CASE REPORT

Autoantibodies coexistence in systemic sclerosis: how to interpret it?

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ABSTRACT

Autoantibodies possibly influence clinical manifestations of systemic sclerosis (SSc). This clinical-serological correlation, associated with the paucity of autoantibodies concomitance, gave rise to the historical paradigm of autoantibodies mutual exclusivity. However, one can question this assumption. Does autoantibodies concomitance mean coexistence of two different entities? On the other hand, if considered a unique disease, is this phenomenon a random event or does it represent a distinct subgroup of patients, with peculiar clinical, pathogenic, and immunogenetic characteristics? The autoantibodies' prevalence in early SSc is high. However, anti-centromere antibody (ACA) and antitopoisomerase 1 antibody (ATA) duplicity is a rare event. Similarly, the ACA, ATA, and anti-RNA polymerase (anti-RNA-P) III coexistence have not been described yet in single patient. In the reported case, with ACA, ATA, and anti-RNA-P III positivity, we have noted early vascular manifestations and late limited cutaneous involvement. This is, to our knowledge, the first report of three concomitant specific autoantibodies in a patient with SSc. We do believe this coexistence represents a rare serologic subgroup of a unique disease, with possible clinical and prognostic value, although this remains to be confirmed.

Keywords: systemic scleroderma, DNA topoisomerases, RNA polymerase III.

INTRODUCTION

The first description of autoantibodies duality in systemic sclerosis (SSc) – anti-centromere antibody (ACA) and antitopoisomerase 1 antibody (ATA) – remains from 1985,[1] and its meaning has yet to be elucidated. This fact may be explained by the rarity of this phenomenon on literature, with a prevalence of 0.05%–5.6%,[2] with significant variations upon the utilized detection methods (indirect immunofluorescence, immunodiffusion, ELISA, or immunoblotting). There is also concomitance of these autoantibodies with anti-RNA polymerase (anti-RNA-P) I, II, and III, anti-Ro, anti-La, anti-Jo, anti-U3-RNP, anti-Th-RNP, anti-Pm-Scl, anti-Ku,[3] anti-histone, and anti-mitochondrial antibodies.[2]

Although it is not proved, there is observational data to support the general agreement that individual autoimmune profile correlates with clinical manifestations in SSc.[2–4] In clinical practice, this concept drives patient's investigation and monitoring, with remarkable prognostic influences in each disease subgroup.[5] This clinical-laboratorial correlation and the paucity of autoantibodies duplicity cases gave rise to the historical paradigm of autoantibodies mutual exclusivity. Nevertheless, one can question this assumption, as well as the idea of coexistence being explained just by chance.

CASE REPORT

A 38-year-old woman presented with arthralgias and Raynaud's phenomenon for the last three years. She had no arthritis, morning stiffness, photosensitivity, dry eyes, dysphagia, urinary complaints, fever, or weight loss, although she had evidence of delayed esophageal emptying. There was no

cutaneous thickening, synovitis, or digital ulcers. Laboratorial tests were normal, except for hypothyroidism with positive antithyroglobulin antibody. Syphilis, hepatitis, HIV, HTLV, and Chagas serologies were negative. Anti-nuclear antibody (ANA) showed anti-centromere pattern (title 1:10240, indirect immunofluorescence method – Hep-2). Anti-Sm, anti-DNA, anti-La, and ATA were negative, but anti-Ro was positive (48 U/mL, cut-off < 10). One year later she presented with sclerodactyly, and the diagnosis of SSc was made. During disease course, other autoantibodies became positive: anti-RNP, 58 U/mL (cut-off < 10); anti-La, 41 U/mL (cut-off < 10); ATA, 21.6 U (cut-off < 20); and anti-RNA-P III, 22.9 (cut-off < 20). All of them were tested by enzyme-linked immunosorbent assay (ELISA).

DISCUSSION

The prevalence of autoantibodies in SSc is high, from 75%[6] to 95%,[7] and its presence precedes clinical manifestations in many cases.[8] On the other hand, the prevalence of ACA + ATA duplicity is rarely reported, varying from 0.05% to 5.6%.[2] These findings, associated with the correlation of autoantibodies, HLA alleles, and clinical damage profile, led to the historical concept of mutual exclusivity and invariant course of autoantibodies titles over time, which was probably based on insensitive techniques of analysis.[8] Immunoblotting technique seems to improve sensitivity of ACA detection when compared to immunofluorescence alone.[9] The case reports of occurrence of concomitant autoantibodies raise the need for this paradigm to be reviewed. There is also description of ATA and anti-RNA-P II association.[3] Nevertheless, to our knowledge triplicity (ATA + ACA + anti-RNA-P III) has not yet been described.

In the context of SSc, clinical-serological association of ACA, ATA, and anti-RNA-P III is proposed.[4] In theory, there is no data to prove a cause-effect relationship between serology and clinical profile of the disease, and one should consider polyclonal activation with consequent hypergamaglobulinemia and also concomitant pathological processes in SSc, such as cancer.[10] Furthermore, different techniques to detect or quantify autoantibodies have distinct sensitivities and specificities, as described above, which can lead to false results. Moreover, since this disease has many clinical presentations and each one represents a combination of genetic and environmental factors, it is hazardous, or maybe impossible, to input to the serologic profile such a strong role.

On the other hand, there is epidemiological data that suggest a correlation between serologic status and clinical profile, as described in Table 1.[2–4,6] Since monitoring and treatment of patients have different approaches upon their autoimmune profile, how would one behave with the group with multiple specific autoantibodies? In the context of concomitant ATA + ACA, in patients with limited or diffuse form of disease, Kikuchi et al.[1] noted a predominance of Raynaud's phenomenon (95% of patients) and esophageal dysfunction, and lower frequency of sclerodactyly, calcinosis and pulmonary fibrosis. This finding could indicate, according to the authors, a reciprocal suppressive effect of these immune products. Jarzabeck-Chorzelski et al.,[9] using three techniques for autoantibody detection (immunofluorescence, immunoblotting and double immunodiffusion), obtained the highest prevalence of ACA and ATA duplicity reported till now (5.6%), with a pronounced vascular involvement – telangiectasia and Raynaud's phenomenon – as well as sclerodactyly, calcinosis, and visceral damage (nine of ten patients). Based on these data, it was suggested that ACA and ATA positivity could indicate an incomplete CREST variant.[1] The presence of anti-RNA-P II does not define a different clinical subtype in patients with ATA.[6]

This work adds to the discussion the case of a patient with SSc who is believed to present, simultaneously, three disease-specific autoantibodies (ACA, ATA, and anti-RNA-P III). To our knowledge, there is no description in the literature of a similar case. Therefore, it is fully justified that the validity of this finding should be carefully considered, not on the premises that it is final, at risk of incurring in error. In this sense, it is noteworthy that the low values (close to the cut-off values) for ATA and anti-RNA-P III antibodies were found by ELISA, a method of higher sensitivity but with a low specificity. Most

Table 1

	Anti-centromere	Anti-topoisomerase	Anti-RNA polimerase (I, II, III)
Clinical manifestations	Limited cutaneous involvement Calcinosis Digital ulcers Pulmonary arterial hypertension	Diffuse skin sclerosis Lung parenchyma involvement Renal vascular injury Myositis/myocarditis	Diffuse and severe skin involvement Renal vascular injury (50% of patients with scleroderma, renal crisis)
Prevalence	50%–90% of CREST patients	40%–90% of patients with diffuse SSc Worse prognosis	Low prevalence (21.9%), but highly specific

CREST: calcinosis, Raynaud's syndrome, esophageal dysmotility, sclerodactyly, telangiectasia; SSc: systemic sclerosis.

kits ELISA express the antibody results as positive, indeterminate, or negative; few kits bring these results as positive, weakly positive, or negative. Therefore, we must keep in mind that the "weakly positive" results found in this work, especially for ATA and anti-RNA-P III antibodies, could be expressed as "indeterminate". With such a result, presented in these terms, we are certainly less prone to error, since we will not consider it, hastily, as a definitely positive finding.

Since we have addressed it, we believe that the issue of autoantibodies in SSc, as in many other autoimmune diseases, is not terminated but, instead, is increasingly more pungent, providing an interesting debate, which is the aim of this study. In our case, in which there was coexistence of ACA, ATA, and anti-RNA-P III, with respect to the above considerations, there was an early vascular involvement (Raynaud) and subsequent limited cutaneous disease (sclerodactyly). However, we must not take conclusions on the absence of greater severity, since isolated cases are not sufficient to predict the clinical profile of these patients.

All these findings stress the participation of the immune system on SSc pathogenesis. Several related mechanisms have been proposed. Examples include topoisomerase-1 enzyme release during endothelial cell apoptosis, with its further binding to fibroblasts surface and then to autoantibodies.[8] Furthermore, Casciola-Rosen[11] suggested that topoisomerase-1 and RNA-P phosphorylation/fragmentation during apoptosis lead to exposure of cryptic epitopes, previously hidden from the immune system. From the presentation of these neo-epitopes, lymphocytes tolerance would be broken. This presentation requires MHC molecules, which explains the suggested association between autoantibodies and HLA alleles: ACA with DR1, 4, and 8 and ATA with DR11.[2] However, in the absence of absolute associations between SSc onset and HLA epitopes, and of definite culprit epitopes for the disease, it could be possible that several distinct mechanisms contribute to similar pathological processes, which, in turn, could express different clinical spectra – the so called "multiple etiology theory".[6]

On the other hand, the autoantibodies multiplicity could mean the existence of different diseases (SSc as a composition of independent clinical entities). Dick et al.[2] reported three cases of ATA + ACA positive patients who exhibited HLA-specific alleles for each of the autoantibodies. Based on this immunogenetic observation, they suggested that duplicity occurs independently. Moreover, the same authors demonstrated reverse fluctuation of the autoantibodies concentrations over time, corroborating the independence hypothesis. Harvey et al.[3] also support the concept that different serological subgroups (ACA, ATA, and anti-RNA-P I) represent, in fact, three distinct diseases, based on HLA alleles analysis. In contrast, associations between class II MHC alleles and serological subgroups in SSc are not strong enough to prove this theory.[5,12]

Rather than occurring by chance or as distinct diseases, distinct autoantibodies could represent a unique disease with its own pathophysiological, clinical, and immunogenetic characteristics. Kikuchi et al.[1] considered that autoantibodies do not coexist by chance, based on the observation that the prevalence of simultaneous ATA + ACA is smaller than the expected probability for them to occur together randomly. Furthermore, the reverse fluctuation found in patients with duplicity could reflect the activity of the same autoimmune disease at different poles. If there are distinct serological spectra over time, it could be useful to measure autoantibodies titles during disease course in order to predict clinical outcomes. In our case, ATA positivity, one year after a limited SSc diagnosis, stresses this approach and could change skin and internal organs involvement and patient's prognosis, although this still has to be proven.

This report demonstrates, for the first time, the coexistence of three specific autoantibodies in SSc and also concomitant thyroid autoimmunity. We support the idea that this triplicity indicates a rare serological subgroup of a unique disease (i.e, it does not occur by chance), with possible, but unproven, clinical and prognostic implications. In addition, we believe that the prevalence of multiplicity of positive autoantibodies in SSc should be reviewed. This could be achieved through more sensitive techniques, already available, and by monitoring autoantibodies titles during the disease course, thereby increasing the probability of detection of this event.

REFERENCES

REFERÊNCIAS

1. Kikuchi M, Inagaki T. Bibliographical study of the concurrente existence of anticentromere and antitopoisomerase I antibodies. Clin Rheumatol 2000; (19):435–41.

2. Dick T, Mierau R, Bartz-Bazzanella P, Alavi M, Stoyanova-Scholz M, Kindler J *et al*. Coexistence of antitopoisomerase I and anticentromere antibodies in patients with systemic sclerosis. Ann Rheum Dis 2002; 61(2):121–7.

3. Harvey GR, Butts S, Rands AL, Patel Y, McHugh NJ. Clinical and serological associations with anti-RNA polymerase antibodies in SS. Clin Exp Immunol 1999; 117(2):395–402.

4. Müller CS, Paiva ES, Azevedo VF, Radominski SC, Lima Filho JHC. Autoantibody profile and clinical correlation in a group of pa*tient*s with systemic sclerosis in southern Brazil. Rev Bras Reumatol 2011; 51(4):319–24.

5. Hamaguchi Y. Autoantibody profiles in systemic sclerosis: Predictive value for clinical evaluation and prognosis. J Dermatol 2010; 37(1):42–53.

6. Bunn CC, Black CM. Systemic sclerosis: an autoantibody mosaic. Clin Exp Immunol 1999; 117(2):207–8.

7. Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum 2005; 35(1):35–42.

8. Walker JG, Fritzler MJ. Update on autoantibodies in systemic sclerosis. Curr Opin Rheumatol 2007, 19(6):580–91.

9. Jarzabek-Chorzelska M, Błaszczyk M, Kołacińska-Strasz Z, Jabłonska S, Chorzelski T, Maul GG. Are ACA and Scl 70 antibodies mutually exclusive? Br J Dermatol. 1990; 122(2):201–8.

10. Airo'P, Ceribelli A, Cavazzana I, Taraborelli M, Zingarelli S, Franceschini F. Malignancies in Italian Patients with Systemic Sclerosis Positive for Anti-RNA Polymerase III Antibodies. J Rheumatol 2011; 38(7):1329–34.

11. Rosen A, Casciola-Rosen L, Wigley F. Role of metal-catalyzed oxidation reactions in the early pathogenesis of scleroderma. Curr Opin Rheumatol 1997; 9(6):538–43.

12. Fanning GC, Welsh KI, Bunn C, Bois RD, Black CM. HLA association in three mutually exclusive autoantibodies subgroups in UK systemic sclerosis patients. Br J Rheumatol 1998; 37(2):201–7.