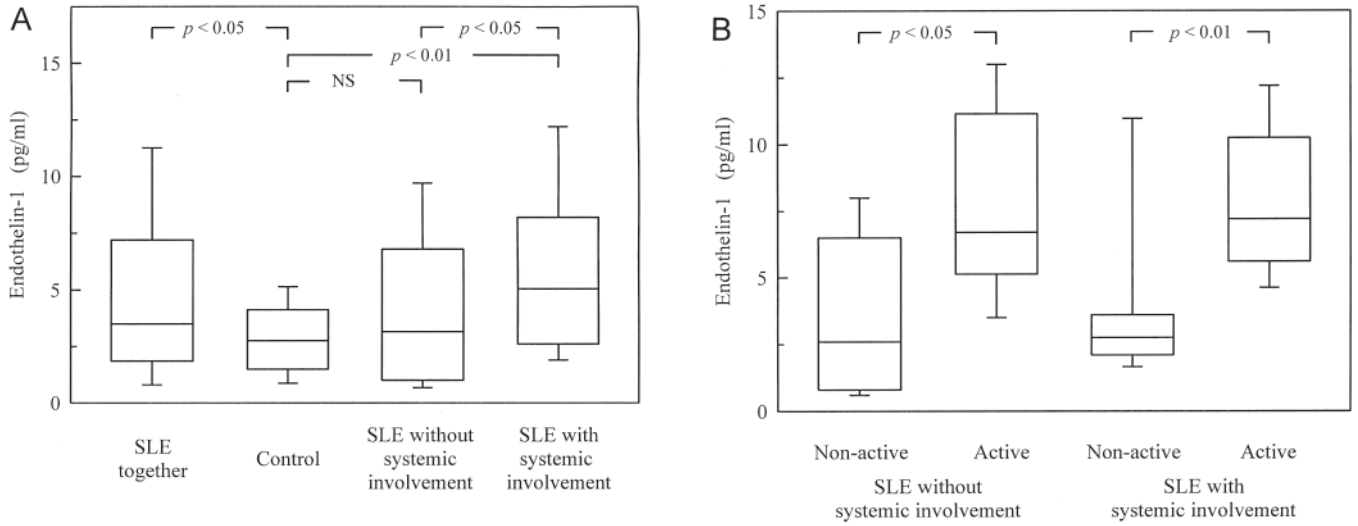

Figure 1. A. Serum concentrations of endothelin-1 in SLE patients with and without internal organ involvement. Box plots represent median, 25th and 75th percentiles (box); whiskers indicate 10th and 90th percentiles. B. Serum concentrations of endothelin-1 in SLE patients with and without internal organ involvement, and with non-active and active disease.

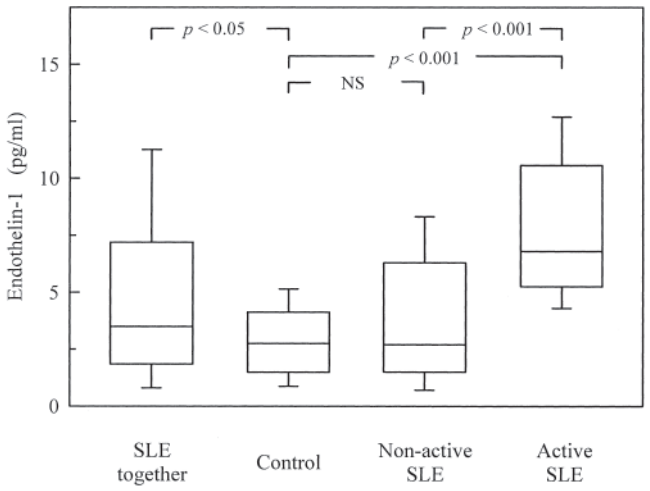

Figure 2. Serum concentrations of endothelin-1 in SLE patients with non-active and active disease. Data presented as in Figure 1.

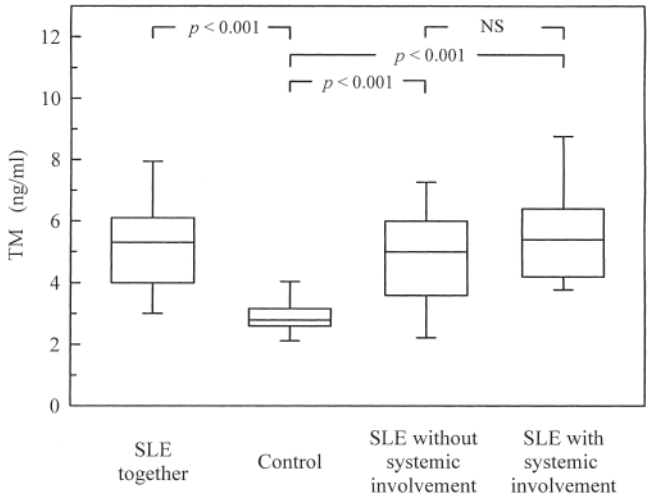

Figure 3. Serum concentrations of thrombomodulin (TM) in SLE patients with and without internal organ involvement. Data presented as in Figure 1.

in endothelial cell injury plays a fundamental role in the pathogenesis of several connective tissue diseases, including SLE[26]. It is therefore useful to measure biological markers of endothelial cell activation *in vivo* since they may provide insight into the clinical manifestations and prognosis of rheumatic diseases.

Previously, we demonstrated the relationship among serum VEGF levels, disease activity, internal organ involvement, and the extent of microvascular capillaroscopic abnormalities in patients with SLE[8]. Our current study investigated whether serum levels of other endothelial activation markers such as ET-1, sTM, and sE-selectin are associated with systemic manifestations and disease activity in SLE.

We found serum ET-1, sE-selectin, and sTM concentrations were significantly elevated in all patients with SLE. Further, significant differences were found in ET-1, sTM, and sE-selectin serum concentrations between SLE patients with systemic involvement and controls. Moreover, patients with internal organ manifestations showed significantly higher serum levels of ET-1 compared to patients without systemic involvement.

ET-1 has been implicated in the development of cardiovascular diseases and kidney damage[27,28]. Several clinical studies have described increased plasma levels of ET-1 in patients with RA[14], SSc[12], and other vascular diseases[28]. Our previous study showed that increased serum level of ET-1 is related to internal organ involvement in the course of

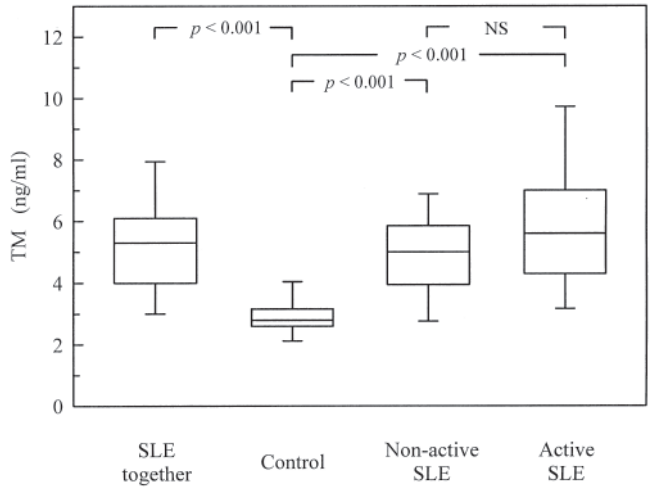

Figure 4. Serum concentrations of thrombomodulin (TM) in SLE patients with non-active and active disease. Data presented as in Figure 1.

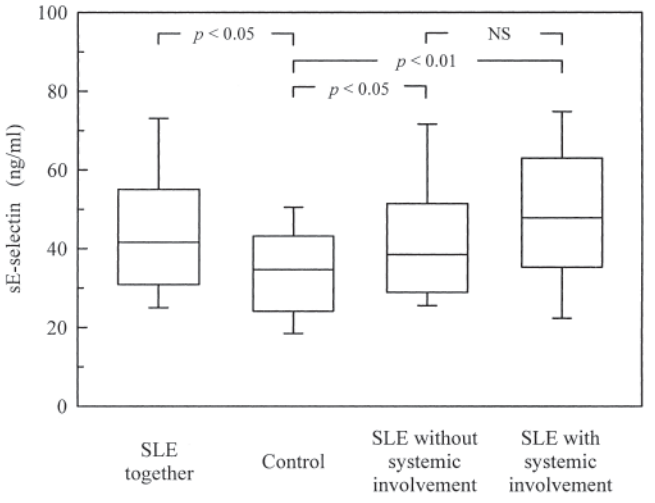

Figure 5. Serum concentrations of sE-selectin in SLE patients with and without internal organ involvement. Data presented as in Figure 1.

RA[9] and SSc[29]. However, few data exist on the role of ET-1 in the pathogenesis of SLE. Several studies have demonstrated increased ET-1 concentrations in SLE patients[30]. It is postulated that overexpression of ET-1 is associated with acute renal failure[31]. In the present study, we observed higher serum ET-1 concentrations in SLE patients with systemic involvement in comparison to the group without internal organ manifestations. Moreover, comparison between patients with active and inactive SLE according to the SLEDAI score showed significantly higher concentration of ET-1 in the sera of patients with active SLE compared to the inactive patients and controls. Further, in both study groups, those with and without systemic involvement, subdivided according to the disease activity, the mean ET-1 serum level remained significantly higher in patients with active SLE than in those with inactive disease. These findings support

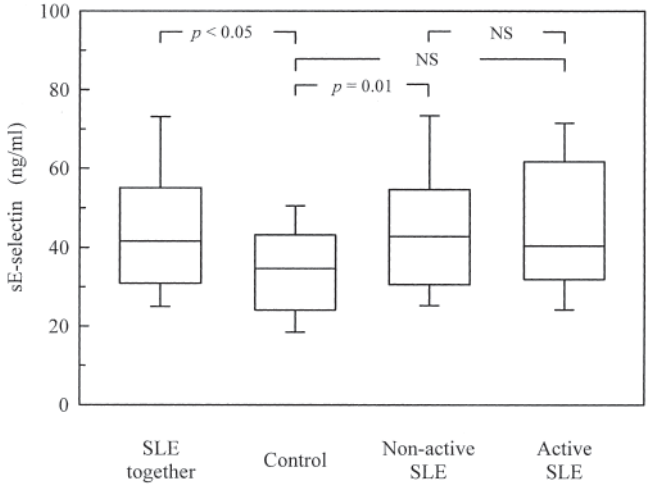

Figure 6. Serum concentrations of sE-selectin in SLE patients with non-active and active disease. Data presented as in Figure 1.

an important role of ET-1 in the pathogenesis of the disease progression in SLE.

The soluble form of TM (sTM), released into circulation after endothelial cell activation, is considered a potential marker of endothelial cell damage. Several studies have shown increased sTM concentrations in patients with SLE compared to healthy controls[15,32,33], which corresponds with the results we describe here.

Moreover, a significant correlation has been observed between serum concentrations of sTM and the disease activity in patients with SLE, RA, SSc, and polymyositis[15]. Although, in our study, serum concentrations of sTM were elevated in SLE patients with systemic involvement compared to those without systemic manifestations, the differences were not significant. Several studies have described increased sTM levels in relation to clinical activity of SLE[34,35]. Moreover, the highest sTM levels were seen in patients with both lupus nephritis and cerebritis[34]. It is postulated that sTM may be released from immunologically mediated inflammatory injuries of vascular endothelial cells[16]. In our study, the highest serum levels of sTM were reached in patients with active SLE, although no significant differences between the SLE groups with active and non-active disease were found. Additionally, serum concentrations of sTM were found to correlate with serum creatinine levels in SLE patients ($p < 0.05$). As well, in patients with lupus nephritis the sTM concentration was higher than in SLE patients without renal involvement, although no significant differences were found (data not shown). It is postulated that increased sTM concentrations are associated with a history of lupus nephritis and reflect increased endothelial synthesis and expression of TM, predominantly in the kidneys[36]. Thus, further prospective studies are needed to confirm whether the serum level of sTM reflects the disease activity and systemic manifestation in patients with SLE.

Endothelial cells stimulated with proinflammatory cytokines express E-selectin, a cell adhesion molecule promoting initial tethering of leukocytes to endothelium, the first step of leukocyte adhesion and emigration into tissues[17]. Soluble E-selectin, released by activated endothelial cells, is considered to be a specific marker of endothelial cell activation during systemic inflammation[21]. Several studies have demonstrated increased sE-selectin levels in serum of patients with RA[37] and SSc[20] compared to healthy controls. Previously, we reported significantly elevated serum levels of sE-selectin in RA patients with distinct variants of RA[38] and in SSc patients[29].

Clinical studies have shown elevated serum levels of sE-selectin in patients with SLE, SSc, and vasculitis[11]. Moreover, sE-selectin has been found to correlate with the disease activity and progression of articular damage in RA[39]. However, in other studies, serum levels of sE-selectin were found not to be elevated in patients with lupus nephritis and Wegener's granulomatosis in comparison to the control group[40], and were not related to the presence of disease exacerbations in SLE patients[41]. In contrast, other authors have shown higher sE-selectin concentrations in patients with SLE[35], and a correlation between serum levels of sE-selectin and prognosis for the disease has been postulated[42]. It was suggested that higher serum levels of sE-selectin may be associated with higher titers of antiphospholipid antibodies and the presence of neuropsychiatric symptoms in the course of SLE[15]. In our study, serum levels of sE-selectin were higher in SLE patients in comparison to control levels, although no significant differences between SLE groups with and without systemic manifestations were found. Moreover, no significant differences were observed in serum sE-selectin levels between the SLE groups with active and those with non-active disease. Further studies are required to confirm the relationship between serum level of sE-selectin and disease activity in patients with SLE.

We demonstrated significantly elevated serum levels of ET-1, sTM, and sE-selectin in SLE patients compared to the control group. Moreover, elevated serum ET-1 concentrations are associated with the disease activity and clinical course of SLE. These findings suggest that persistently elevated levels of endothelial activation markers may reflect a more severe form of SLE, and failure to normalize these measures leads to greater microvascular damage and poorer prognosis. Recent studies have recognized the occurrence of accelerated atherosclerosis in SLE patients[43]. Premature vascular disease may be responsible for a broad spectrum of clinical symptoms, and its presence is associated with increased mortality. Better understanding of the circumstances of vascular complication may be crucial to improved treatment.

Further prospective studies are necessary to confirm the usefulness of other serum endothelial activation markers in evaluation of the severity and prognosis of SLE, and to assess the risk of severe systemic organ manifestations in asymptomatic patients. Proper diagnosis prior to the onset of irreversible organ damage has essential clinical implications and may be a potential indication to initiate early, aggressive treatment.

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