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Characteristics of Systemic Sclerosis patients with positive anti-Th/To antibodies: about 6 patients and literature review

Caractéristiques des patients souffrant de Sclérodémie Systémique avec positivité des anticorps anti-Th/To : au sujet de 6 patients et revue de la littérature

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Résumé

Parmi les anticorps décrits associés à la Sclérodémie Systémique (ScS), les anticorps anti-Th/To (anti-Th/To) sont rares et ont été peu étudiés. Ainsi, nous n'avons que peu de connaissance sur le profil des patients positifs pour les anti-Th/To. A partir d'une Biobanque locale (Marseille, France), nous avons sélectionné de façon rétrospective 6 patients positifs pour les anti-Th/To, en test Immunodot. Tous souffraient de ScS, et partageaient des caractéristiques cliniques et biologiques communes comme la forme cutanée limitée de la ScS, une baisse de la diffusion alvéolocapillaire pulmonaire et un aspect moucheté nucléolaire en immunofluorescence indirecte au test de dépistage des anticorps anti-nucléaires sur cellules Hep-2.

Afin de caractériser davantage les patients positifs pour les anti-Th/To, nous avons réalisé une revue de la littérature sur le sujet. A partir de l'étude de 402 patients positifs pour les anti-Th/To, nous confirmons que ces anticorps sont principalement associés à la forme cutanée limitée de la maladie (88% de l'ensemble des patients), et à une atteinte pulmonaire de la ScS (50% de l'ensemble des patients). Cette analyse de la littérature illustre la rareté des anti-Th/To avec une fréquence moyenne estimée de 3,4% chez tous les patients souffrant de ScS dans le monde entier, leur caractère habituellement exclusif vis-à-vis des autres autoanticorps spécifiques de la Sclérodémie, ainsi que leur grande spécificité (environ 98%) pour le diagnostic de la ScS.

Mots clé : Sclérodémie systémique; anti-Th/To autoanticorps; forme cutanée limitée; atteinte interstitielle pulmonaire

Abstract:

Among the antibodies described in Systemic Sclerosis (SSc), anti-Th/To antibodies (anti-Th/To) are rare and have been poorly studied. Thus, little is known about the profile of anti-Th/To positive patients. From our local Biobank (Marseille, France), we retrospectively selected data for 6 patients positive for anti-Th/To with an Immunodot assay. All of them suffered from SSc, sharing clinical and biological common features such as a limited cutaneous form of SSc, a decreased lung diffusing capacity and a speckled nuclear nucleolar immunofluorescence pattern of antinuclear antibodies screening on HEp-2 cells.

In order to further characterize patients positive for anti-Th/To, we performed a thorough literature review. From 402 studied patients positive for anti-Th/To, we confirmed that these antibodies are associated with the limited cutaneous form of the disease (88% of the patients), and with an SSc related-pulmonary involvement (50%). The review analysis pointed out the rarity of the anti-Th/To with an estimated mean frequency of 3.4% of all SSc patients worldwide, their usual exclusivity with respect to the specific antibodies of scleroderma, and their high specificity (around 98%) for the diagnosis of SSc.

Keywords: Systemic Sclerosis; anti-Th/To antibodies; limited cutaneous form; interstitial lung disease

Introduction

Systemic sclerosis (SSc, scleroderma) is a rare connective-tissue disease characterized by skin and internal organ fibrosis, vascular alteration and a specific auto-immune profile [1,2]. For patients suspected to suffer from scleroderma, based on clinical and morphological features, characterization of their immunological profile offers a crucial lead in the final diagnosis. To date, three autoantibodies have been considered as specific of SSc: the anti-topoisomerase I (anti-topo 1), anti-centromere (ACA) and anti-RNA Polymerase III (anti-RNAPolIII) antibodies. Clinical characteristics and prognosis associated with anti-topo 1, ACA and anti-RNAPolIII antibodies are well characterized [1]. Indeed, anti-RNAPolIII antibodies are mainly associated with a diffuse cutaneous subtype, a higher risk of scleroderma renal crisis and a risk of cancer in close temporal relationship to SSc onset [3-6]. Anti-topo1 antibodies are usually associated with a diffuse cutaneous form, pulmonary fibrosis, severe peripheral vascular disease and cardiac involvement, all resulting in a high mortality rate [7-10]. On the contrary, anti-centromere (ACA) are associated with the limited cutaneous type, and are protective for Interstitial Lung Disease (ILD), cardiomyopathy, and death [3,11,12]. Other antibodies associated with SSc such as anti-Th/To are rarely observed and their impact on clinical characteristics and prognosis have been poorly described [1,2,3].

Th/To antigens are located in the nucleolus of the cell nucleus. Anti-Th/To antibodies recognize two endoribonucleases: the ribonucleoprotein 7-2/MRP-RNP (Th) and the ribonuclease 8-2/RNP (To); the antigenic complex is 40kD. They display a nucleolar staining by indirect immunofluorescence on HEp-2 cells.

In this article, we report the characteristics of 6 French patients positive for anti-Th/To, and we propose a review of the clinical characteristics associated with this type of antibody.

Patients and methods:

Sera were collected from a Biobank (DC 2012-1704) with respect of ethical directives in the laboratory of immunology of APHM in Marseilles (South of France). Antinuclear antibodies (ANAs) were detected by indirect immunofluorescence on HEp-2 cells (Bio-Rad Laboratories, Hercules, CA) at a screening dilution of 1:160.

Over a one-year period (January 2017 to December 2018), we selected 64 samples positive for ANAs associated with a clinical suspicion of SSc and without positivity of specific SSc autoantibodies (anti-topo 1, ACA and anti-RNAPolIII). These sera were tested for anti-Th/To

IgG antibodies using BlueDiver® Combi Immunodots (Alphadia SA/SN, Wavre, Belgium) commercialized by Eurobio-Ingen®, Les Ulis, France.

We retrospectively collected the following clinical data on patient's medical records: i/ clinical characteristics of patients positive for anti-Th/To comprised demographic characteristics (age, sex), ACR/EULAR 2013 criteria fulfillment, associated diagnosis in case of overlap syndromes, Raynaud's phenomenon, sclerodactyly, calcinosis, digital pitting scar, and telangiectasias. Upper digestive signs included esophageal reflux, dysphagia, or dyspepsia; lower digestive signs included malabsorption, diarrheas, or anal incontinence. Pulmonary function tests (Total Lung Capacity, DLCO) and echocardiography (Left Ejection Ventricular Fraction, pericardial effusion, Pulmonary Arterial Hypertension) were recorded. Interstitial Lung disease (ILD) on chest CT scan was defined by with qualitative criteria consisting in the presence of honey combing, ground glass opacities, reticular abnormalities, traction bronchiectasis and septa thickening. Muscle involvement was defined by muscular pain or CPK elevation. ii/ immunological parameters were also recorded: ANAs, anti-dsDNA. ACA/anti-RNApol III/anti-topo I, anti-U1-RNP, anti-SSA/SSB, anti-Jo1/PM-Scl, anti-CCP, Rheumatoid factor level, anti-mitochondrial, Lupus Anticoagulant and antiphospholipid antibodies, cryoglobulinemia.

Secondly, we questioned the research banks Pubmed and Google scholar, with keywords "scleroderma", "systemic scleroderma", "auto-antibodies profiles", "Th/To" in order to collect a large population of patients anti-Th/To positive and gather their clinical and phenotypic characteristics. We then extracted statistical information regarding these antibodies.

For the statistical analysis, weighted arithmetic means were calculated from the literature review to estimate the frequency of Th/To antibodies among SSc patients, and the frequency of limited cutaneous form or pulmonary involvement among Th/To patients. Weighted arithmetic means were obtained by weighting for each study the result of the frequency of analyzed data by the number of included patients.

Results:

1) Clinical and biological characteristics of 6 anti-Th/To antibody positive patients from the BioBank

Among the 64 sera referred to the laboratory for the routine detection of SSc-related autoantibodies, 6 were found positive for anti-Th/To and negative for the SSc classical autoantibodies (anti-topo 1, ACA and anti-RNAPolIII). The 6 patients were diagnosed with SSc according to the 2013 ACR/EULAR criteria. **Table 1** shows their characteristics.

The six patients shared clinical and biological characteristics. The main clinical features included: Raynaud's syndrome (6/6) and sclerodactyly (6/6), telangiectasia (6/6), gastroesophageal reflux (5/6), digital pitting scar (2/6) and calcinosis cutis (1/6). All of these patients had a cutaneous limited form. Moreover, all of the six sera displayed positive ANAs with a speckled nuclear nucleolar immunofluorescence pattern (**Figure 1**), corresponding to the nucleolar localization of the antigen Th/To.

Two patients had auto-immune associated diseases: Sjogren's syndrome (patient 1), characterized by a sicca syndrome, typical salivary glands biopsy histological analysis and anti-SSA (60 KD) antibodies, and primary biliary cirrhosis (patient 2) with anti-mitochondrial antibody and a mixed cryoglobulinemia.

None of the patients had a restrictive pulmonary syndrome, but all had a decreased diffusing lung capacity of carbon monoxide (DLCO), and 5 had an ILD on chest CT-scan. The echocardiography noted a normal left ventricular ejection fraction (6/6), pulmonary arterial hypertension (PAH) (1/6 confirmed with a right heart catheterization), and pericarditis (2/6). One patient suffered from myalgia with normal CPK levels.

Three anti-Th/To positive patients also had anti-SSA (60 kD) antibodies. When performed ($n=3$), the search for anti-phospholipid antibodies was negative.

2) Literature review on anti-Th/To antibodies

Results of the literature research regarding the positivity of anti-Th/To antibodies in SSc patients are summarized in **Tables 2-4**.

Firstly, in order to estimate the frequency of anti-Th/To positivity in SSc patients, we collected the data from 25 articles including 8727 SSc patients (**Table 2**): a total of 300 patients were found positive for anti-Th/To antibodies. The estimated frequency of anti-Th/To among SSc patients ranged between 0 to 13%, with a mean of 3.4% (300/8727, median = 3%, first and third interquartile = 2 and 4%). The positivity of anti-Th/To did not seem to be

affected by the dosage method, since 3.3% of the patients were positive using a DOT and 3.4% using radioimmunoprecipitation.

Secondly, we also studied the frequency of anti-Th/To according to the continent on which the studies were carried out (**Table 3**). Interestingly, these antibodies seem to be more frequent in Asia and Oceania (4.7%) than in Europe (1.5%). It is difficult to determine the frequency of anti-Th/To in South America from a single study.

Literature review on the cutaneous form among SSc patients with anti-Th/To antibodies, based on 313 patients from 16 articles, highlighted the high frequency of the limited cutaneous form, respecting 87.5% of the patients (median = 89%, from 55% to up to 100% depending of the case series).

Finally, in order to estimate the frequency of pulmonary fibrosis among SSc patients with anti-Th/To, we collected the data from 10 articles including 264 SSc patients with anti-Th/To (**Table 4**). Pulmonary involvement was screened with different methods; chest radiography, chest CT scan, or pulmonary function testing (PFT). As example, PFT could be considered pathological either by an anomaly of the DCLO < 75%, or a total lung capacity (TLC) < 80%, or sometimes not specified at all in the article. In this review, 47.3% of SSc patients with anti-Th/To suffered from pulmonary fibrosis (median = 42%, first and third interquartile = 35 and 53%).

Discussion

We report here 6 patients diagnosed with an SSc for whom the only immunological marker for the SSc disease was the positivity of anti-Th/To. The presence of anti-SSA (60kD) antibody was found in 3/6 patients. One patient completed the international classification criteria for Sjögren's syndrome [13] and was diagnosed with an overlap syndrome. Another patient suffered from an associated auto-immune cholangitis. The association between SSc and auto-immune cholangitis has been described as Reynold's syndrome [14].

In our series, all of the patients had a limited cutaneous form of the disease, and most of them had interstitial lung disease and a decreased DLCO value. Consistently, the literature review

revealed an association between anti-Th/To and the limited cutaneous form of the disease and the ILD, defining a specific clinical pattern or phenotype associated with this antibody.

ACA represent the main antibodies associated with the limited cutaneous form of SSc. Thus, the comparison of clinical and prognostic characteristics of anti-Th/To and ACA patients appears interesting. Mitri et al. [15] described the characteristics of 96 anti-Th/To positive patients in the larger cohort reported in literature. They concluded that, in comparison to ACA positive patients, anti-Th/To positive patients had a lower cutaneous extension of skin sclerosis.

Our literature review also suggested less digital pitting scars, digital tip ulcers, and gangrene in anti-Th/To positive SSc patients [16-21], provided that such clinical manifestations were heterogeneously assessed based on scleroderma skin score (Rodnan Scleroderma Skin Score, Cochin Hand score, Kapandji index..), Raynaud's phenomenon severity score, frequency of ulcers or digital scars. Conversely, ACA are known to be related with a high risk of severe digital vascular disease [3,11,12] .

Mitri et al. [15] also noticed that anti-Th/To antibodies were associated with a higher risk of ILD (47%) compared to ACA (13%). This result was supported by Ceribelli et al. [19] in a smaller cohort of patients. Our literature review estimated that half of anti-Th/To positive patients have a pulmonary involvement. This percentage was higher than the one found in the literature for ACA which was around 30-40% [3,21].

Interestingly, anti-Th/To have been retrospectively detected in 13/285 patients with a clinical diagnosis of idiopathic pulmonary fibrosis [3,21]. In this study, among the 13 anti-Th/To positive patients, 9 were retrospectively diagnosed as having SSc *sine scleroderma*, based on immunologic characteristics, Raynaud's phenomenon and pulmonary involvement. This latter article highlighted a clinical pattern associated with anti-Th/To, characterized by a high frequency of pulmonary disease, mild skin sclerosis and peripheral vascular involvement.

The specificity of anti-Th/To for the diagnosis of SSc appears very high, estimated between 90 and 99% from articles assessing the anti-Th/To among cohorts of scleroderma and non-scleroderma patients. Indeed, Kuwana et al [22] found that anti-Th/To had a 99% specificity for SSc diagnosis among a cohort of 1048 patients with various auto-immune diseases. Similar results were obtained in Villalta et al [23], Van Praet et al [24] and Mahler et al [20], with a specificity of respectively 99, 98 and 99.5%. In Mitri et al [15], the specificity was a

little lower, calculated at 90%. Among patients without SSc, anti-Th/To were most often detected in patients with isolated Raynaud's phenomena andILD, and exceptionally with systemic lupus erythematosus, polymyositis, rheumatoid arthritis, primary Sjogren's syndrome and primary immunologic thrombocytopenia.

Autoantibodies related to SSc are known to be mutually exclusive [25]. In most of the reported studies [19,23,24,26,27], anti-Th/To were not associated with other classical SSc antibodies and cases of co-positivity appear very rare [22].

Anti-Th/To have an excellent specificity for SSc and their research appears useful, given their association with ILD. However, their frequency is low (3% of SSc patients), in particular in Europe (1% of the patients) and indeed does not sustain their systematic screening in the routine immunological assessment. Mahler et al [20] suggested a higher relevance of anti-Th/To in SSc patients negative for ACA, anti-topo1, anti-PM-Scl, anti-RNP, anti-RNAPIII, anti-fibrillarine, and anti-NOR 90. Thus, screening of Th/To seems more useful after a negative search for the most common known antibodies.

Interestingly, in our little series of anti-Th/To positive SSc patients, a speckled nucleolar immunofluorescence pattern on HEp-2 cells was observed. Nucleolar pattern of Th/To antibodies was also described in others studies [15,16,18–20,28]. This pattern was reported as homogenous [29] or speckled [16], as in our cases. Considering these data, a screening strategy based on the nuclear immunofluorescence pattern could increase their sensitivity and could be more cost-effective than a multiantigen Immunodot.

Conclusion

Based on an exhaustive literature review, anti-Th/To appear as relevant markers of SSc, more frequently observed in patients with a limited cutaneous form of the disease and with an interstitial lung involvement. Their accuracy for SSc diagnosis is of great interest for patients with a suspected SSc disease and with negative SSc-specific antibodies, particularly in case of a speckled nucleolar immunofluorescence pattern observed at the screening of ANAs.

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251 detecting anti-Th/To autoantibodies

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253 **Conflict of interest**

254 The authors declare non conflict of interest.

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Legend of the Figure

Figure 1: Anti-nuclear antibody pattern observed on HEp-2 cells by indirect immunofluorescence (400 x magnification): anti-Th/To autoantibodies are associated with a speckled-nucleolar pattern that displays a homogeneous staining of nucleoli associated with numerous fluorescent speckles of variable size distributed throughout the nucleoplasm. Metaphase chromosomal plates remain unstained.

265 **Legend of the tables**

266

267 **Table 1: Clinical and biological characteristics of patients positive for anti-Th/To**
 268 **antibodies**

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	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (years)	57	53	67	60	62	57
Sex	Female	Female	Female	Female	Male	Female
Associated diagnosis	Sjogren's syndrome	Primary biliary cirrhosis	No	No	No	No
Age at diagnosis	55	30	64	45	58	40
ACR/EULAR Criteria	14	16	11	11	11	15
Raynaud's phenomenon	Yes	Yes	Yes	Yes	Yes	Yes
Sclerodactyly	Yes	Yes	Yes	Yes	Yes	Yes
Calcinosis cutis	No	Yes	No	No	No	No
Digital pitting scar	Yes	Yes	No	No	No	No
Telangiectasias	Yes	Yes	Yes	Yes	Yes	Yes
Cutaneous form	Limited	Limited	Limited	Limited	Limited	Limited
Pericarditis	No	No	Yes	No	Yes	No
Muscle damage	Yes	No	No	No	No	No
Upper digestive signs	Yes	Yes	Yes	No	Yes	Yes
Lower digestive signs	No	No	No	No	No	No
Total LungCapacity (%)	91%	101%	86%	97%	93%	102%
DLCO (%)	48%	24%	64%	74%	39%	60%
ILD on chest CT-scan	Yes	Yes	Yes	NC	Yes	Yes
Left Ventricular Function	Normal	Normal	Normal	Normal	Normal	Normal
PAH	No	No	No	Yes	No	No
Level of ANAs (dilutions titers)	>1/1280	>1/1280	>1/1280	>1/1280	>1/1280	>1/1280
Immunofluorescence pattern	SN	SN	SN	SN	SN	SN
Type of auto-antibodies						
Anti-dsDNA (IU/ml)	No	Yes (90)	No	No	No	No
ACA/anti-RNAPol III/anti-topo I (IU/ml)	No	No	No	No	No	No
Anti-Jo1/PM-Scl (IU/ml)	No	No	No	No	No	No
Anti-U1-RNP (IU/ml)	No	No	No	No	No	No
Anti-SSA/SSB (IU/ml)	>131 / 0	62 / 0	0	30 / 0	0	0
Anti-CCP (IU/ml)	0	0	NC	NC	NC	No
Rheumatoid factor level (IU/ml)	60	0	0	0	0	25
Anti-mitochondrial (IU/ml)	0	800	NC	NC	NC	NC
LA or antiphospholipid (UI/ml)	0	0	NC	0	NC	NC
Cryoglobulinemia	0	Positive mixed	NC	NC	NC	NC

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271 NC: Not communicated; DLCO: diffusing capacity of the lung for carbon monoxide; PAH:
 272 Pulmonary arterial hypertension confirmed with a right heart catheterization; LA: Lupus
 273 anticoagulant; Anti-phospholipid antibodies included anti-cardiolipin and anti β_2 -glycoprotein
 274 1 antibodies; SN: Speckled nuclear nucleolar.

275 **Table 2: Literature review on the frequency of Th/To antibodies in Systemic Sclerosis**
276 **(SSc)**

Articles	Location	Number of SSc	Dosage method	Anti-Th/To positivity (N) %
Kipnis1990 [28]	USA	84	DOT	(11) 13%
Okano1990 [16]	USA	371	RadioIP	(15) 4%
Falkner 2000 [29]	USA	292	DOT	(28) 9.6%
Jacobsen 2001 [17]	Denmark	174	DOT	(4) 2.3%
Yamane 2001 [18]	Japan	70	RadioIP	(7) 10%
Kuwana 2002 [22]	Japan	303	RadioIP	(14) 4%
Van Eenennaam H 2002 [30]	Netherlands	14	RadioIP	(2) 14.3%
Steen 2005 [31]	USA	1432	DOT	(72) 4.9%
Meyer 2007 [32]	France	127	DOT	(1) 1%
Meyer 2007 [32]	USA	247	DOT	(10) 4%
Hamagushi 2008 [33]	Japan	203	DOT	(7) 3%
Ceribelli 2010 [19]	Italy	261	RadioIP	(8) 3%
Mierau 2011 [34]	Germany	863	DOT	(2) 0.2%
Rodriguez-Reyna 2011 [35]	Mexico	139	DOT	(0) 0%
Van Praet 2011 [24]	Belgium	145	DOT	(3) 2.1%
Villalta 2012 [23]	Italia	210	DOT	(7) 3.3%
Graf 2012 [36]	Australia	129	DOT	(8) 6%
Poormoghim 2013 [37]	Iran	100	DOT	(4) 4%
Mehra 2013 [38]	Canada	528	DOT	(15) 2.8 %
Wielosz 2014 [39]	Poland	97	DOT	(3) 3.4%
Hudson 2014 [40]	Canada	874	DOT	(20) 2%
Malher 2014 [20]	Canada	873	RadioIP	(19) 2%
Chang 2015 [41]	New Zealand	60	DOT	(1).7%
Nandiwada 2016 [42]	USA	1000	DOT	(39) 3.9%

Liaskos 2017 [43]	Greece	131	DOT	0
TOTAL		8727		(300) 3.4%

277 DOT: Multi antigen Immunodot test of anti-Th/To antibodies

278 RadioIP: Radioimmunoprecipitation

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Table 3: Frequency of anti-Th/To antibodies in Systemic Sclerosis (SSc) patients by continent

Continent	Number of studies	Number of SSc patients	Th/To positivity (N) %
North America	9	5701	(229) 4.0%
South America	1	139	(0) 0%
Europe	9	2022	(30) 1.5%
Asia	4	676	(32) 4.7%
Oceania	2	189	(9) 4.7%

Table 4: Literature review on the frequency of pulmonary fibrosis among Systemic Sclerosis (SSc) patients with anti-Th/To antibodies

Articles	Location	Definition of pulmonary fibrosis in the article	Number of pulmonary involvement among anti-Th/To positive patients (%)
Okano 1990 [16]	USA	CT-scan or PFT	6/14 (42%)
Falkner [29]	USA	CR or CT-scan	6/23 (26%)
Kuwana 2002 [22]	Japan	CT-scan	5/14 (35%)
Mitri 2003 [15]	USA	CR or PFT	46/96 (48%)
Steen 2005 [31]	USA	CR or PFT	45/72 (63%)
Hamagushi 2008 [33]	Japan	CT-scan or PFT	2/7 (29%)
Ceribelli 2010 [19]	Italy	CR or CT-scan	3/8 (38%)
Graf 2012 [36]	Australia	CT-scan or PFT	2/8 (25%)
Malher 2014 [20]	Canada	CT-scan or PFT	10/19 (53%)
Muller 2019	France	CT-scan or PFT	5/6 (83%)
TOTAL			125/264 (47.3%)

CT-scan = Computerized Tomography scanner

CR = Chest Radiography

PFT = Pulmonary Function Testing

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