

The level of anti-topoisomerase I antibodies highly correlates with metacarpophalangeal and proximal interphalangeal joints flexion contractures in patients with systemic sclerosis

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Abstract

Background

It is found that an antibody directed against DNA topoisomerase I (anti-topo I abs) is detected almost exclusively in systemic sclerosis (SSc). These antibodies are predictors of pulmonary fibrosis and peripheral vascular disease.

Objective

Metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints flexion contractures are assessed as markers of active SSc. The aim of this study was to find out is there any relationship between anti-topo I abs and MCP and PIP joints flexion contractures.

Methods

Twenty-eight patients with active disease who fulfilled the American College of Rheumatology criteria for SSc were included in this study. Twenty eight healthy control subjects were also investigated. Clinical and radiological assessments of the hands were carried out. The flexion ranges in the 8 finger joints by goniometric measurement were obtained. Anti-topo I abs with an enzyme linked immunosorbent assay (ELISA) were measured.

Results

MCP and PIP joints flexion contractures and the levels of anti-topo I abs were significantly higher in patients with systemic sclerosis than in healthy control. The anti-topo I abs were found in 16 of 28 patients with systemic sclerosis. Sixteen of 28 patients with active disease had MPC and proximal PIP joints flexion contractures. In 16 SSc patients with anti-topo I abs, 13 had metacarpophalangeal and proximal interphalangeal joints flexion contractures. In only 3 patients of 16 with the flexion contractures the levels of anti-topo I abs were negative. The patients with MPC and PIP joints flexion contractures had higher mean value of anti-topo I abs titers (53.718 ± 50.977 vs 8.127 ± 8.915 , $P < 0.0001$) than did those with no contractures. Furthermore, the titers of anti-topoisomerase I antibody positively correlated with the flexion contractures ($r = 0.4252$, $P = 0.0241$). Radiologically, joint space narrowing and flexion contractures of the fingers were seen significantly more frequently in the SSc patients with anti-topo I abs ($P < 0.05$).

Conclusion

Serum level of anti-topoisomerase I antibodies is in direct relationship with MPC and PIP joints flexion contractures.

Key words

Systemic sclerosis, anti-topoisomerase I antibody, contracture.

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Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by fibrosis and vascular obliteration affecting the skin and certain internal organs, including the lung, heart, gastrointestinal tract, and kidneys (1). The etiology and pathogenesis of SSc remain unknown. Signs of vascular injury and devascularization of involved organs in association with evidence of profound endothelial dysfunction are well documented. Fibroblast activation leading to tissue fibrosis and immune involvement constitute the two other fundamental pathologic processes in the disease in addition to the vascular disorder (2). Numerous *in vivo* studies indicate that SSc fibroblast acquire their activated status very early during disease progression, but the underlying mechanisms responsible for the switch to activated status remains unknown (3). In diffuse SSc (dSSc), there may be initial diffuse puffiness of the finger, but with time the skin thickens, and flexion contractures often develop (4). During the disease course, many patients develop joint involvement which is manifest clinically as arthralgia and/or arthritis and/or flexion contractures, and radiologically as joint space narrowing, erosions and subluxatio (4, 5). Articular involvement in SSc could depend either on periarticular fibrosis or synovitis or even on an overlapping rheumatoid arthritis (6). Musculoskeletal symptoms range from mild arthralgias to frank nonerosive arthritis with synovitis resembling rheumatoid arthritis. The sclerosis of the skin of the fingers or limbs is often associated with contractures of the joints. Deeper tissue fibrosis can also involve the fascia and underlying muscle. If areas around the tendons are involved, active and passive range of motion of the joints are limited and painful (4). Flexion contractures in SSc develop secondary to abnormal tightening of the skin. Indeed, flexion contractures occur the most often at the hands, especially at MCP and PIP joints, but they can also occur elsewhere (4).

SSc is associated with several autoantibodies, each of which is useful in the

diagnosis of affected patients and in determining their prognosis. The autoantibodies classically associated with SSc include anti-centromere antibodies (ACA) and anti-topo I abs (otherwise known as anti-Scl-70 antibodies). ACA and anti-topo I abs are very useful in distinguishing patients with SSc from healthy controls, from patients with other connective tissue disease, and from unaffected family members (7, 8). ACA often predict a limited skin involvement and the absence of pulmonary fibrosis (9). Antinuclear antibodies (ANA) are found in the sera of more than 95% of SSc patients. Previous studies have established an association between ANA and certain clinical subsets of SSc.

Antibody directed against DNA topoisomerase I is found only in SSc (5). Anti-topo I abs has been associated with peripheral vascular disease, pulmonary intestinal fibrosis (8), cardiac involvement, coexisting malignancies (9), and HLA-DRB1*11 (10-12).

Kuwana *et al.* (13) reported the association of clinical improvement with loss of anti-topo I abs in a small group of 28 patients with SSc. Results of this study indicate that there is a distinct subset of anti-topo I-positive SSc patients who lose anti-topo I antibody during the disease course and have a favorable outcome. *In vivo* production of anti-topo I autoantibody may require antigenic stimulation that activates topo I-reactive T and B cells (13). Some other investigators have identified correlations of anti-topo I abs levels with certain clinical features, including gastrointestinal disease, skin thickness, and pulmonary function (14). In patients with SSc anti-topo I abs correlate positively with the disease severity in the skin (total skin score) and with disease activity (15).

MCP and PIP joints flexion contractures are assessed as markers of active SSc. The aim of this study was to find out is there any relationship between anti-topo I abs and MPC and PIP joints flexion contractures.

Patients and methods

Between July 2000 and August 2004, 28 female patients with SSc fulfilling

the American College of Rheumatology criteria for diagnosis (1) and 28 female healthy controls (HC) were enrolled into the study. All HC subjects were clinically examined and they were free from finger deformity, joint swelling or tenderness, or other evidence of rheumatic disease. The University hospital Split Local research Ethic Committee approved the study and all subjects provided informed consent to participate. 28 SSc patients (mean age $49 \pm 9,529$ years, range 32–73) were studied and compared with 28 HC, matched for sex, race and age (mean age $44 \pm 8,826$, range 30–68). Disease duration was 3.3 ± 3.5 years (range 0.08–10 years). The duration of the disease was calculated from the time of onset of the first clinical event (other than Raynaud's phenomenon) that was a clear manifestation of SSc. None of the SSc patients had received immunosuppressive therapy. 28 new sera from patients with SSc and from HC were examined for anti-topo I abs, ANA, ACA, RF, presence or absence of digital amputations, active or healed ulcers, arthralgia, arthritis and tendon friction rubs. (Table I). Sixteen of 28 SSc patients were positive for anti-topo I abs. All 28 patients were classified as having diffuse SSc (1). The activity of SSc was based on the European Scleroderma Treatment and Research (EUSTAR) criteria (16).

ESR was measured according to the Westergren method, C-reactive protein (CRP) (cut-off level 0.5 mg/mL) and rheumatoid factor (RF) were tested by immunonephelometric assay (using a Behring nephelometer) (cut-off level 18 U/mL) (17). ANA were detected by immunofluorescence, using HEp-2 cells as the substrate, and double immunodiffusion (ANA with titers $\geq 1:40$ were considered positive) (18). ACA were detected by indirect immunofluorescence assay using HEp-2 cells (19). Anti-topo I abs were measured by an enzyme linked immunosorbent assay (ELISA) (20). Anti-topo I abs titer below 15 units was considered negative. Assessments include measurements of MPC and PIP joints range of motion (degrees). In each patient the passive flexion range of the finger

Table I. Characteristics of healthy controls and SSc patients.

	Healthy controls	SSc patients with flexion contracture	SSc patients without flexion contracture
Number of subjects	28	16	12
Sex (female/male)	28/0	16/0	12/0
ANA positive	0	16	12
ACA positive	0	1 (6.25)	2 (16.6)
Anti-topo I abs positive	0	13 (81.25)	3 (25)
RF positive	0	1 (6.25)	1
Presence/absence of digital amputations	NA	2/14	1/11
Active/healed ulcers	NA	1 (6.25)	0
Arthralgias	NA	14 (87.5)	2 (16.6)
Arthritis	NA	1 (6.25)	1 (8.3)
Tendon friction rubs	NA	2 (12.5)	1 (8.3)

joints was measured in both hands using the zero axis technique recommended by the American Academy of Orthopedic Surgeons (21). From the measurements in the controls, standards for normal joint motility were defined: PIP joint flexion 110 degrees, extension 5 degrees, entire motility 130 degrees; MP joint flexion 90 degrees, extension 20 degrees, entire motility 110 degrees (21). Joint motility was correlated to the level of anti-topo I abs (Table II). To avoid interobserver error all measurements were made by the same investigator (MR), using a standard digital goniometer. Patients were categorized as having joints contractures or not having joints contractures. MCP and PIP joints flexion contracture were correlated with anti-topo I abs, ANA, ACA, RF, presence or absence of digital amputations, active or healed ulcers and disease duration. Radiographic evaluation included posteroanterior and oblique views of the

Table II. Anti-topo I abs titers in the 28 systemic sclerosis patients grouped according to the presence/absence of MPC and PIP joint involvement*.

	Severity of joint involvement	
	absence of joint contractures (n = 12)	presence of joint contracture (n = 16)
Mean anti-topo I abs titer	8.127 ± 8.915	53.718 ± 50.977

*Values are the mean \pm SD units, $p < 0.0001$ (see text for details).

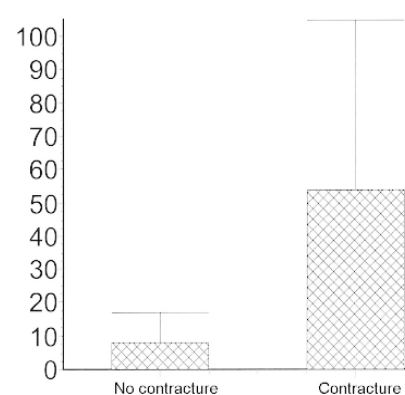


Fig. 1. Higher levels of anti-topo I abs in SSc patients with MPC and PIP joints flexion contractures. The mean anti-topo I abs titer were significantly higher in patients with flexion contractures than in patients with no contracture ($P < 0.001$ by the Mann-Whitney test).

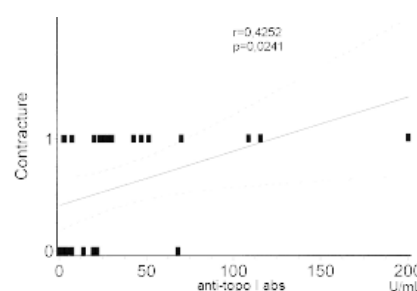


Fig. 2. The MPC and PIP joints flexion contractures are correlated with level of anti-topo I. Spearman's correlation analyses were performed to determine the level of statistical significance.

hands. All radiographs were evaluated without knowledge of the clinical or serological data of the patients examined (Fig. 3). All MPC and PIP joints flexion contractures are recorded with digital camera (Fig. 4).



Fig. 3. Posteroanterior radiographic view of the hands shows MPC and PIP joint flexion contractures, acro-osteolysis and radiological demineralization in a SSc patient.



Fig. 4. Hands of SSc patients with presence of digital amputations, active and healed ulcers and flexion contractures.

Statistical analysis

The analysis of results was performed by the statistical Package for Social Science (SPSS) for Windows release 8.0. Data were expressed at the median and range or mean \pm standard deviation (SD) and 95% confidence interval (95% CI), when appropriate. Differences between groups were analyzed using nonparametric methods. Linear correlation between continuous vari-

ables was evaluated using Spearman's rank coefficient. The significance was set at a P value < 0.05 using two-tailed tests.

Results

All 28 patients were classified as having active dSSc. Arthralgias and MPC and PIP joints contractures were identified more frequently than frank arthritis. No significant correlations were

found between the clinical features of joint involvement and the levels of ANA, ACA, and RF. No significant correlation was found among the clinical features of joint involvement and presence/absence of digital amputations or active and healed ulcers. Whereas the level of anti-topo I abs significantly correlated with the MPC and PIP joint contractures. Sixteen SSc patients (57,14%) were found to be positive for anti-topo I abs. The anti-topo I abs titer directly correlated with the metacarpophalangeal and proximal interphalangeal joints flexion contractures (Table II). The mean anti-topo I abs titers in patients with joint contractures and in patients with no joint contractures were 53.718 ± 50.977 units and 8.127 ± 8.915 units, respectively. The mean anti-topoisomerase I antibody titer was significantly higher in the first group ($P < 0.0001$) (Fig. 1). The patients with a negative anti-topo I abs titer (< 15 units) were primarily those with no joint contractures. Nine of twelve patients with no joint contractures (75.0%) had an anti-topo I abs titer below 15 units. In contrast, three of sixteen (18.7%) patients with joint contractures had anti-topo I abs below 15 units. The anti-topo I abs titer significantly correlated with the MPC and PIP joint contractures: a coefficient of correlation is ($r = 0.4252$) (Fig. 2) and the P value is 0.0241. No significant correlation was found among the clinical features of joint involvement and disease duration. The prevalence of tendon friction rubs was higher in anti-topo I abs positive SSc patients, but the difference did not reach statistical significance. Radiographic abnormalities of joints in dSSc patients were: joint space narrowing (31%), radiological demineralization (23%), acro-osteolysis (14%), flexion contracture (55%) and calcinosis (23%).

Discussion

SSc is a heterogenous disorder in terms of disease symptoms and clinical course. Clinical rheumatic complaints have long been recognized in SSc patients. Most patients show arthralgias, particularly of the hands and feet. Occasionally, joint swelling occurs at

the onset or during the course of the disease, or as a manifestation of an overlapping rheumatoid arthritis, this last association being quite uncommon (4-6). There were no cases that satisfied the classification criteria of rheumatoid arthritis (22) and rheumatoid factor was present at a low titer only in one patient who did not show any contractures. The SSc-specific autoantibody profiles associate strongly with distinct clinical phenotypes and disease symptoms, making serologic testing of great diagnostic aid (7). Anti-topo I abs are very useful in the diagnosis of SSc, they predict pulmonary fibrosis and are associated with a poorer prognosis (5). The presence of anti-topo I abs increases the risk for diffuse skin involvement (23). A positive correlation between the anti-topo I abs level and the total skin score (TSS) (14) and other clinical features was reported previously (13). Up to now, we have not found in the literature any report concerned with positive or negative correlation between level of anti-topo I abs and joint contractures in patients with SSc. We have identified more frequently arthralgias and MPC and PIP joint contractures than frank arthritis. In this study we have found that in patients with dSSc, serum level of anti-topo I abs correlate positively with MPC and PIP joints flexion contractures. However, none of the patients received immunosuppressive therapy or high-dose steroids (which have been reported to decrease the level of anti-topo I abs) (13). Previous studies reported conflicting results concerning the association of increasing anti-topo I abs level with some clinical manifestation. Hildebrandt *et al.* (24) described two patients who developed myositis and worsening cardiac dysfunction with arrhythmia accompanied by a rise in the anti-topo I abs level. In contrast, another study reported that changes in the anti-topo I abs level was not associated with any particular clinical manifestations (25). This study has shown that the level of anti-topo I abs can correlate with some other clinical changes in systemic sclerosis such as MPC and PIP joints contractures. The anti-topo I abs level correlated posi-

tively with MPC and PIP joints contractures (Fig. 2), suggesting that the antibody level may be related to the severity of MPC and PIP joint contractures.

A potential bias in our study may have been the fact that all the enrolled SSc patients were suffering from the diffuse form of the disease, often presenting both MCP and PIP contractures and high titer of anti-topo I abs, thus, the correlation found between these two items could be just a coincidence. However, La Montagna *et al.* (26) did not find any significant correlation between levels of anti-topo I abs and MPC and PIP joint contractures. According to presented results it can be expected that increasing of anti-topo I abs level will be positively related to the severity of MPC and PIP joint contractures. Serum levels of anti-topo I abs correlate positively with disease severity and disease activity in dSSc (13). According to this, MCP and PIP joints contractures could be assessed as markers of active dSSc. Longitudinal study with larger numbers of SSc patients positive for anti-topo I abs will be needed to confirm our findings and expectations.

The question remains whether the high titers anti-topo I abs occurs coincidentally with joint damage or whether it appears earlier and it can be the predictor of contractures in systemic sclerosis.

In conclusion, the serum level of anti-topo I abs is in direct relationship with MPC and PIP joints flexion contractures.

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