

Prevalence of anti-histone antibodies, their clinical significance and correlation with other autoantibodies in a cohort of Italian scleroderma patients

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Abstract

Purpose The aim of our study was to determine the prevalence, clinical significance of antibodies to individual histone components and to evaluate their correlation with other autoantibody specificities in a cohort of Italian SSc patients. Some authors, demonstrated high prevalence of anti-histone antibodies in Italian SSc patients, associated with cardiac and renal involvement, suggesting a prognostic value of these autoantibodies; however, these data need to be confirmed.

Methods Serum from 112 adult SSc patients, classified as diffuse (dc) and limited cutaneous (lc) SSc subsets were analyzed for autoantibodies by indirect immunofluorescence, fluoroenzyme immunoassay and enzyme immunoassay.

Results AHA were found in 13 patients (11.6%), nine with lcSSc and four with dcSSc. Among them, five patients were anti-Sc170+ and four were anti-CENP B+. The presence of AHA was not associated with multi-organ involvement or with diffuse subset, as already described. Anti-Sc170 was detected in 43% of patients, anti-CENP B in 32% and anti-RNA polymerase III in 7.1%. We

confirmed the association between anti-Sc170 antibodies and pulmonary fibrosis (OR 15.75, $p < 0.0001$).

Conclusion In our experience, the very low prevalence of AHA in Italian SSc patients and the lack of association with clinical manifestations suggest that this test is of little clinical use; however, it would be worthwhile extending the study to a larger population of patients.

Keywords Systemic sclerosis · Anti-histone antibodies · Prevalence · Clinical manifestations

Introduction

The highest prevalence of anti-histone antibodies (AHA) is in drug-induced systemic lupus erythematosus (SLE), however, AHA are found also in other diseases, such as systemic sclerosis (SSc). SSc is a systemic connective tissue disease characterized by microvascular injury and abnormalities of the immune system leading to fibrosis of the skin and major internal organs. Antinuclear autoantibody (ANA) specificities are associated with certain clinical features of the disease. Moreover, differences in the frequencies of ANA specificities correlated with ethnic origin and geographic locations are characteristic of the disease [1–6]. Some authors [6] have shown differences in antibody specificities and their clinical manifestation, between French and American Caucasian patients; others [7] have shown that serologic profiles of Swedish SSc patients did not differ significantly from those of Caucasian populations from other parts of the world. Large geographical variations in SSc prevalence and incidence have been described and differences in individual disease presentations were recently studied [8–10]. The latter analysis suggested that eastern European centers see SSc patients

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with more severe manifestations than other centers focusing on the major autoantibodies [10]. In diffuse SSc, positivity for IgG antibodies against inner core molecules of histone, such as H2B, is linked to severe lung fibrosis [7, 11, 12]. Parodi et al. [13] 15 years ago demonstrated 41.8% of AHA prevalence in Italian SSc patients, associated with cardiac and renal involvements, suggesting a prognostic value for these autoantibodies; the same group in 2002 [14] demonstrated that AHA alone or associated with anti-centromere antibodies characterized a disease subset with more frequent visceral involvement and a probably poor outcome. The aim of our study was to determine the prevalence and clinical significance of antibodies to individual histone components and their correlation with other autoantibody specificities, in a cohort of Italian SSc patients.

Materials and methods

Our study included 112 adult Caucasian patients with SSc consecutively diagnosed in two Italian Rheumatology Units (Siena and Bari) in 2007 and 2008. All patients (99 females, 13 males) fulfilled the American College of Rheumatology criteria and were clinically grouped according to the classification of Leroy and Medsger [15, 16]. Mean age at the time of diagnosis was 56.3 ± 12.2 years and mean duration of disease was 8.1 ± 4.7 years. Serum samples were analyzed by routine tests and stored at -40°C until serological assay. All patients gave written informed consent for anonymous storage of serum. Clinical data were obtained by retrospective review of the records; the study was performed according to guidelines issued by local ethical committee. Onset of the disease was regarded as the time of the first non-Raynaud symptom. Esophageal motility was assessed by double-contrast esophagography and manometric measurements. Lung fibrosis was studied by conventional radiography and functional tests. Pulmonary hypertension was defined according to the ESC guidelines of Venice, 2003 [17]. Electrocardiography and echocardiography were used to investigate pulmonary hypertension. The echocardiographic cut-off was 35 mmHg at rest, which is higher than 25 mmHg considered in the definition. Right heart catheterisation was performed only in those patients with values higher than 40 mmHg to identify other cardiac problems and to prescribe treatment with ETRA. Renal function was determined by routine biological tests and by echography. Radiographs of hands, feet, elbows and knees were used to investigate joint involvement and calcinosis. Muscular involvement was evaluated by CPK and specific electromyographic alterations. Multi-organ involvement was recorded when more than one organ was involved without considering Raynaud phenomenon.

Serological tests

The sera were tested by indirect immunofluorescence for ANA at a serum dilution of 1:160, using human HEp-2 cell line as substrate (INOVA, San Diego, USA) and by Western immunoblot (HEp-2 Marblot strips, MarDx, CA, USA). In particular, the last technique allows detection also of anti-Ku and anti-fibrillarin specificities. Anti-ENA and anti-Scl70 was detected by fluoro-enzyme immunoassay (FEIA) (PHADIA, Germany). Anti-PM/Scl, anti-RNA polymerase III and anti-histone components were tested by ELISA (AESKU Diagnostics, Germany; MBL, Japan; and AESKU, respectively). Anti-histone components (H1, H2A, H2B, H3 and H4) were derived from human native protein extracted from HeLa cell lines, as declared by AESKU.

Statistical analysis

Chi-square test or Fisher's exact test were used to determine significant differences between sets of categorical data. The odds ratio was calculated using Woolf's method to establish associations between autoantibodies and organ involvement.

Results

Out of 112 patients, 47 (42%) had diffuse cutaneous SSc (dcSSc) and 65 (58%) had limited cutaneous SSc (lcSSc). Frequent clinical features were lung fibrosis (65%), gastrointestinal involvement (63%), pulmonary hypertension (39%), and multi-organ involvement (52%) (Table 1). None of our patients was diagnosed with SSc and other connective tissue diseases. Four patients died. Two had diffuse disease (anti-Scl70+, one died of kidney failure, one of severe lung fibrosis), while the other two had limited disease (anti-Scl70+, one patient died of lung neoplasm and one of pulmonary hypertension). A total of 109 (97.4%) patients were ANA positive. The most common ANA patterns were "diffuse grains" (42%), centromeric (32%), speckled (8.9%), nucleolar (9.8%), and homogeneous (4.5%); we also found cytoplasmic pattern (3.6%) and mitotic spindle apparatus pattern (1.7%) (Table 2). All anti-centromere positive sera by IIF were confirmed by FEIA as anti-CENP B positive. AHA were found in 13 patients (11.6%), 9 with limited disease and 4 with diffuse disease; 5 were also anti-Scl70+ and 4 were anti-CENP B+. AHA antibodies were not significantly associated with lung fibrosis or related to multi-organ involvement or any other autoantibody specificity. In the 13 AHA-positive patients, antibody specificities were: nine H1 (2 dcSSc, 7 lcSSc), seven H2B (3 dcSSc, 4 lcSSc), one H2A (lcSSc), two H3

Table 1 Clinical features frequent in 112 Italian patients

Clinical manifestations	N/total (%)	Diffuse 47 (41.9) N/diff (%)	Limited 65 (58) N/lim (%)	<i>p</i>
Gastrointestinal involvement (esophagus)	71 (63)	29 (62)	42 (65)	ns
Lung fibrosis	73 (65)	40 (85)	33 (50.7)	<0.01
Pulmonary hypertension	44 (39)	23 (49)	21 (32)	0.08(ns)
Renal involvement ^a	4 (3.6)	4 (8.5)	–	–
Myositis	4 (3.6)	4 (8.5)	–	–
Multi-organ involvement	59 (52)	31 (65.9)	28 (43)	<0.01
Renal crisis	2 (1.7)	2 (4.2)	–	–
Deceased	4 (3)	2 (4.2)	2 (3)	–

^a Hypertension and/or raised serum creatinine level

Table 2 Distribution of IIF patterns and specificities of autoantibodies in 112 Italian patients

IIF patterns and specificities of autoantibodies	N/total (%)	Diffuse N/total (%)	Limited N/total (%)	<i>p</i>
Positive IIF ANA	109 (97)	45 (96)	64 (98)	ns
Diffuse grains	47 (42)	23 (49)	24 (37)	ns
ACA	36 (32)	8 (17)	28 (43)	<0.01
Speckled	10 (8.9)	6 (13)	4 (6)	ns
Nucleolar	11 (9.8)	5 (11)	6 (9)	ns
Homogeneous	5 (4.5)	3 (6)	2 (3)	ns
Mitotic spindle apparatus	2 (1.7)	1 (2)	1 (1.5)	–
Cytoplasmic	4 (3.6)	1 (2)	3 (5)	–
More than one positive IIF pattern	11 (9.8)	3 (6)	8 (12)	ns
Specificities identified				
Anti-Sc170	48 (43)	24 (51)	24 (37)	ns
Anti-CENPB	36 (32)	8 (17)	28 (43)	<0.01
Anti-histones	13 (11.6)	4 (8.5)	9 (14)	ns
Anti-RNA pol III	8 (7.1)	4 (8.5)	4 (6)	ns
Anti-PM/SCL	4 (3.5)	3 (6.3)	1 (1.5)	–
Anti-fibrillarin	2 (1.8)	1 (2)	1 (1.5)	–
Anti-Ku	1 (0.9)	1 (2.1)	–	–

(lcSSc) and one H4 (lcSSc). Anti-Sc170 and nucleolar pattern were associated with multi-organ involvement (OR = 3.92, $p < 0.01$ and OR = 4.79, $p < 0.01$, respectively). Anti-Sc170 antibodies were associated with lung fibrosis (OR = 15.75, $p < 0.0001$). There was a difference in organ involvement and ANA specificities between patients with dcSSc and lcSSc ($p < 0.01$) and of 17/28 lcSSc patients with multi-organ involvement were anti-Sc170 positive (61%, OR = 5.6, $p < 0.01$).

Discussion

In this study of Italian SSc patients, anti-histone autoantibodies did not show a remarkable prevalence. These antibodies were not statistically associated with lung fibrosis, multi-organ involvement or any other autoantibody specificity, conflicting with results obtained by

other authors [7, 11, 13, 14]. In fact, in the study by Parodi et al. on the prevalence of AHA in SSc patients, AHA was associated with ACA and anti-Sc170 more frequently than with other ANA, and Sato et al. reported AHA in 27 of 92 patients with SSc. Our results did not support these previous results suggesting that it is unproductive to look for AHA in scleroderma, probably because these antibodies are only an epiphenomenon of the disease. Moreover, we need to consider that since the 1950s when autoantibodies that bind to histones have been demonstrated, a variety of assays have been used to detect AHA. Initially, immunofixation or complement fixation methods were used; more recently, RIA, immunoblotting, and EIA methods have been developed. Different analytic methods have yielded discrepant results. Thus, outside a research setting, there are insufficient data to support the use of testing for AHA for the diagnostic or prognostic purposes.

Concerning other autoantibodies, the frequency of ANA in our cohort of patients was 97.4%, in line with previous studies [6]. Anti-Sc170 was positive in 43% of patients with SSc. This frequency was approximately equal to that found in the EUSTAR study [10] for the Italian subgroup (from Milan) and to previous studies on French Caucasian patients, and was higher than in US Caucasian patients [6]. Conversely, our patients with SSc had a relatively higher percentage of ACA (32%) than patients of other studies (16–22%) [6, 18]. In particular, statistically significant differences in the distribution of major antibodies (anti-Sc170, anti-centromere and anti-RNA pol III) were observed between French and American Caucasian patients. In the latter population, the three autoantibodies had quite similar distribution, whereas in the French population, as well in the present study population, anti-Sc170 was more frequent than anti-centromere, and anti-RNA pol III was less prevalent [6]. We also found that “diffuse grains” was the typical fluorescence pattern of anti-Sc170 antibodies, not the homogeneous/nucleolar pattern described by other authors. This may explain the different prevalence of nucleolar pattern found in our study; the association with multi-organ involvement, however, was in line with other studies [7]. The prevalence of anti-fibrillar antibodies was low and comparable with that reported in other studies [6, 18].

Anti-Sc170 and anti-RNA pol III are representative SSc-specific antibodies linked to dSSc. Both are associated with diffuse skin thickening, but vasculopathy and pulmonary fibrosis are more frequently seen in patients with anti-Sc170 [18]. Results of the present study confirm these findings. In the literature, the rate of renal crisis has been significantly associated with anti-RNA pol [18], whereas in our sample we observed two renal crises, both in patients with anti-Sc170 antibodies, as previously showed in other study in Italy [19]. When we compared clinical features with SSc-related ANAs, in line with previous reports, we found strong associations between SSc-related ANA and clinical features: anti-Sc170 with dSSc, and lung fibrosis and multi-organ involvement with dcSSc and lcSSc. In our cohort of patients, pulmonary hypertension was often secondary to lung fibrosis, and we therefore did not find correlations with anti-centromere antibodies.

In conclusion, our data indicate that: AHA have very low prevalence in Italian SSc patients and it is therefore unproductive to look for them in this disease; due to low prevalence, anti-fibrillar and anti-PM-Scl autoantibody tests should be performed only in the presence of clinical symptoms strongly suggestive of SSc and/or in reference centers. Nowadays the lack of standardization of methods (Western blot/immunodot, etc.) could increase the risk of false positive and clinical misinterpretation. More studies

with larger samples could be useful to standardize the measurement of these autoantibodies, basically because their detection can be helpful for diagnosing patients who test negative for anti-Sc170 and anti-centromere antibodies.

Conflict of interest None.

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