Different contributions of angiostatin and endostatin in angiogenesis impairment in systemic sclerosis: a cohort study

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ABSTRACT

Objective. *To determine the concentrations of circulating endostatin and angiostatin in patients with systemic sclerosis (SSc) and to assess its relationship to disease subsets, evolution phase, organ involvement and nailfold capillaroscopic changes.*

Methods. *Endostatin and angiostatin serum levels were measured by ELISA in a cohort of 57 patients with SSc, and correlated with disease subsets, evolution phase, organ involvement and nailfold capillaroscopic changes.*

Results. *Endostatin and angiostatin serum levels were significantly higher in patients with SSc than in healthy controls. Also, angiostatin was elevated in diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc), but not in pre-SSc, while endostatin was increased in all SSc subsets. Moreover, endostatin was augmented in lcSSc, with or without CREST syndrome, whereas angiostatin was increased exclusively in patients with CREST. Analysis according to disease evolution phase found that endostatin was elevated in all phases while angiostatin was only significantly higher in intermediate and late phases of disease. Analysis regarding organ involvement revealed that angiostatin was significantly higher in patients with osteoarticular involvement and with more serious lung affection; no significant differences were found for endostatin. Finally, endostatin was significantly increased in all nailfold capillaroscopy stages, while angiostatin was only elevated in active and late phases.*

Conclusion. *In accordance with previous studies, we found that endostatin and angiostatin concentrations are elevated in SSc patients. Additionally, we recognised the important role that endostatin might play as an early disease marker and realised that angiostatin is a marker of late disease and relates to lung disease severity.*

Introduction

Systemic sclerosis or scleroderma (SSc) is an autoimmune connective tissue disease characterised by vascular injury and widespread fibrosis involving the skin and various internal organs. Angiogenesis and vasculogenesis impairment plays a major role, although disease pathogenesis remains not fully elucidated (1-3).

Angiogenesis is a complex process dependent on the tight balance between pro-angiogenic and angiostatic factors, which is seen normally in healthy tissues. Pathological angiogenesis occurs when a hypoxic environment or inflammatory state induces a deregulation of this homeostasis and angiogenic growth factors outweigh the inhibitors (1).

Proteolytic fragments of several extracellular proteins have shown to have antiangiogenic activity, contributing to abnormal wound healing and vascular repair in SSc patients. Angiostatin, a cleavage product of plasminogen, is one of these anti-angiogenic factors; endostatin, a heparin sulphate proteoglycan found in almost all epithelial and endothelial basement membranes that results from the cleavage of type XVIII collagen, is another angiostatic factor (1).

Several studies have previously investigated endostatin concentrations in SSc patients, with contradictory results (4-10). While most found that endostatin levels are elevated in SSc patients (4, 6-10), one failed to achieve this relation (5). On the contrary, we found only one study in the MEDLINE database (PubMed) relating angiostatin with SSc, and it concluded that angiostatin is increased in SSc patients (11).

The aim of this study was to determine the concentrations of circulating endostatin and angiostatin in patients with SSc and to assess a relationship between these concentrations and disease subsets, evolution phase, type of

organ involvement (skin, peripheral vascular, lung, heart, gastrointestinal, kidney, muscle, and osteoarticular) and nailfold capillaroscopic changes.

Patients and methods

Patients

Sixty-one consecutive patients were selected from a 190-patients-population with SSc, at the Clinical Immunology Unit of Centro Hospitalar do Porto, Portugal, between September 2010 and March 2011. Four patients were later excluded, three because of overlapping pathologies (mixed connective tissue disease, infection with human immunodeficiency virus, B non-Hodgkin lymphoma) and a fourth one who missed the blood sampling. The study was approved by the Ethics Committee and the Board of Directors of the Hospital and all patients signed an informed consent form.

Forty-seven patients fulfilled the American College of Rheumatology (ACR) criteria for SSc (12) while the remaining ten did not present skin involvement and were classified as pre-scleroderma (pre-SSc), as explained below.

The ACR/EULAR (American College of Rheumatology/European League Against Rheumatism) criteria of 2013 (13) were met by those 47 patients that had met the ACR criteria, and by 1 of the 10 without skin involvement due to having digital ulcers. For practical reasons, the analysis of this last patient was made together with those that did not present skin involvement.

In the study population, 55 were women and 2 were men, with a median age of 54 years, ranging from 18 to 82 years. Patients' characteristics are summarised in Tables I and II. Twenty-five healthy individuals were used as controls.

Clinical assessment

Patient disease subset was classified as pre-scleroderma (pre-SSc) according to LeRoy and Medsger (14), and limited cutaneous (lcSSc) or diffuse cutaneous (dcSSc) scleroderma, as previously described by LeRoy *et al*. (15). Over time, several authors and, more recently, the EULAR Scleroderma Trial and Research group (EUSTAR) have recognised that there is an early stage of disease in which there is no skin involvement but that may evolve to limited or diffuse SSc, and named it differently: "limited scleroderma" (14), "pre-scleroderma" (16), "early SSc" (17), and "very early SSc" (18); even though, they represent nearly the same concept (19). We used the definition of pre-SSc according to LeRoy and Medsger criteria, where patients must have Raynaud's phenomenon plus scleroderma-type nailfold capillary changes and/or scleroderma-type autoantibodies (14).

Table I. Clinical characteristics of systemic sclerosis patients (n = 57).

Age (years)	54 (18-82)
Gender	
Females	55 (96.5%)
Males	2 (3.5%)
Disease subset	
Pre-SSc	10 (17.5%)
dcSSc	13 (22.8%)
lcSSc	34 (59.6%)
CREST	17 (29.8%)
Non-CREST	17 (29.8%)
Disease phase	
Early	7 (14.9%)
Intermediate	10 (21.3%)
Late	30 (63.8%)
Capillaroscopic pattern	
Normal/ minor alterations	4 (7.3%)
Early	12 (21.8%)
Active	21 (38.2%)
Late	18 (32.7%)

Age is presented as median (range). Other results are presented as n (%).
SSc: Scleroderma; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; CREST: calcinosis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia.

Limited and diffuse SSc were then subdivided into early, intermediate and late SSc, according to the time since first symptom related to the disease and in consonance with subset of disease (20). Early phase of lcSSc has less than five years of evolution and late phase has at least ten years. On the contrary, early phase of dSSc has less than three years of evolution and late phase a duration superior to six years. Intermediate values correspond to intermediate phases of limited and diffuse SSc.

Additionally, limited SSc was further separated in CREST (calcinosis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia) and non-CREST groups according to Lonzetti *et al*. (21).

There is a lack of a standardised method to assess disease severity. We decided to use Medsger's severity scale (22), which classifies involvement of each of nine organ system (general, peripheral vascular, skin, joints/tendons, muscles, gastrointestinal (GI) tract, lungs, heart and kidneys) from 0 (no documented involvement or without need of treatment, *e.g*. Raynaud) to 4 (end stage disease), without an overall score. It has limitations as a measure of activity due to its lack of sensitivity to change score in the presence of SSc severity improvement (23). Even though, it is a quite useful tool as a prognostic measure (24).

A complete clinical profile was established for each patient at the time of study enrolment and the degree of organ involvement was determined by medical history, physical examination, and complementary tests.

Organ involvement was evaluated using the Medsger scale cut-off point, except for osteoarticular evaluation, since it does not value joint involvement but only the distance between thumb and thenar eminence, which was not evaluated systematically in our patients.

The Modified Rodnan Skin Score was the tool chosen for evaluating skin involvement (25).

Joint evaluation was made through physical exam, and complemented by image exams (radiography and/ or joint echography) whenever justified. DAS 28 (Disease Activity Score Calculator for Rheumatoid Arthritis) was used to classify the activity of this involvement (26).

Muscular involvement was evaluated by physical examination and complemented by biochemical study (creatine phosphokinase, aldolase and glutamate oxaloacetate transaminase).

Gastrointestinal tract involvement was documented by upper gastrointestinal endoscopy, oesophageal manometry, and, in symptomatic patients, by gastric scintigraphy. Involvement of the lower GI tract, whenever justified, was documented using barium tests, glucose hydrogen breath test, ultrasound, colonoscopy and anorectal manometry.

Table II. Organ involvement according to Medsger Severity Scale*.

Organ	Score	Pre-SSc [n=10]	Limited SSc [n=34]	Diffuse SSc [n=13]
Skin	0	10 (100)	-	-
	1	-	33 (97.1)	3 (23.1)
	2	-	1 (2.9)	9 (69.2)
	3	-	-	1 (7.7)
Peripheral vascular	0	5 (50.0)	-	-
	1	4 (40.0)	18 (52.9)	1 (7.7)
	2	-	3 (8.8)	4 (30.8)
	3	1 (10.0)	11 (32.4)	8 (61.5)
	4	-	2 (5.9)	-
Gastrointestinal tract	Non-classified	2 (20.0)	2 (5.9)	1 (7.7)
	0	5 (50.0)	17 (50.0)	5 (38.5)
	1	3 (30.0)	15 (44.1)	7 (53.8)
Lung	Non-classified	1 (10.0)	-	-
	0	6 (60.0)	10 (29.4)	2 (15.4)
	1	3 (30.0)	11 (32.4)	6 (46.2)
	2	-	11 (32.4)	2 (15.4)
	3	-	1 (2.9)	1 (7.7)
	4	-	1 (2.9)	2 (15.4)
Heart	Non-classified	3 (30.0)	4 (11.8)	1 (7.7)
	0	6 (60.0)	18 (52.9)	8 (61.5)
	1	1 (10.0)	2 (5.9)	2 (15.4)
	2	-	5 (14.7)	2 (15.4)
	3	-	5 (14.7)	-
Osteoarticular	Non-classified	-	1 (2.9)	-
	0	8 (80.0)	24 (70.6)	12 (92.3)
	1	2 (20.0)	9 (26.5)	1 (7.7)

Results presented as n (%). *S*Sc: Scleroderma.
*From "0" (no documented involvement or without need of treatment) to "4" (end stage disease).

Table III. Angiostatin and endostatin levels in study population and healthy controls, and according to systemic sclerosis subsets.

	Angiostatin (ng/ml)	*p*-value (patients *vs.* controls)	Endostatin (ng/ml)	*p*-value (patients *vs.* controls)
Healthy controls (n=25)	34.6 (0.0-127.0)	0.005	1.6 (0.0-11.5)	<0.001
Study population (n=57)	48.5 (19.3-126.5)		20.2 (0.0-178.8)	
pre-SSc (n=10)	46.9 (21.3-76.7)	0.108	17.7 (1.8-69.8)	<0.001
lcSSc (n=34)	48.5 (19.3-126.5)	0.014	20.6 (0.0-73.0)	<0.001
non-CREST (n=17)	48.5 (19.3-126.5)	0.069	18.8 (9.8-40.3)	<0.001
CREST (n=17)	54.6 (21.3-85.4)	0.023	21.8 (0.0-73.0)	<0.001
dcSSc (n=13)	54.6 (24.4-101.1)	0.025	21.5 (6.3-178.8)	<0.001

Results presented as median (range) values.
SSc: Scleroderma; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; CREST: calcinosis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia.

Renal impairment was evaluated by biochemical study.

Chest teleradiography, high resolution computed tomography, spirometry, diffusing capacity of the lungs for carbon monoxide (DLCO) corrected for haemoglobin and alveolar volume, and two-dimensional echocardiogram with evaluation of pulmonary artery systolic pressure (PSAP) were used to evaluate pulmonary involvement. PSAP was considered abnormal when greater than 35 mmHg. Right heart catheterisation was performed in patients with PSAP above or equal to 40 mmHg in order to confirm pulmonary hypertension. Forced vital capacity (FVC) and DLCO were considered abnormal when lower than 80%. Patients with lung fibrosis and/or alveolitis affecting at least the lung bases were considered to have major pulmonary alterations; criteria for minor alterations included septal hypertrophy and limited zones of fibrosis and/or ground glass opacities.

Conduction disturbances and/or arrhythmias were detected by electrocardiogram and/or Holter, and systolic dysfunction was evaluated with two-dimensional echocardiogram, namely through calculation of the left ventricular ejection fraction.

Finally, a score introduced by Cutolo *et al*. (27) was used to classify nailfold microvascular changes as observed by capillary microscopy (early, active and late capillaroscopic patterns of microvascular damage).

Laboratory analysis

Serum concentrations of endostatin and angiostatin were measured using ELISA assays from USCN Life Sciences Inc. (Wuhan, China), accordingly to the instructions provided by the manufacturer.

Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for Social Sciences (SPSS 22.0) software (SPSS Inc, IL, USA). Descriptive statistics include the presentation of frequencies, medians, minimums and maximums. The Kolmogorov-Smirnov test was used to assess the normality of distributions.

Once studied variables did not present a normal distribution and given the small size of the statistical sample, non-parametric tests were used. The Mann-Whitney test was used to compare mean ranks of two independent samples. Although corresponding to an ordinal variable, the small number of phases of disease led to the analysis using Kruskal-Wallis tests to compare the 3 disease phases instead of the use of correlations. Regarding the relationships of each organ's Medsger severity scale with angiostatin and endostatin serum levels, despite this scale having a sufficient number of points in order to be used to compute correlation coefficients, patients exhibited a very diverse number of different scores (from 2 to 4); therefore, for each organ we compared patients at the lowest score (0 or 1) with the remaining. The null hypothesis was rejected when $p<0.05$.

Results

Statistically significant differences were found in serum levels of angiostatin ($p=0.005$) and endostatin ($p<0.001$)

Table IV. Angiostatin and endostatin serum levels according to the scleroderma evolution phase.

	Angiostatin (ng/ml)	*p*-value (patients *vs*. controls)	Endostatin (ng/ml)	*p*-value (patients *vs*. controls)
Healthy controls (n=25)	34.6 (0.0-127.0)	NA	1.6 (0.0-11.5)	NA
Early SSc (n=7)	40.4 (30.9-115.5)	0.138	15.0 (9.8-28.6)	<0.001
Intermediate SSc (n=10)	54.0 (21.3-85.3)	0.037	34.4 (16.4-38.3)	<0.001
Late SSc (n=30)	48.5 (19.3-126.5)	0.015	20.1 (0.0-178.7)	<0.001

Results presented as median (range) values. SSc: Scleroderma.
Angiostatin: Early *vs*. Intermediate (p=0.922); Early *vs*. Late (p=0.954); Intermediate *vs*. Late (p=0.988); Early *vs*. Intermediate + Late (p=0.489).
Endostatin: Early *vs*. Intermediate (p=0.003); Early *vs*. Late (p=0.535); Early *vs*. Intermediate + Late (p=0.169); Intermediate *vs*. Late (p=0.04).

between the study population and healthy controls (Table III).

Disease subsets analysis revealed that angiostatin concentration was significantly higher in patients with lcSSc (p=0.014) and dcSSc (p=0.025), but not in patients with pre-SSc, in comparison with controls, while elevated serum endostatin concentrations were noted in all SSc subsets (p<0.001) (Table III).

Serum levels of angiostatin were significantly higher in CREST patients when compared with healthy controls (p=0.023); however, no statistically significant differences were found between lcSSc patients without CREST syndrome and controls. In contrast, serum endostatin levels were higher in both groups of lcSSc, with or without CREST (p<0.001) (Table III).

Analysis according to disease evolution phase revealed that angiostatin serum levels were significantly higher in intermediate and late SSc (p=0.037 and p=0.015, respectively), but not in patients with early SSc (p>0.05), as compared to controls, while endostatin concentrations were significantly higher in all disease evolution phases (p<0.001) (Table IV). Moreover, we realised that the endostatin serum levels were higher in intermediate and late SSc as compared to early SSc, although differences reached statistical significance only for patients with intermediate SSc (p=0.003).

Analysis comparing angiostatin and endostatin levels in patients presenting organ involvement (score ≥1) *versus* without organ involvement (score=0), revealed that angiostatin levels were significantly higher in patients with osteoarticular disease; no significant results were found on the other organ analysis or regarding to endostatin. A zero Medsger skin score corresponds indeed to the pre-SSc subset (Table V). Respecting to the severity of organ involvement, we observed that angiostatin serum levels were higher in patients presenting more serious lung involvement (score ≥2) (p<0.05). Likewise, no significant differences were found on the other organ analysis or regarding to endostatin (Table V). Sub-analysis for lung involvement also found no significant differences between normal and abnormal CT-scan, PSAP and pulmonary function tests.

Fifty-one patients from the study population presented changes on nailfold capillaroscopy. Analysis of angiostatin and endostatin concentrations in its three different stages showed that endostatin was significantly increased in all the 3 stages compared to controls, while angiostatin was only elevated in active and late phases (Table VI).

Discussion

Systemic sclerosis is associated with a disruption of vascular homeostasis and an unbalance between proangiogenic and antiangiogenic factors (1-3).

The present study shows that endostatin, a proteolytic fragment of type-XVIII collagen that acts as an angiogenesis inhibitor, is significantly higher in the

Table V. Angiostatin and endostatin levels in pre-scleroderma and scleroderma patients, according to organ involvement and Medsger severity scale.

	Angiostatin (ng/ml)						Endostatin (ng/ml)					
	Involvement			Severity			Involvement			Severity		
Organ	Score = 0	Score ≥1	*p*	Score = 1	Score ≥2	*p*	Score = 0	Score ≥1	*p*	Score = 1	Score ≥2	*p*
Skin	46.8 (21.3-76.7)	50.0 (19.3-26.5)	0.495	46.3 (19.3-115.6)	70.9 (24.4-126.5)	0.092	17.7 (1.8-69.8)	20.9 (0.0-178.8)	0.413	20.6 (0.0-73.0)	24.4 (9.8-178.8)	0.642
Peripheral vascular	39.8 (21.3-71.1)	49.2 (19.3-126.5)	0.225	53.3 (19.3-81.3)	48.1 (21.3-126.5)	0.699	19.3 (1.8-69.8)	20.6 (0.0-178.8)	0.966	20.0 (0.0-73.0)	21.8 (3.8-178.8)	0.513
GastrointestinaI tract	48.1 (19.3-115.6)	54.6 (24.4-126.5)	0.264	- -	- -	-	19.4 (0.0-38.3)	20.9 (1.8-178.8)	0.714	- -	- -	-
Lung	50.7 (21.3-101.1)	48.5 (19.3-126.5)	0.965	42.7 (19.3-126.5)	70.9 (24.4-115.6)	0.037	32.4 (2.5-69.8)	18.4 (0.0-178.8)	0.138	18.2 (1.8-178.8)	19.6 (0.0-73.0)	0.579
Heart	51.6 (19.3-115.6)	54.6 (23.5-126.5)	0.395	47.0 (39.8-80.0)	60.6 (23.5-126.5)	0.673	20.4 (2.5-178.8)	19.3 (0.0-40.3)	0.585	19.3 (11.9-21.8)	24.8 (0.0-40.3)	0.752
Osteoarticular	45.8 (21.3-101.1)	58.4 (40.4-126.5)	0.024	- -	- -	-	20.8 (0.0-73.0)	18.6 (8.3-178.8)	0.632	- -	- -	-

Results presented as median (range) values. The number of participants in each group can be obtained from the results presented in Table II.
The patients included in the study showed no renal manifestations or deterioration in general health and only one patient showed changes in muscle strength, so these scores are not presented.

Table VI. Relation between capillaroscopic patterns and angiostatin and endostatin serum levels in pre-scleroderma and scleroderma patients (n=51).

Capillaroscopic pattern	Angiostatin (ng/ml)	*p*-value (patients *vs*. controls)	Endostatin (ng/ml)	*p*-value (patients *vs*. controls)
Early (n=12)	48.3 (19.3-76.7)	0.119	27.9 (1.8-69.8)	<0.001
Active (n=21)	46.1 (23.5-115.6)	0.024	19.4 (2.5- 178.8)	<0.001
Late (n=18)	70.9 (23.5-126.5)	0.010	21.0 (5.2-44.0)	<0.001

Results presented as median (range) values.

sera from patients with SSc than in healthy controls, supporting previous studies which had already established this relationship (4, 6-10), including two studies that assessed larger cohorts of SSc patients (8, 10), and in opposition to the results obtained by Distler *et al*. (5). With respect to angiostatin, and in agreement with the only study we found in the literature (11), we observed that its serum levels were significantly higher in SSc patients than in healthy controls, suggesting that this molecule, a cleavage product of plasminogen with anti-angiogenic properties, also plays an important role in SSc pathogenesis. In addition to confirm these observations, our study add relevant information to what is already present in the literature.

Concerning the disease subsets, in addition to confirm prior findings of higher endostatin serum levels in limited and diffuse SSc subsets than in healthy controls (7), we realised that endostatin levels are also increased in Pre-SSc. In contrast, we also observed, for the first time, that angiostatin was significantly higher in patients with lcSSc and dcSSc, but not in patients with pre-SSc.

Previous studies have also tried to establish a relationship between the concentration of these antiangiogenic factors and SSc disease phases (5, 9). We observed that endostatin was elevated in all clinical phases of disease, in opposition to Distler *et al*. (5). Furthermore, we realised that there was a significant difference between endostatin levels in early and intermediate disease phases in a similar manner of Farouk *et al*., who found significantly increased levels in late stage of disease defined as disease duration superior to 3 years (9). Moreover, we found, for the first time, that serum endostatin levels were increased in patients with lcSSc, with and without CREST, while angiostatin concentrations were only increased in patients with CREST, making us wonder if there could be different pathogenic mechanisms in this specific clinical picture of the SSc spectrum.

As expected and, once more, in opposition to Distler *et al*. (5), a positive correlation was achieved between serum levels of endostatin and all the three stages of nailfold capillaroscopic changes, and between angiostatin and active and late stages of capillaroscopic changes.

Some studies had already tried to establish a relationship between endostatin levels and lung, heart, kidney, skin and peripheral vascular involvement, but not with other organ involvement or with angiostatin (4, 5, 7, 9).

In regard to endostatin and lung disease, our results are in consonance with previous studies found in literature, which did not find a significant interrelation between endostatin levels and lung involvement (5, 7). In addition, we found that high serum angiostatin levels were positively correlated with the severity of lung involvement (Mesdger score ≥2). Hebbar *et al*. established a positive correlation between endostatin and abnormalities on chest x-ray; however, the comparison of this population with all the other patients (including those with no alterations and with altered pulmonary function tests), may have influenced the results (4). Other study found a positive correlation between endostatin levels and lung disease, defined as DLCO<75% and FVC<80% (9); we conducted a similar analysis, which showed no significant results. Using different methodological approaches, an inverse correlation was achieved between endostatin concentration and FVC (8) and DLCO (10). As for pulmonary hypertension, two studies found a positive relationship with endostatin levels (8, 10). In accordance, Hummers *et al*. recognised that patients presenting high right ventricular systolic pressure (≥40 mmHg) and pulmonary arterial hypertension confirmed by right heart catheterisation, had higher concentrations of endostatin (our similar analysis showed no significant differences) (8); and supporting these findings, Reiseter *et al*. observed that increased circulating endostatin was independently associated with pulmonary arterial hypertension (10).

Some studies have also analysed the relationship between skin score and peripheral vascular involvement and endostatin, with contradictory results (4, 5, 7, 9). Regarding to skin, two failed to achieve a significant correlation between skin involvement and endostatin (5, 7), as we observed, but two others concluded that endostatin levels were higher in patients with higher skin scores (4, 9), although a different classification for cutaneous sclerosis (Barnett classification) was used for the first one and a histopathological evaluation in the second one. As for cutaneous ulcers, only one found a positive correlation (4).

In contrast to our results regarding to cardiovascular system, a prior study demonstrated endostatin concentrations in SSc patients with heart involvement were significantly higher than in those without such changes (6). Surprisingly, attending to the antiangiogenic effect of angiostatin in synovial membrane (28), we realised that angiostatin serum levels, but not endostatin, were higher in patients presenting osteoarticular involvement.

Taken altogether, these achievements allow us to conclude that endostatin might play an important role as an early marker of disease and that persists over disease evolution. In addition, the systematic observation of elevated angiostatin serum levels in more advanced stages of disease – in limited and diffuse SSc, in intermediate and late phases of disease, with active and late capillaroscopic changes, or in a global view in patients with and more severe organ involvement, allow

us to hypothesise that angiostatin has a greater impact in disease progression, is a marker of late disease and relates to lung disease severity.

In summary, we demonstrated that SSc is associated with the elevation of two anti-angiogenic factors, endostatin and angiostatin. Endostatin increases in an early stage of disease, highlighting its possible role as an early marker that might help us in the clinical assessment of of SSc – may it predict evolution from pre-scleroderma phase to *true* systemic sclerosis? Prospective longitudinal studies are required to assess this hypothesis. Furthermore, angiostatin is not only a marker of late disease and severer lung involvement but also it is related with disease progression and it might be implicated in a distinct pathogenic mechanism responsible for the development of CREST in patients with limited SSc. Likewise further studies are needed to assess this relationship.

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