More severe nailfold capillaroscopy findings and anti-endothelial cell antibodies. Are they useful tools for prognostic use in systemic sclerosis?

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Abstract

Objective

Anti-endothelial cell antibodies (AECA) have been described in systemic sclerosis (SSc) but their clinical relevance is unclear.

Methods

Aim of this study was to measure serum levels of AECA in 62 SSc patients, examining the main clinical and laboratory features, including nailfold capillaroscopy (NC) abnormalities and looking for any significant association.

Results

Fourteen patients (23%) were AECA positive. An "early" NC pattern was observed in 21 patients (34%), an "active" pattern in 24 (39%) and a "late" pattern in 17 cases (27%). In those patients with AECA, a "late" NC pattern was significantly more frequent respect to the "early" and "late" patterns ($p<0.05$); besides AECA serum levels were significantly higher in the "late" group of patients respect to the other two ($p<0.04$ and $p<0.02$ respectively), also showing a significantly more severe modified skin score (mSS) (≥15) ($p<0.04$), while those cases with more aggressive NC patterns ("active" and "late") had a more frequent finding of arterial hypertension ($p<0.05$) and cardiac involvement ($p<0.05$) respect to those with "early" NC pattern.

Conclusion

Thus, advanced NC findings were more frequently found in those patients with higher levels of AECA and their contemporary presence may consent to identify specific SSc subsets i.e., those with higher skin scores and cardiovascular involvement. These data suggest that AECA may have a role in the progression of the endothelial damage and their presence and titer should be considered as an adjunctive risk factor for a more severe disease. We also confirm the diagnostic and prognostic validity for NC in SSc, underlying the importance for an accurate capillaroscopic assessment. The contemporary assessment of these two diagnostic tools can be useful to better define different subset of SSc patients.

Key words

Systemic sclerosis, anti-endothelial cell antibodies, nailfold capillaroscopy.

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Introduction

Microvascular involvement seems to be one of the earliest event in systemic sclerosis (SSc) and many different factors, including humoral autoimmunity, may contribute to the endothelial cell damage (1). Thus it is not surprising that anti-endothelial cell antibodies (AECA) have been frequently described in this connective tissue disease although their clinical relevance is still debated (2) and no exaustive data are available on their real prevalence, varying from 20 to 70% according to different reports (3-6).

AECA seem to be more frequent in the diffuse subset and in those patients presenting pulmonary involvement, mainly pulmonary fibrosis or hypertension and alveolo-capillary impairment, or with digital ulcers, conditions clearly related to some kind of vascular or, more correctly, microvascular impairment (5-11). Besides AECA have been also proposed as serologic markers of disease severity in SSc (5).

To clinically evaluate the microvascular involvement in SSc patients, nailfold capillaroscopy (NC) proved to be a non-invasive technique able to show early microcirculatory abnormalities that may have diagnostic and prognostic value (12, 13). Typical NC features have been described (12, 14, 15, 16), and their evaluation has been proposed to increase the diagnostic sensitivity, at least for the limited form of the disease (17). However there is still a surprising discrepancy between NC potential application and its still too limited use in rheumatological practice, considering that it seems a reliable diagnostic and prognostic tool for SSc (18).

It is well known that SSc may present a multi-organ involvement and its clinical features may vary a lot from patient to patient, thus having variable outcome, with different prognostic evolution and, what is more important, requiring different therapeutical approaches (19).

To further implement our knowledge on the possible outcome measures, we focused on specific microvascular variables thus evaluating the NC abnormalities together with AECA serum levels in a group of SSc patients. Furthermore we examined the main cutaneous, pulmonary, cardiac, renal and capillaroscopic features in these patients, looking for any possible correlation among the different clinical, instrumental and laboratory parameters.

Materials and methods

Sixty-two consecutive patients (57 women and 5 men; mean age = 52.8 yrs; mean disease duration = 108.7 months) fulfilling the SSc classification criteria proposed by the American College of Rheumatology (ACR) (20) were recruited from the Division of Rheumatology in Rome University, giving their informed consent. The clinical, capillaroscopy and laboratory data reported in this study were obtained at the time the blood samples were drawn. Control sera were obtained from 20 healthy subjects, matched for sex and age.

Patients had a detailed clinical assessment and their organ system involvement was defined as previously described (21): lung = bibasilar pulmonary fibrosis on chest radiography; isolated pulmonary hypertention = clinical evidence of pulmonary hypertention and increased mean pulmonary arterial pressure (> 35 mmHg), indirectly assessed by echocardiography, in the absence of severe pulmonary interstitial fibrosis; esophagus = hypomotility shown by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arhythmias requiring treatment.

The cutaneous evaluation included: modified skin score (mSS) (22), presence/absence of Raynaud's phenomenon, digital pitting scars, teleangectaesia, calcinosis.

All serum samples were stored at -70°C until used. Antinuclear antibodies (ANA) including anti-centromere antibodies were detected by indirect immunofluorescence using HEp-2 cell line as substrate (Binding Site). Antibodies against topoisomerase I (anti-Scl70) and anticardiolipin antibodies (IgG and IgM isotypes) were measured using enzyme-linked immunosorbent assays (ELISA) (Diamedix, Miami, FL).

AECA serum levels were also measured according to an ELISA method. Briefly, human umbilical-vein endothelial cells were isolated by collagenase perfusion

from normal-term umbilical cord veins as previously described (23) and were cultured in M199 medium (Sigma Chemical Co, St. Louis, MO, USA) supplemented with 20% FCS. These cells (third to fourth passage) were used to detect AECA of IgG isotype using a cell-surface ELISA on living cells allowed to grow to confluence in microtiter plates. After three washes with Hank's balanced salt solution (HBSS), non-specific binding sites were blocked for 2 hours at room temperature with 3% bovine serum albumin/HBSS. After two washes with HBSS, the wells were incubated with 100 ml of each patient serum, diluted 1:50 in HBSS, for 2 hours at room temperature. After three washes with HBSS, the bound antibodies were detected with alkaline-phosphatase-conjugated goat antibodies anti-human IgG (Sigma), using 1mg/ml *p*-nitrophenylphosphate. Optical density (OD) was read at 405 nm wave length and AECA were expressed as a binding index (BI), equal to 100 x (S-A)/(B-A), where S is the OD of the tested sample, A is the OD of a negative control, and B that of a positive reference serum. AECA were considered positive when the BI was higher than the cut-off value (mean + 2 SD of 66 healthy controls) corresponding to 50% of a positive reference serum from an SLE patient (24). Each serum sample was tested in duplicate.

Nailfold videocapillaroscopy

Each patient was acclimatized for 20 minutes at a room temperature of 20-24°C prior to NC. NC was carried out using a Wild stereomicroscope with interchangeable lenses giving magnifications of x100, x160, x250 and x400. A fibreoptic light source and filter provided cold illumination. The optical microscope was connected to a JVC colour video camera. This allowed the image of the NC to be projected on to a Sony 14 inch television screen which was connected to a colour video printer, in order to obtain data as homogeneous and comparable as possible (25).

In each patient, the nailfolds of all fingers were examined after a drop of immersion oil was placed on the nailfold bed to improve resolution. Fingers affected by recent local trauma were not analyzed. NC was performed according to the standard method (14). Only capillaries in the distal row of the nailfold were analyzed and scored. Haemorrhages were evaluated near the distal row.

The following morphological parameters were considered, according to previous classifications: the presence of enlarged and giant capillaries, haemorrhages, loss of capillaries (avascularity), disorganization of the vascular array, ramified/bushy capillaries and sludging of blood (14, 25). A semiquantitative rating scale was adopted to score these changes, according to previous studies (15, 26): score 0 = no changes; 1 = few (< 4) alterations; 2 = some (between 4 and 6) alterations; 3 = frequent (6 or more) alterations per linear millimetre. The mean score for each subject was obtained from analysis of all fingers.

The rating system for avascular areas (avascularity of the capillary bed) was classified as grade 0 = no obvious avascular areas; grade 1 = mild (one or two discrete areas of vascular deletion); grade 2 = moderate = more than two discrete areas of vascular deletion; grade 3 = severe (the presence of large, confluent avascular areas) (27).

Patients were distributed into a proper NC pattern, as already reported (15). The patterns were (A) "early" (few giant capillaries, few haemorrhages, relatively preserved capillary distribution, no evident loss of capillaries); (B) "active" (frequent giant capillaries, frequent haemorrhages, moderate loss of capillaries with some avascular areas, mild disorganization of the capillary architecture, absent or some ramified capillaries); (C) "late" (irregular enlargement of the capillaries, few or absent giant capillaries, absence of haemorrhages, severe loss of capillaries with large avascular areas, severe disorganization of the normal capillary array, frequent ramified/bushy capillaries).

Statistical analysis

Categorical variables were analysed by χ^2 test or Fisher's exact test and differences between the means were determined using Mann-Whitney test for

Table I. Main clinical-demographic characteristics of 62 SSc patients.

M/F	5/57
Mean age (range)(years)	52.8 (20-77)
Mean disease duration (range)(months)	108.7 (3-420)
dSSc/lSSc (n/%)	35/27 (56/44)
Modified skin score ≥15 (n/%)	14/23
"early" NC pattern (n/%)	21/34
"active" NC pattern (n/%)	24/39
"late" NC pattern (n/%)	17/27
AECA+ (n/%)	14/23
Anti-centromere antibodies+(n/%)	25/40
Anti-topoisomerase I antibodies+ (n/%)	20/32
Anti-phospholipid antibodies+ (n/%)	7/11
Pulmonary fibrosis (n/%)	25/40
Pulmonary hypertension (n/%)	7/11
Gastrointestinal involvement (n/%)	34/55
Cardiac involvement (n/%)	11/18
Digital pitting ulcers (n/%)	33/53
Treatments (n/%)	
*Low-dose steroids**	22/35
*Immunosuppressive agents***	25/40
*Vasodilators****	36/58
None	19/30

* ≤8 mg/day of 6-methylprednisolone.
** cyclophosphamide, methotrexate, azathioprine, cyclosporine.
*** Calcium channel blockers, e.v. prostanoids.

unpaired samples. *P*-values less than 0.05 were considered statistically significant.

Results

The main clinical-demographic and laboratory parameters of our 62 SSc patients are shown in Table I.

Thirty-five (56.4%) of them had a diffuse cutaneous form of SSc (dSSc) and 27 (43.6%) had a limited cutaneous SSc (lSSc). All patients complained for Raynaud's phenomenon. 14 patients (23%) had a modified skin score (mSS) ≥15 while pulmonary fibrosis was found in 25 cases (40%) and pulmonary hypertension in 7 (11%). Anti-topoisomerase I antibodies were present in 32% of cases, anti-centromere antibodies in 40.3%, anti-phospholipid antibodies in 11%.

In the 14 SSc patients (23%) with AECA, 5 (35%) had a mSS ≥15, 6 (43%) had pulmonary fibrosis, 9 (64%) had gastrointestinal abnormalities, 6 (43%) had digital pitting scars and 11 (78%) had an articular involvement; 7 of them (50%) had also xerophtalmia and xerostomia. The main features of the patients with and without AECA are shown in Table II.

A capillaroscopic score = 1 was found in 16 cases (26%), score = 2 in 28 (45%) and score = 3 in 18 (29%). An avascular area score grade 1 was present in 15 patients (24%), grade 2 in 17 (27%) and grade 3 in 16 (26%). An "early" NC pattern was observed in 21 SSc patients (34%), an "active" pattern was found in 24 patients (39%) and a "late" pattern was recognized in 17 cases (27%). Those patients with a late capillaroscopy pattern had a more frequent diffuse subset of the disease, in 12 (71%) cases respect to 5 cases (29%) with a limited subset.

In those patients with AECA, a "late" NC pattern (Fig. 1) was significantly more frequent respect to the "early" and "active" patterns (p <0.05); besides AECA serum levels were significantly higher in the "late" group of SSc patients respect to the "early" and "active" groups (p=0.04 and p<0.02, respectively) (Fig. 2). Furthermore, the same group of patients showed a significantly more severe mSS (≥15) (p<0.04), while those cases with more aggressive NC patterns ("active" and "late") had a more frequent finding of arterial hypertension (p<0.05) and cardiac involvement (p<0.05) respect to those with "early" NC abnormalities (Table III).

We did not find any significant association concerning age, disease duration or treatments.

Table II. Comparison of the main clinical-demographical and laboratory parameters in SSc patients with and without AECA.

	AECA+ patients (n=14)	AECA- patients (n=48)
M:F	2:12	3:45
Mean age (range)(years)	59.4 (30-74)	50.8 (20-77)
Mean disease duration (range)(months)	73.5 (3-204)	118.9 (11-420)
dSSc/lSSc (n)	8/6	26/22
Modified skin score ≥15 (n/%)	5/36	9/19
Pulmonary fibrosis (n/%)	6/43	19/40
Articular involvement (n/%)	11/78.5	30/62.5
Gastrointestinal involvement (n/%)	9/64	25/52
Cardiovascolar involvement (n/%)	3/21	8/17
Pulmonary hypertension (n/%)	3/21	4/8
ESR > 30mm/hr (n/%)	6/43	8/17
CRP > 6mg/L (n/%)	7/50	9/19
Anti-topoisomerase I+ (n/%)	5/36	15/31
Anti-centromere+ (n/%)	7/50	18/37.5
Capillaroscopic score = 3* (n/%)	6/43	12/24
Avascolar areas score > grade 1 (n/%)	8/57	25/52

*Frequent alterations (> 6 per linear millimeter).

Discussion

AECA are a rather heterogeneous group of autoantibodies and can be found in a wide variety of diseases, where endothelial damage is a relevant aspect (3, 4). They have been proposed to have a pathogenetic role, and to be able to induce a vascular inflammation of autoimmune origin (28).

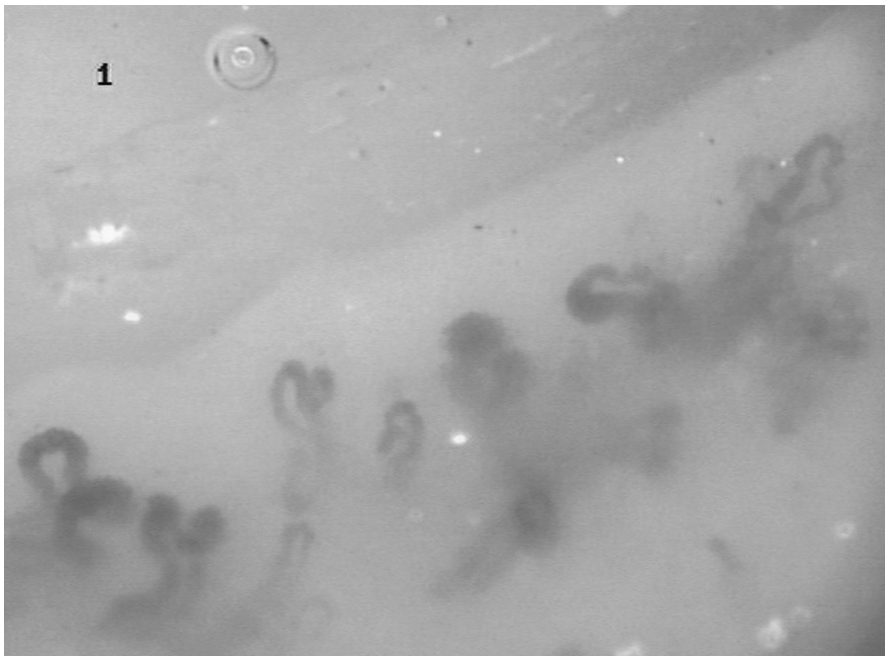

Fig. 1. "late" nailfold capillaroscopy pattern in a SSc patient with high serum levels of AECA. Irregular enlargement of the capillaries with few giant capillaries, avascular areas and a severe disorganization of the capillary array with ramified capillaries are shown.

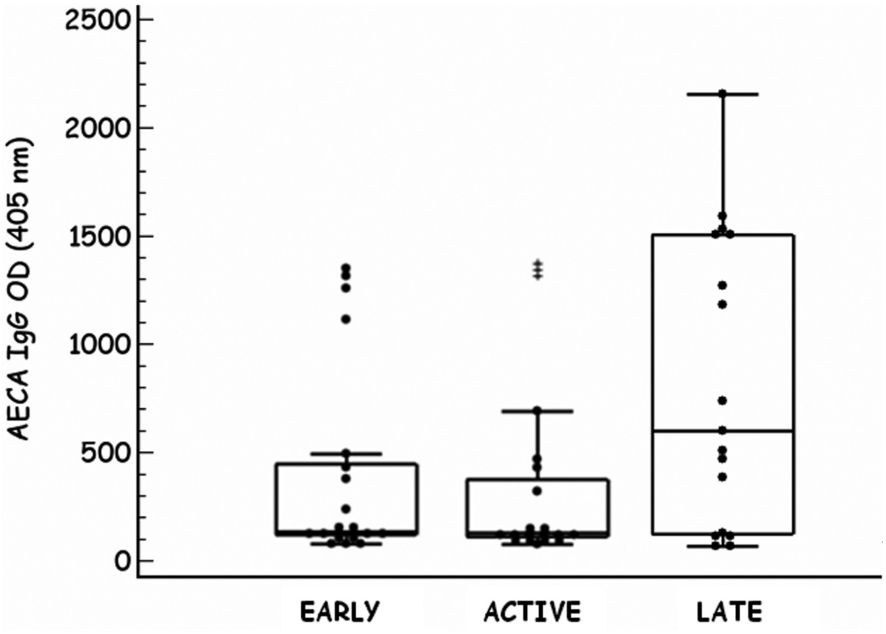

Fig. 2. Graphical representation of significantly higher AECA serum levels in those SSc patients with "late" NC pattern respect to "early" (p=0.04) and "active" (p<0.02) groups.

Table III. Main differences among 62 SSc patients divided on the basis of the three NC patterns (early, active, late).

	Early NC pattern	Active NC pattern	Late NC pattern
Patient numbers (M/F)	21 (0/21)	24 (4/20)	17 (1/16)
Mean age (years)	50.4	50.7	57.7
Mean disease duration (months)	100	118.8	103.4
Diffuse SSc (n/%)	12/57	11/46	12/71
Limited SSc (n/%)	9/43	13/54	5/29
AECA + (n/%)	4/19	3/12.5	7/41
Anti-topoisomerase I Ab+ (n/%)	6/29	7/29	6/35
Anti-centromere Ab+ (n/%)	8/38	11/46	6/35
mRodnan Skin Score ≥15 (n/%)	3/14	3/12.5	8/47*
Arterial hypertension (n/%)	0/0	6/25**	6/35**
Cardiovascular involvement (n/%)	1/5	5/21**	5/29**

* p<0.04 with respect to "early" and "active" pattern
** p<0.05 with respect to "early" pattern

Several investigators have reported the occurrence of AECA in SSc, whereas the prevalence was highly variable depending on patient selection and on the type of laboratory tests. Besides in SSc many different factors seem to be involved in the determinism of the endothelial damage and AECA have been frequently associated with the presence of significant microvascular lesions and disease severity (7-10). But discrepancies still arise concerning the role for these autoantibodies in SSc and there is still meaning concerning the real value for AECA in SSc as well as for their possible correlation with clinical and laboratory features (4, 5, 29).

Actually NC proved to be a useful instrument for the assessment of microvascular involvement in SSc patients, able to show early microcirculatory abnormalities that may have diagnostic and prognostic values in this disease (12-16, 18).

In the present study we investigated the role for AECA in the determinism of the endothelial damage in SSc and to see if two different parameters of microvascular involvement, such as AECA and NC, taken together could be of help for the clinician in the evaluation of SSc and could otherwise implement its assessment.

Although the small number of cases in each group does not permit us to reach any final conclusion, our findings showed that more severe microvascular NC findings were more frequently found in those AECA positive cases with higher levels of these antibodies, regardless the disease duration. Besides in our patients, more severe NC abnormalities significantly correlated with some of the main clinical characteristics of the disease such as the cutaneous and the cardiovascular ones.

Till now the question has been whether AECA should be considered only another potential disease marker or if they can have a pathogenetic role in SSc, as speculated by others (5, 9). Our findings seem to give additional evidence for AECA effects on the microvascular changes, as assessed by NC. The presence and higher levels of AECA, associated with more severe NC abnormalities may be able to identify specific SSc subsets, *i.e.*, those with higher skin scores and cardiovascular involvement. It seems that more severe NC changes are seen in those cases with a more severe cutaneous extension of the disease, such as diffuse rather than limited forms, and with the presence of other vascular aspects, such as arterial hypertension and cardiac abnormalities.

Thus our data suggest that AECA, although not directly linked with the progression of the disease, may have a role in the endothelial damage of SSc and their presence and titer should be considered as an adjunctive risk factor for a more severe disease.

We also confirm the diagnostic and prognostic validity for NC in SSc, underlying the importance for an accurate capillaroscopic assessment in this disease. The already cited more frequent positivity and higher levels of AECA, in those subjects presenting more severe NC patterns, led us to sustain that the contemporary assessment of these two diagnostic tools can be useful to better define different subset of SSc patients.

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