Significance of repeated measurements of autoantibodies and endothelin-1 in systemic sclerosis: a longitudinal study

Sirs,

Serum autoantibodies are considered a hallmark of systemic sclerosis (SSc) (1, 2). Some of them are considered highly specific for SSc, including anti-centromere (ACA), anti-topoisomerase I (anti-topo I) and anti-RNA polymerase III (anti-RNAP III) antibodies (3, 4), and have been included in the 2013 American College of Rheumatology/European League against Rheumatism (ACR/EULAR) classification criteria for SSc (5). In addition, endothelial dysfunction, represented by increased levels of endothelin 1 (ET-1) and others mediators, play a key role in the pathogenesis of SSc (6). Despite the well-known association of SSc-related autoantibodies and ET-1 with distinctive clinical manifestations, few studies have evaluated the longitudinal behaviour and the variability of these biomarkers throughout the evolution of the disease. The present study aimed to analyse the longitudinal levels of SSc-specific autoantibodies and ET-1, as well as the association between them and the microvascular abnormalities detected by capillaroscopy in a 12-month follow-up in patients with SSc.

In this longitudinal study, 70 patients with SSc attending the Rheumatology Division of the Federal University of Mato Grosso Sul and of the Federal University of São Paulo, Brazil, were consecutively selected from March 2016 to April 2017 (baseline data previously published) (7). Patients should meet the ACR/EULAR 2013 classification criteria for SSc (5) and were followed for 12 months. All subjects signed informed consent approved by the institutional ethical committee of both institutions.

Demographic and clinical features were collected. Serum ACA, anti-topo I, anti-RNAP III autoantibodies, and ET-1 levels were measured using enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite Centromere CENP-A & CENP-B, QUANTA Lite TM Scl-70 and QUANTA Lite RNA Pol III, INOVA Diagnostics, San Diego, CA, USA; ELISA Endothelin-1 kit, R&D systems, Minneapolis, MN, USA), at baseline and after 6 and 12 months of follow-up, according to the manufacturers' instructions. Videocapillaroscopy was performed using an optical videocapillaroscopic probe under a 200× magnification lens at the 3 visits (Optilia Medical, Sweden), as previously described (8).

Among the 70 patients (92.9% women, mean age of 46.8±12.5 years, and mean disease duration of 9.41±6.26 years), ACA was present in 28 patients (40%), anti-topo I in 21 patients (30%), and anti-RNAP III in 5 patients (7.1%) at baseline. The serum levels and the number of patients who were positive for ACA and anti-topo I remained stable during the follow-up. Anti-RNAP III showed significantly variability in its levels and frequency throughout the study, but most of their serum levels were low. ET-1 levels significantly increased at 12 months compared to baseline (p=0.047). Videocapillaroscopy showed a significant decrease in the number of capillaries/mm and microhaemorrhages and an increase in the number of enlarged capillaries and giant capillaries and in the avascular score throughout the 12-month follow-up (Table I). Patients with persistently high levels of ET-1 presented a higher frequency of active and recurrent digital ulcers, and a worse microangiopathy on capillaroscopy than those with normal ET-1 levels at all evaluations (p<0.05). Our study has several limitations, including the sample size and the lack of a longitudinal analysis of internal organ involvement.

In conclusion, the stability of ACA and anti-topo I levels during follow-up indicate that they are not useful as disease activity biomarkers. Anti-RNAP III showed considerable variability in their levels throughout the study, but the clinical significance of this finding still needs further investigation since most of these patients presented low levels of this antibody, indicating that low levels of anti-RNAP III, specially using ELISA, should be interpreted with caution (9, 10). The association of ET-1 levels with more severe microangiopathy on videocapillaroscopy and the presence of digital ulcers, suggests a possible role of ET-1 as a peripheral vascular biomarker in SSc.

Table I. Longitudinal data of systemic sclerosis-related antibodies, endothelin-1 serum levels and nailfold capillaroscopy of the 70 SSc patients.

Variable	Follow-up			*p*
	Baseline	6 months	12 months	
ACA				
Positive, n (%)	28	33	33	0.556
Weak/moderate/strongly reactive	3/10/15	5/12/16	3/16/14	0.779
Mean value (U/mL) in the 28 patients with positive ACA in the first evaluation	78.5 ± 31.0	81.7 ± 33.6	75.9 ± 26.7	0.232
Anti-topo I				
Positive, n (%)	21	19	20	0.925
Weak / moderate / strongly reactive	4/1/16	1/3/15	3/4/13	0.450
Mean value (U/mL) in the 21 patients with positive anti-topo I in the first evaluation	160.0 ± 90.5	141.1 ± 73.8	142.8 ± 88.4	0.078
Anti-RNAP III				
Positive, n (%)	5	1	9	0.028
Weak / moderate / strongly reactive	5/0/0	1/0/0	4/5/0	0.082
Mean value (U/mL) in the 5 patients with positive anti-RNAP III in the first evaluation	31.8 ± 5.0	16.9 ± 10.8	41.8 ± 15.6	0.009
Endothelin-1				
Mean value (pg/ml), ± SD	2.0 ± 1.8	2.1 ± 2.2	2.5 ± 1.6	0.047
High ET-1 levels, n (%)	20	18	34	0.005
Nailfold videocapillaroscopy				
Number of capillaries/mm	7.32 ± 1.01	7.09 ± 0.98	6.59 ± 1.16	<0.001
Enlarged capillaries	1.12 ± 0.79	1.32 ± 0.82	1.60 ± 0.89	<0.001
Giant capillaries	0.22 ± 0.24	0.29 ± 0.30	0.45 ± 0.36	<0.001
Microhaemorrhages	0.64 ± 0.60	0.57 ± 0.68	0.37 ± 0.42	<0.001
Avascular score	0.97 ± 0.72	1.08 ± 0.72	1.26 ± 0.75	<0.001

Results are presented as mean ± standard deviation or absolute number and frequency.
ACA: anti-centromere; anti-topo I: anti-topoisomerase I; anti-RNAP: anti-RNA polymerase III.
ACA, anti-Scl-70 and anti-RNA polymerase III: negative <20 units/mL (U/mL); weakly reactive 20-39 U/mL, moderately reactive: 40-80 U/mL; strongly reactive > 80 U/mL
Generalised linear models and Bonferroni *post hoc* tests were carried out to compare differences among different times of assessment (baseline, 6 months and 12 months) and between groups.

A.M.C. HORIMOTO[1,3], *MD, PhD*
A.S. DE SOUZA[2], *MD, PhD*
S.H. RODRIGUES[3], *MD*
C. KAYSER[3], *MD, PhD*

[1]Department of Medicine, Rheumatology Division, Universidade Federal do Mato Grosso do Sul, Campo Grande;
[2]Department of Biological and Health Sciences, Institute of Biosciences, Universidade Federal do Mato Grosso do Sul, Campo Grande;
[3]Rheumatology Division, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil.

Please address correspondence to:
Alex M.C. Horimoto,
Department of Medicine,
Rheumatology Division,
Universidade Federal do Mato Grosso do Sul,
Av. Senador Filinto Muller s/n°,
Campo Grande, MS, 79080-190, Brazil.
E-mail: clinicaactivite@gmail.com

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