Correlation of Biomarkers of Endothelium Dysfunction and Matrix Remodeling in Patients with Systemic Sclerosis

SOPHIE BLAISE, RENKE MAAS, CANDICE TROCME, GHAINSOM D. KOM, MATTHIEU ROUSTIT, PATRICK H. CARPENTIER, and JEAN-LUC CRACOWSKI

ABSTRACT. ***Objective.*** Systemic sclerosis (SSc) is a multisystem disease characterized by microvascular dysfunction and excessive fibrosis. However, the relationship between these 2 features remains unclear. Endothelial dysfunction can be assessed by quantifying plasma asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase. Matrix remodeling can be assessed by quantifying serum tissue inhibitor of matrix metalloproteinases-1 (TIMP-1). Both biomarkers are elevated in patients with SSc. Our objective was to test whether plasma ADMA is correlated with serum TIMP-1.

Methods. We enrolled 91 subjects, 39 patients with SSc, 28 patients with primary Raynaud's phenomenon (RP), and 24 healthy volunteers. Plasma ADMA concentrations were measured by liquid chromatography-tandem mass spectrometry. Serum TIMP-1 concentrations were determined by ELISA.

Results. Mean ADMA concentrations were higher in patients with SSc (0.68 μM ± 0.12) than in patients with primary RP or healthy volunteers (respectively, 0.56 μM ± 0.14 and 0.62 μM ± 0.12; p = 0.002). Median serum TIMP-1 concentrations were increased in patients with SSc compared to primary RP and healthy volunteers [12 (9–15), 11 (8–13), and 10 (7–13) nM, respectively; p = 0.05]. In the SSc group, we observed a statistically significant correlation between plasma ADMA and serum TIMP-1 (r = 0.34, p = 0.035).

Conclusion. These data are consistent with our hypothesis of an association of endothelial dysfunction and matrix remodeling in scleroderma spectrum disorders. (First Release April 1 2009; J Rheumatol 2009;36:984–8; doi:10.3899/jrheum.080924)

Key Indexing Terms:
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Microvascular dysfunction is an early event in the pathogenesis of systemic sclerosis (SSc). It is characterized by an increased lymphocyte binding as well as a decreased endothelium-dependent flow-mediated relaxation[1]. Endothelial dysfunction can be investigated by functional testing[2] or by measurement of biomarkers. Recently, asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide (NO) synthase, has emerged as a promising biomarker of endothelial dysfunction in cardiovascular diseases[3-5]. Further, plasma ADMA is increased in patients with SSc when compared to patients with primary Raynaud's phenomenon (RP)[6,7].

SSc is characterized by an increased collagen deposition. The net accumulation of extracellular matrix is the result of the balance between synthesis and degradation of extracellular components, partly regulated by enzymes called matrix metalloproteinases (MMP). The tissue fibrosis observed in SSc may be due to an inhibition of MMP activity by MMP-specific inhibitors called tissue inhibitors of metalloproteinases (TIMP). Serum concentration of MMP-1 is increased in patients with SSc[8,9]. Both MMP-1 and MMP-9

are inhibited by TIMP-1, and serum concentrations of TIMP-1 are consistently high in SSc[8-11].

While microvascular dysfunction and matrix remodeling have been widely assessed in SSc, there is a lack of data about the pathophysiological link between the 2 processes. Our objective was to test whether ADMA, a biomarker of endothelial dysfunction, is correlated to TIMP-1, a biomarker of matrix remodeling.

MATERIALS AND METHODS

Study population. This was a descriptive single-center case-control study performed as described[12]. All subjects gave written informed consent, and the study was approved by the institutional review board of Grenoble University Hospital, France, in January 2004. This study is part of a larger cohort study about the vascular phenotype enrolling patients with SSc, patients with primary RP, and healthy volunteer controls. Inclusion and exclusion criteria are described elsewhere[12].

Laboratory evaluations. Determination of ADMA and symmetric dimethylarginine (SDMA): prechilled EDTA vacutainers were used to collect blood samples and immediately placed on ice. They were centrifuged at 4°C within a few minutes and plasma samples were stored at –80°C until analysis. Then, all samples were sent in dry ice by an express carrier to the Institute of Experimental and Clinical Pharmacology and Toxicology (Hamburg, Germany). ADMA concentrations were measured by liquid chromatography-tandem mass spectrometry, as described and validated[13].

ELISA for serum MMP-1, MMP-9, and TIMP-1: samples were centrifuged at 1800 g for 10 min at 15°C, serum was stabilized with a protease inhibitor cocktail without EDTA (Roche Diagnostic, Meylan, France), and aliquots were stored at –80°C. Serum TIMP-1 concentrations were determined by a commercial ELISA assay (Amersham Pharmacia Biotech, Buckinghamshire, UK). This assay quantifies both free and bound TIMP-1 with a detection limit of 1.25 ng/ml. Serum MMP-1 and MMP-9 concentrations were also evaluated by commercial ELISA (R&D Systems, Abingdon, UK). The minimum detectable levels were 0.021 ng/ml for proMMP-1 and 0.516 ng/ml for total MMP-9.

Laser Doppler measurements. Cutaneous blood flow was assessed using laser Doppler flowmetry (Periflux System 5000; Perimed, Järfälla, Sweden) as described[13]. The amplitude of the post-occlusive reactive hyperemia response was expressed as peak cutaneous vascular conductance raw value in mV/mm Hg.

Data analysis. The number of subjects was calculated on the hypothesis that we would detect a correlation coefficient of 0.4 between ADMA and TIMP-1, with a power of 80%, and an alpha risk of 0.05 for a 1-tailed analysis.

Data distribution was analyzed prior to quantitative data analysis. When data did not follow a normal distribution, nonparametric

Table 1. Demographic, clinical, and biological characteristics of patients with cutaneous systemic sclerosis (SSc), primary Raynaud's phenomenon (RP), and healthy controls (HC).

	Controls, n = 24	RP, n = 28	SSc, n = 39
Mean age, yrs (SD)	52 (10)	49 (9)	52 (11)
Female, n (%)	21 (87)	25 (89)	35 (90)
Raynaud's phenomenon, n (%)	0 (0)	28 (100)	39 (100)
Median Raynaud's disease duration, yrs, (10th–90th percentiles)	0 (0)	14 (5–42)	10 (3–27)
RP, median no. of fingers involved (10th–90th percentiles)	0 (0)	8 (6–10)	10 (8–10)
RP, thumbs involved, n (%)	0 (0)	14 (50)	31 (80)
RP, feet involved, n (%)	0 (0)	13 (46)	30 (77)
Median SSc disease duration, yrs (10th–90th)	NA	NA	5 (1–16)
Digital pitting scars (%)	0 (0)	0 (0)	22 (56)
Sclerodactyly, n (%)	0 (0)	0 (0)	29 (74)
Median Rodnan modified skin score (10th–90th)	0 (0)	0 (0)	6 (0–24)
ISSc/lcSSc/dSSc, n	0/0/0	0/0/0	8/22/9
Pulmonary fibrosis, n (%)	0 (0)	0 (0)	11 (28)
Pulmonary arterial hypertension, n (%)	0 (0)	0 (0)	1 (2)
Esophageal dysmotility, n (%)	0 (0)	0 (0)	21 (54)
Mean creatinine clearance, ml/min (SD)	86 (22)	79 (12)	91 (25)
Mean microalbuminuria, mg/l (SD)	15 (11)	17 (19)	16 (17)
Mean cardiac rate, beat/min (SD)	62 (10)	67 (13)	71 (13)
Mean systolic/diastolic blood pressure, mm Hg (SD)	113 (13)/66 (9)	110 (14)/66 (9)	119 (20)/69 (11)
Mean body mass index, kg/cm^2 (SD)	24 (4)	20 (2)	23 (3)
Autoantibodies			
Anticentromere, n (%)	0 (0)	0 (0)	16 (41)
Antitopoisomere I, n (%)	0 (0)	0 (0)	11 (28)
Mean plasma LDL cholesterol, g × l^{-1} (SD)	1.11 (0.3)	1.03 (0.3)	1.11 (0.3)
Mean plasma glycemia mmol × l^{-1} (SD)	4.6 (0.6)	4.6 (0.9)	4.6 (0.5)

NA: not applicable; ISSc/lcSSc/dSSc: limited/limited cutaneous/diffuse systemic sclerosis; LDL: low density lipoprotein.

statistical methods were performed. p values less than 0.05, corrected for multiple comparisons, were considered significant. Quantitative data were expressed as the mean ± standard deviation or median and 10th and 90th percentiles.

RESULTS

The characteristics of the 91 patients enrolled in our study are listed in Table 1.

Mean ADMA concentrations were significantly higher in patients with SSc than in patients with primary RP or controls (0.68 ± 0.12, 0.56 ± 0.14, and 0.62 ± 0.12 μM, respectively; p = 0.002; Figure 1). Mean SDMA concentration was also significantly higher in patients with SSc than in patients with primary RP or controls (0.59 ± 0.12, 0.54 ± 0.10, and 0.53 ± 0.11 μM; p = 0.038; Figure 1). No difference was shown in ADMA and SDMA concentrations between the different SSc subsets. No significant difference was observed between controls and patients with primary RP.

Median TIMP-1 concentration was higher in patients with SSc [12 nM (range 9–15)] than in patients with primary RP [11.7 nM (8–13)] and controls [10.1 nM (7–13)] (p = 0.05; Figure 2). Similarly, median MMP-1 levels were higher in patients with SSc [0.09 nM (0.04–0.20)] than in controls [0.05 nM (0.03–0.11)] (p = 0.0263; Figure 2). There was no difference in MMP-9 level between patients with SSc, patients with primary RP, and controls [4.61 nM (2.79–9.31), 4.16 nM (2.56–7.48), and 4.54 nM (3.15–6.19)].

In the SSc group, we observed a statistically significant correlation (r = 0.34; p = 0.034) between ADMA, a biomarker of endothelial dysfunction, and TIMP-1, a biomarker of matrix remodeling (Figure 3). No correlation between ADMA or TIMP-1 and Rodnan skin score, scleroderma subset, pulmonary status, or autoantibody status was found.

Post-occlusive reactive hyperemia peak CVC was inversely correlated with serum TIMP-1 concentration (r = –0.36; p = 0.004) but not with ADMA. Moreover, TIMP-1 concentration was higher in patients with [12.1 nM (9.5–15.2)] versus those without [10.4 nM (7.7–13.7)] digital pitting scars (p = 0.026). We also observed a nonsignificant trend towards higher ADMA concentration in patients with digital pitting scars.

DISCUSSION

Rajagopalan, *et al* showed that ADMA was increased in patients with secondary RP compared to primary RP[6]. Similar results were obtained by Dooley, *et al*, who also demonstrated that ADMA concentrations in patients with primary RP were not different from those of healthy controls[7]. Further, Dooley, *et al* showed that patients with diffuse cutaneous SSc exhibited increased ADMA concentration, whereas it was similar to controls in patients with

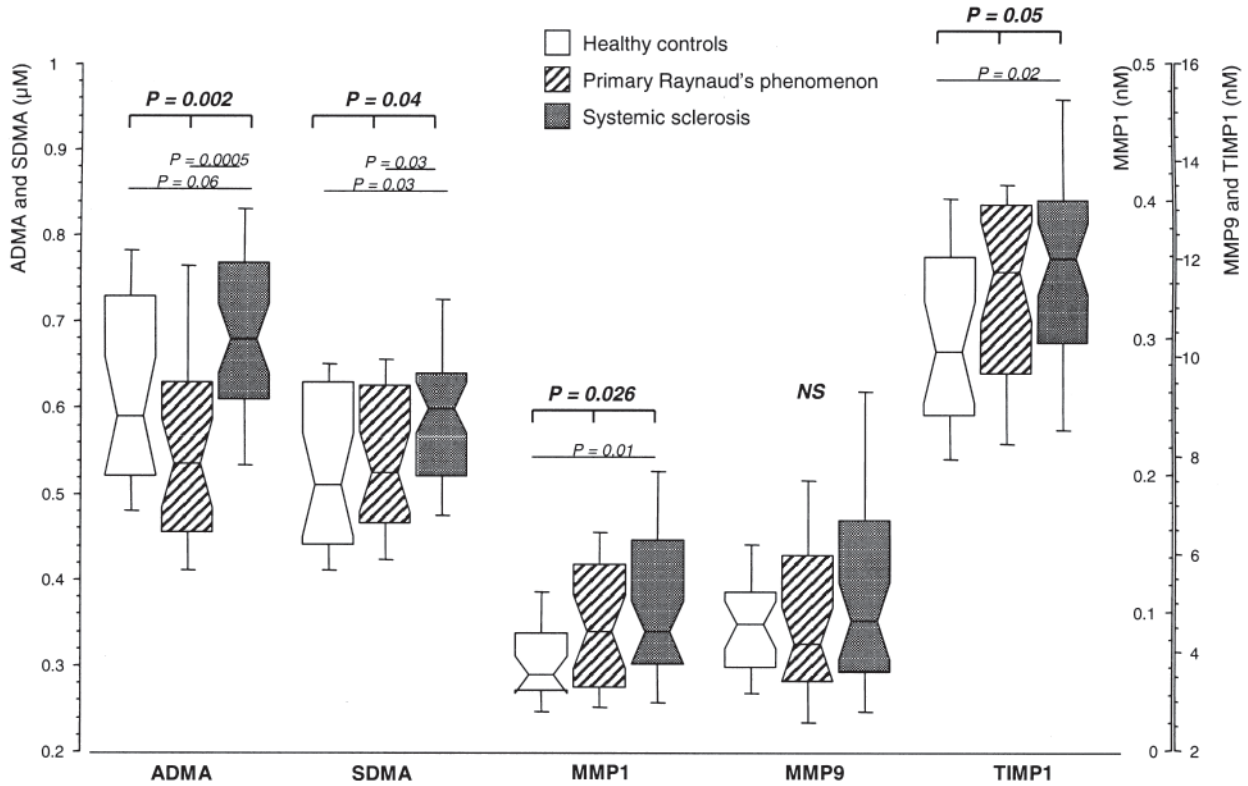

Figure 1. Plasma ADMA, plasma SDMA, serum MMP-1, serum MMP-9, and serum TIMP-1 concentrations in patients with SSc, primary Raynaud's phenomenon, and controls.

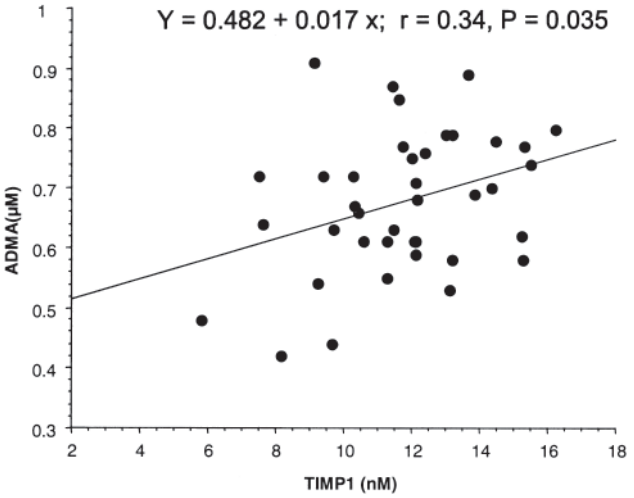

Figure 2. Correlation between plasma ADMA and serum TIMP-1 concentrations in 39 patients with SSc.

limited SSc[7]. In contrast, we observed no effect of disease stage on ADMA concentration in our study. Taken together with the observation that creatinine clearance was similar in controls and in patients with SSc, this suggests that the observed increase in ADMA concentrations is not due to an abnormal renal function.

Several studies showed that TIMP-1 levels are consistently high in patients with SSc, and may be associated with disease activity[8-11]. Thus, our finding of increased TIMP-1 serum concentration in the SSc group agrees with previous results.

While serum MMP-1 concentration was low in healthy controls, higher values were observed in the SSc group, while no significant difference was observed between SSc and primary RP patients. Studies of lung fibrosis support the notion that MMP-1 is upregulated in fibrotic diseases[14]. Thus, our study provides further evidence of a paradox whereby excessive accumulation of collagen in fibrotic disease is associated with an overexpression of the cleaving enzyme, and not absence of it, as might have been expected.

Divergent results have been observed concerning serum MMP-9 concentrations[10,15], suggesting that quantification of MMP-9 is not a useful biomarker in SSc.

From a pathogenic point of view, SSc is characterized by an early endothelial-dependent vasodilatation dysfunction, and an increased lymphocyte transmigration, leading to increased production of cytokines[1]. The latter modulates MMP and TIMP expressions. We characterized a correlation between ADMA, a biomarker of endothelial dysfunction, and TIMP-1, a biomarker of matrix remodeling, in SSc. In addition, serum TIMP-1 concentration was inversely correlated to post-occlusive reactive hyperemia, and was increased in patients with digital pitting scars. The link between NO and MMP in SSc remains unclear. NO inhibits collagen secretion in normal fibroblasts, but such regulation is lost in SSc fibroblasts. It has been suggested that this phenomenon may be due to the upregulation of MMP-1 and/or inhibition of prolyl hydroxylase[16]. Another explanation implicated anti-fibroblast antibodies in SSc that may increase collagenolytic activity and MMP-1 production in dermal fibroblasts[17]. More functional experiments are required to provide a definitive conclusion, especially in the different subgroups of SSc.

We showed for the first time that in patients with SSc, ADMA, a biomarker of endothelial dysfunction, was increased and was positively correlated with TIMP-1, a biomarker of matrix remodeling. Further, we observed a correlation between TIMP-1 concentration and a global test of microvascular dysfunction. These data suggest an association between endothelial dysfunction and matrix remodeling in scleroderma spectrum disorders. However, further studies are required to test whether there is a direct pathophysiological link.

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