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Immunoblotting on HEp-2 cells increases the detection of antitopoisomerase 1 antibodies in patients with systemic sclerosis☆

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Abstract In order to improve the detection of antitopoisomerase 1 antibodies in a cohort of 111 systemic sclerosis patients, we have performed immunoblots on protein extracts of HEp-2 cells. Using indirect immunofluorescence and ELISA, 27 patients (24.3%) had antitopoisomerase 1 antibodies, 32 (28.8%) had anticentromere antibodies, 31 (27.9%) had antinuclear antibodies with none of these two antibodies and 21 (18.9%) had no antinuclear antibody. IgG from 24/27 (88.9%) patients with antitopoisomerase 1 antibodies bound to 2 protein bands of 63 and 100 kDa identified as topoisomerase 1 by N-terminal sequencing. Antitopoisomerase 1 antibodies were detected in 9 (8.1%) patients who had no antitopoisomerase 1 antibody as determined by ELISA. Patients with antitopoisomerase 1 antibodies had an almost similar phenotype without distinction between ELISA or immunoblot approaches. Our results provide evidence for the use of a combination of ELISA and immunoblot approaches for the detection of antitopoisomerase 1 antibodies.

Introduction

Systemic sclerosis (SSc) is a connective tissue disorder characterized by excessive collagen deposition in the dermis and internal organs, by vascular hyperreactivity and obliteration phenomena [1]. Among the multiple autoantibodies identified in the serum of SSc patients, antinuclear antibodies are present in 80–90% of the patients and provide helpful information [2]. Thus, when

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antinuclear antibodies are identified in a given SSc patient, further characterization of these antibodies is warranted. Three autoantibodies are specific for SSc and mutually exclusive: anticentromere antibodies, associated with limited cutaneous SSc (lc-SSc) [3]; antitopoisomerase 1 antibodies, related to diffuse SSc (d-SSc) and interstitial lung disease [4] and anti-RNA-polymerase III antibodies that are associated with scleroderma renal crisis [5]. Although their pathogenic role is not documented, disease-specific autoantibodies are helpful to determine the prognosis. Thus, antitopoisomerase 1 or anti-RNA-polymerase III antibodies are related to visceral involvement, whereas anticentromere antibodies are usually associated with a good prognosis, except for a minority of patients who will develop pulmonary arterial hypertension [6]. Disease specific autoantibodies can be investigated through different methods. Usually, anticentromere antibodies are detected by indirect immunofluorescence (IIF) on HEp-2 cells. Generally, antitopoisomerase 1 antibodies are investigated in patients with antinuclear antibodies without anticentromere specificity, through different techniques, including Enzyme Linked Immunosorbent Assay (ELISA) and counter immunoelectrophoresis. These two techniques are commonly used, although none of them can be considered as the reference. Nevertheless, 5% of SSc patients do not have antinuclear antibodies and 20 to 40% of SSc patients with antinuclear antibodies do not express anticentromere or antitopoisomerase 1 antibodies. Therefore, it is difficult to determine the prognosis of these patients.

By using a quantitative immunoblotting technique, we have observed an IgG reactivity with a 100 kDa endothelial cell antigen, identified as topoisomerase 1 [7]. In that study, we also observed that IgG from all patients with antitopoisomerase 1 antibodies and from 50% of patients with no anticentromere or antitopoisomerase 1 antibody (investigated by IIF and ELISA) bound to topoisomerase 1 [7]. The 100 kDa IgG reactivity was also present and even stronger when HEp-2 cells were used as a source of antigens [7]. These results impelled us to use an immunoblotting technique in order to document the presence of antitopoisomerase 1 antibodies in the serum of SSc patients.

In consequence, we were prompted to analyze serum IgG reactivities of a cohort of SSc patients with HEp-2 cell antigens with the immunoblot approach in order to increase the detection of antitopoisomerase 1 antibodies.

Methods

Patients

All patients fulfilled the American College of Rheumatology and/or Leroy and Medsger criteria [8,9]. Lc-SSc was defined by skin thickening in areas solely distal to the elbows and knees, with or without facial involvement; d-SSc was defined by the presence of skin thickening proximal, as well as distal, to the elbows and knees, with or without facial or truncal involvement [9]. One hundred and eleven SSc patients including 52 (46.8%) with d-SSc and 59 (53.2%) with lc-SSc were included into the study. Their clinical characteristics are detailed in Table 1. Interstitial lung disease (ILD) was defined upon high-resolution computed tomography of the chest including one or more of the following features: isolated ground glass opacities; honeycombing; and concurrent presence of ground glass attenuation and traction bronchectasis and/or bronchiolectasis. Pulmonary arterial hypertension (PAH) was detected by echocardiography in 33 patients. We chose a tricuspid gradient of 30 mm Hg (i.e. an estimated systolic PAP $\geq 30 + 10 = 40$ mm Hg assuming a right atrial pressure of 10 mm Hg in all patients) to suspect PAH. PAH was confirmed by right-heart catheterization in 13 patients, defined as mean pulmonary artery pressure at rest >25 mm Hg. Among the 33 patients with PAH detected by echocardiography, 21 had ILD and forced vital capacity $\leq 75\%$ of normal values. Heart involvement was defined by cardiac insufficiency and/or conduction block or arrhythmia. Scleroderma renal crisis was defined by rapidly progressive oliguric renal insufficiency with no other explanation and/or rapidly progressive hypertension occurring during the course of SSc [10]. Digestive involvement included esophagus and bowel involvement. Esophagus involvement was defined by dysphagia, heartburn and regurgitation. Bowel involvement was defined by malabsorption, pseudo-obstruction and/or bacterial overgrowth. Four patients had a past history of cancer; three patients had anti-RNP antibodies and responded to mixed connective tissue disease criteria, although dominant

Table 1 Clinical characteristics of SSc patients

Groups of patients	ATA	ACA	ANA with no ACA or ATA	ANA negative
Number of patients (female/male)	27 (22/5)	32 (31/1)	31 (21/10)	21 (17/4)
Mean age ± SD (year)	46.2 ± 14.3	54.2 ± 19.3	43.7 ± 13.7	49.1 ± 15.1
Disease duration ± SD (months)	89.4 ± 95.1	71.1 ± 91.8	63.0 ± 106.3	69.5 ± 89.7
Limited cutaneous (%)	4 (14.8)	29 (90.6)	16 (51.6)	10 (47.6)
Diffuse (%)	23 (85.2)	3 (9.4)	15 (48.4)	11 (52.4)
Interstitial lung disease (%)	22 (81.5)	7 (21.9)	14 (45.2)	9 (42.9)
PAH (%)	12 (44.4)	6 (6.8)	9 (29.1)	6 (28.6)
Digestive involvement (%)	24 (88.8)	18 (56.2)	19 (61.3)	13 (61.9)
Heart (%)	5 (18.5)	1 (3.1)	3 (9.6)	1 (4.7)
Renal crisis (%)	5 (18.5)	2 (6.2)	12 (38.7)	2 (9.5)

ACA: anticentromere antibodies; ANA: antinuclear antibodies; ATA: antitopoisomerase 1 antibodies; PAH: pulmonary arterial hypertension. SD: standard deviation.