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Incidence of anti-topoisomerase I antibody seroconversion and clinical association in systemic sclerosis

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Abstract

Anti-topoisomerase I antibody (ATA) is an autoantibody associating with a poor prognosis in systemic sclerosis (SSc). The changing of ATA from negative to positive or vice versa might have an effect on the outcome. We aimed to define the incidence and to determine the association between clinical parameters and ATA seroconversion. A historical cohort study of adult SSc patients who attended the Scleroderma Clinic, Khon Kaen University, Thailand was conducted. We included all patients tested at least twice for ATA during January 2008-May 2018. ATA positive seroconversion was set when ATA was initially negative but switched to positive over the period of follow-up while ATA negative seroconversion was fulfilled when ATA changed from positive to negative. Forty-eight SSc patients were tested for ATA at least twice. ATA positive seroconversion was not detected during follow up while 2 cases (4.2%) were defined as ATA negative seroconversion with the incidence of 1.0 per 100 person-years (95%CI 0.02-0.4). Both patients presented regression of skin thickness and pulmonary arterial hypertension (PAH). There was no parameter associated with ATA negative seroconversion. There was a low incidence of both ATA negative and positive seroconversion among SSc patients and no significant clinical association by repeating test was detected. Repeating test for ATA may have low clinical utility for SSc patients.

Keywords: Scleroderma, Anti-Scl70, Anti-topoisomerase I antibody, Systemic sclerosis

1. Introduction

Systemic sclerosis (SSc) is characterized by fibrosis of tissue, dysfunction of vascular endothelial and immunologic abnormalities. The pathogenesis of SSc is complex and uncertain [1]. Sclerosis of the skin or skin thickness is the classical feature of SSc and is the definitive criterion for diagnosis of SSc in majority of cases; however, the patient may have internal organ involvement without skin tightness [2].

Anti-topoisomerase I antibody (ATA), a well-known specific autoantibody among SSc, is reported between 15 and 80% in SSc patients [3-5]. ATA is practically never seen in robust controls or in non-affected families of patients with SSc nor in the patients with Raynaud's phenomenon or other connective tissue diseases [3,4]. ATA is more frequently found in the diffuse cutaneous SSc (dcSSc) subset which is a more severe form of SSc than limited cutaneous SSc (lcSSc).[6-9] The presence of ATA in SSc is associated with pulmonary fibrosis [6-13], cardiac involvement [6,7], tendon friction rub [6], hand deformity [5,6], and acro-osteolysis [6]; notwithstanding, it cannot be used to differentiate dcSSc from lcSSc among Thai SSc [5].

A longitudinal study of 28 Japanese SSc patients-positive for ATA at their first visit using double immunodiffusion-revealed that 6 of 28 lost ATA during the course of the disease (i.e., during 10 years of follow-up). The finding was associated with a favorable outcome, less-progressive of pulmonary interstitial fibrosis, and a better survival rate compared with SSc patients who had persistence of ATA [14]. The study, however, included a relatively small population and provided no detail of clinical characteristics such as clinical features at onset and when repeated antibody tests were performed. In addition, the clinical characteristics and

features of Thai SSc are distinct from those of Japanese sufferers; such as having a greater incidence of dcSSc, a higher prevalence of male, and a poorer outcome [5,7,15]. Our aims were to define the incidence and clinical parameters association with ATA conversion among Thai SSc.

2. Materials and methods

Adult SSc patients who attended at the Scleroderma Clinic, Khon Kean University, Thailand and prospectively followed the cohort were analysed. We included and reviewed the clinical characteristic of all the patients who were tested for ATA at least twice between January 2008 and May 2018 with a testing interval of more than 1 year. The patients who had ATA testing by other methods or from other centers were excluded from the study.

2.1 Laboratory method

Serum specimens from the first and follow up visits were used. ATA was investigated by EUROIMMUN AG (Lübeck, Germany) method. The serum was initially 30 minutes incubation. Then the fluid was sucked and rinsed off with working-strength wash buffer. The specimen was then re-incubated with alkaline phosphatase-labelled anti-Human IgG at room temperature for 30 minutes. After that the fluid was re-aspirated and rinsed off again as described. The specimen was incubated with substrate solution at room temperature in the third step for 10 minutes and the fluid was re-aspirated and rinsed off again with distilled water. The last step, the specimen was placed on the strip, air dried, assessed and interpreted the result [5,13].

2.2 Operational definitions

SSc is diagnosed on the basis of the criteria of 1980 American College of Rheumatology (ACR) [16] and/or fulfillment of the ACR/EULAR 2013 criteria [17]. SSc is divided and classified into 2 subsets; lcSSc and dcSSc per definition of LeRoy et al [18]. The start date of the study is the date of any first non-Raynaud SSc symptom. Duration of disease was calculated by the date from disease onset of non-Raynaud's symptoms to the date of second ATA test.

The definition of persistent ATA was fulfilled when ATA remained negative or positive at the initial test and follow up test. ATA positive seroconversion was set when ATA was initially negative but switched to positive over the period of follow-up. By contrast, ATA negative seroconversion was fulfilled when ATA changed from positive to negative. Pulmonary arterial hypertension (PAH) was definite diagnosed when a mean pulmonary arterial pressure > 20 mmHg confirmed by right heart catheterization. Pulmonary fibrosis was fibrosis of lungs detection either by chest radiographic or by HRCT. Renal crisis was determined by rapidly progressive renal impairment and/or accelerated arterial hypertension occurrence over the course of SSc. Esophageal involvement was determined by dysphagia, reflux symptoms, or heartburn [19]. Stomach involvement included dyspepsia, early satiety, and bloating [20]. Intestinal involvement included bloating, diarrhea, malabsorption, ileus obstruction, constipation or pseudointestinal obstruction. Myocardial involvement was determined when left ventricular ejection fraction was ≤ 50%.

2.3 Statistical analysis

Clinical features of SSc were classified into continuous, dichotomous or polytomous variables. The incidence of ATA seroconversion was described together with the 95% confidence intervals (95%CI). The clinical characteristics of all patients, patients with persistent ATA, and with ATA seroconversion are presented as percentage or proportion and mean±SD or median (interquartile range; IQR) as appropriate. The clinical difference between persistent ATA positive, persistent ATA negative, and ATA seroconversion were tested using the ANOVA or the Kruskal-Wallis test (for continuous data) and the χ^2 or Fisher's Exact test (for categorical data) as appropriate. Statistical significant was defined when a p-value was less than 0.05. STATA version 16.0 was used as the software for statistical analysis.

3. Results and discussion

Totally 481 SSc patients who attended the Scleroderma Clinic who were tested for ATA were reviewed; 48 of these were tested for ATA at least twice. From the 193.1 person-months, 37 patients were ATA persistent positive, 9 were ATA persistent negative, 2 were ATA negative seroconversion, and none were ATA positive seroconversion: the respective incidence was 19.2 per 100 person-years (95%CI 13.9-26.4), 4.7 per 100 person-years (95%CI 2.4-9.0), and 1.0 per 100 person-years (95%CI 0.02-0.4).

The patients who were ATA persistent positive commonly had a salt-pepper skin at onset and during follow-up compared to patients who were ATA persistent negative or ATA negative seroconversion (p=0.04 and 0.04, respectively). ATA persistent positive patients also had more frequent tendon friction rub at onset, and during follow-up more hand deformity, muscle weakness, and renal crisis than the other groups (not statistically significant: p=0.21, 0.23, 0.72 and 0.99, respectively). While the patients negative for ATA seroconversion trended to have digital ulcer, stomach involvement and weight loss, PAH at onset, and PAH during follow-up than the other groups (not statistically significant: p=0.67, 0.34, 0.73, 0.06, 0.05, respectively). The clinical differences vis-à-vis ATA persistent negative, ATA persistent positive, and ATA negative seroconversion are presented in Table 1.

Table 1 Clinical difference of ATA persistent negative, ATA persistent positive and ATA negative seroconversion.

Clinical characteristic	ATA persistent negative N = 9	ATA persistent positive N = 37	ATA negative conversion N = 2	*P*-value
Sex: female	7 (77.8)	22 (59.5)	0	0.18
dcSSc subset	4 (44.4)	29 (78.4)	1 (50.0)	0.09
Age at onset (years); mean±SD	52.9±9.2	53.1±10.8	53.8±3.4	0.99
Age on study date (years); mean±SD	57.0±9.2	57.0±10.4	59.3±4.5	0.95
Duration of disease (years); mean±SD	4.1±2.6	3.9±3.3	5.5±0.8	0.76
Clinical feature at onset				
Raynaud's phenomenon	8 (88.9)	30 (81.1)	2 (100.0)	1.00
Digital ulcer	2 (22.2)	9 (24.3)	1 (50.0)	0.67
Digital gangrene	0	0	0	
Telangiectasia	2 (22.2)	5 (13.5)	0	0.72
Calcinosis	0	0	0	
Salt-pepper skin	3 (33.3)	25 (67.6)	0	0.04*
Edematous skin	2 (22.2)	17 (45.9)	1 (50.0)	0.39
Tendon friction rub	0	10 (27.0)	0	0.21
Hand deformity	2 (22.2)	3 (8.1)	0	0.40
Synovitis	3 (33.3)	7 (18.9)	0	0.62
Muscle weakness	3 (33.3)	4 (10.8)	0	0.26
Esophageal involvement	6 (66.7)	15 (40.5)	1 (50.0)	0.41
Stomach involvement	1 (11.1)	10 (27.0)	1 (50.0)	0.34
Intestinal involvement	0	1 (2.7)	0	0.99
Weight loss	5 (55.6)	22 (59.5)	2 (100.00)	0.73
mRSS	10 (4-12)	10.5 (2-17.5)	4 (4)	
Pulmonary fibrosis	3 (33.3)	6 (14.7)	1 (50.00)	0.19
PAH	1 (11.1)	1 (2.9)	1 (50.00)	0.06
Renal crisis	0	0	0	-
Clinical feature during follow-up				
Raynaud's phenomenon	5 (55.6)	21 (56.8)	1 (50.00)	0.99
Digital ulcer	1 (11.1)	7 (18.9)	0	0.99
Digital gangrene	0	1 (2.7)	0	0.99
Telangiectasia	2 (22.2)	12 (32.4)	0	0.85
Calcinosis	0	0	0	-
Salt-pepper skin	1 (11.1)	19 (51.4)	0	0.04*
Edematous skin	0	5 (13.5)	0	0.65
Tendon friction rub	1 (11.1)	5 (13.5)	0	0.99
Hand deformity	2 (22.2)	18 (48.7)	0	0.23
Synovitis	2 (22.2)	1 (2.7)	0	0.20
Muscle weakness	0	4 (10.8)	0	0.72
Esophageal involvement	3 (33.3)	19 (51.4)	1 (50.0)	0.79
Stomach involvement	1 (11.1)	4 (10.8)	0	0.99
Intestinal involvement	1 (11.1)	6 (16.2)	0	0.99
Weight loss	2 (22.2)	10 (27.0)	0	0.99
mRSS	0 (0-2)	6 (2-16)	0	0.99
Pulmonary fibrosis	4 (4.4)	15 (40.5)	1 (50.0)	0.99
PAH	1 (11.1)	7 (18.9)	2 (100.0)	0.05
Renal crisis	0	3 (8.1)	0	0.99

ATA: anti-topoisomerase I antibody; SSc: systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; SD: standard deviation; mRSS: modified Rodnan skin score; PAH: pulmonary arterial hypertension.

The two patients negative for ATA seroconversion were both males: one was diagnosed with dcSSc and the other with lcSSc. Both had mild SSc symptoms and regression of skin thickness but developed PAH by the study date (Table 2.).

Table 2 Clinical characteristics of patients with ATA negative seroconversion.

Clinical characteristic	Case 1	Case 2
Sex	Male	Male
Age at onset (years)	57	51
Age on study date (years)	63	56
Duration of disease on study date (years)	6.1	5.0
SSc subset	dcSSc	lcSSc
Clinical features on study date		
Raynaud's phenomenon	No	No
Digital ulcer	No	No
Digital gangrene	No	No
Telangiectasia	No	No
Calcinosis	No	No
Salt-pepper skin	No	No
Edematous skin	No	No
Tendon friction rub	No	No
Hand deformity	No	No
Synovitis	No	No
Muscle weakness	No	No
Esophageal involvement	Yes	No
Stomach involvement	No	No
Intestinal involvement	No	No
Weight loss	No	No
mRSS	0	0
Pulmonary fibrosis	Yes	No
PAH	Yes	Yes
Renal crisis	No	No

ATA: anti-topoisomerase I antibody; SSc: systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; lcSSc limited cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; PAH: pulmonary arterial hypertension.

ATA is a common specific antibody for diagnosis of SSc and the its presence is associated with a bad prognosis according to the association with dcSSc and internal organ involvement [6]. ATA can, however, be negative despite having overt SSc clinical features [13]. Anti-centromere antibody (ACA) is an another specific antibody that has been frequently detected in the lcSSc subset [21] and it is reported to be associated with PAH [6] but has a negative association with cardiac involvement and renal crisis [21]. The absence of both specific antibodies in SSc has been reported in around 15% of cases and the finding is related to overlap syndrome and less severity of skin thickness [5]. The loss of the antibody especially ATA has been reported in Japanese SSc patients after serial ATA testing [14] but the incidence of changing ATA titer or seroconversion has not been well defined.

The incidence of seroconversion in Thai SSc is low with the incidence of 1.0 per 100 person-years. In our study, 2 of 48 SSc patients had ATA negative seroconversion and none had ATA positive seroconversion. The number of ATA seroconversions during follow-up was low, agreeing with Kuwana et al [14] from Japan and Hildebrandt et al [22] from Germany; albeit the number of ATA seroconversions among the Thais was lower than among the Japanese (6 of 28) [14] and Germans (1 of 13) [22] The reason could be related to the various techniques for ATA testing which have different reliabilities, sensitivities, and specificities. We used the Western immunoblot (IB) technique whereas the other studies used immunodiffusion (ID) [14,22], Western immunoblot [22], and/or enzyme-linked immunosorbent assay (ELISA) technique [14,22]. According to a study on the clinical relevance of ATA,[23] ATA as determined by the IB technique had a higher sensitivity (41% vs 20-28%) and lower specificity (90-100% vs 99-100%) than the ID technique for diagnosing SSc. Meanwhile, the ELISA technique has the highest sensitivity (43-75%) for diagnosis of SSc compared to the ID and IB techniques [23]. The specificity of ELISA is, however, between 76 and 100%, which is lower than the other two techniques Another reason for the low ATA seroconversion rate among Thai SSc might be the high proportion of ATA positive and the dcSSc subset in Thai with SSc [5,13] compared to Caucasians and some Asians [7,11,12,24-29]. Once ATA is positive in Thai SSc, it usually remains positive. According to the low incidence

of ATA seroconversion, we propose that it is unnecessary to repeat the ATA test or to do serial ATA testing during follow-up; instead clinical monitoring is more meaningful.

In our study, the patient with ATA negative seroconversion trended to have favorable outcomes compared to those who are persistent ATA positive. The mean disease duration in this study was about 4-5 years. It might be indicated that we included the SSc patients with nearly regression by disease itself or with treatment given. Kuwana et al. found that patients with ATA negative seroconversion had nominally less dcSSc, hand deformity, joint and cardiac involvement than those who had persistent ATA [14]. Hildebrandt et al. also found stable skin and lung disease in a patient with ATA negative seroconversion [22]. Likewise, in our study, the two patients who had ATA negative seroconversion had skin regression and less gastrointestinal involvement during follow-up than those who were ATA persistent positive albeit all later developed PAH. The clinical outcomes are consistent with the previous studies. Although all ATA negative seroconversion patients had PAH during follow-up and the association between ATA negative and PAH has been previously reported [30], the association between ATA negative seroconversion and PAH cannot be confirmed due to a low statistical power of our study. According to the low incidence of both negative and positive ATA seroconversion, no significant clinical association by repeating test among SSc patients, and it is no evidence whether or not it should be retested in the patients with disease flare after regression, repeated ATA test might not be useful for clinical practice.

Our study has some limitations; a) The low numbers of patients included in the study might influence the statistical power; b) Patient selection for repeated ATA testing depended on the attending physician; c) The interval of ATA repeated testing for each patient was not the same, so we excluded patients who had an test interval of than 1 year in order to achieve a more or less equal test interval; and d) We did not repeat the ATA test in all of the SSc patients in our cohort because of budget limitations. Strengths of our research include:
a) We included clinical parameters of interest such as severity of skin tightness by mRSS, internal organ involvement both at the onset and on the study date.
b) The incidence rate of ATA seroconversion with 95%CI was calculated, so the result provides some understanding of the epidemiology of ATA seroconversion among SSc patients that can be applied for improvement the health care of the patients. Nevertheless, our result does provide preliminary data of SSc with ATA seroconversion that is fundamental for better care in daily practice.

4. Conclusion

In our study, ATA negative seroconversion presented in Thai SSc while ATA positive seroconversion did not. ATA negative seroconversion trended to recovery skin fibrosis and evolvement of vasculopathy especially PAH. Due to a low incidence of both ATA negative and positive seroconversion in SSc and no significant clinical association by repeating test, therefore repeating test for ATA may have low clinical utility for SSc.

5. Conflict of interest

The authors declare no conflicts of interest.

6. Acknowledgements

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8. Author contributions

SS did the data collection and drafted the manuscript. CF conceived and designed the study, and proofread the manuscript**.** PP, AM, SS, and RN proofread the manuscript**.**

9. Ethic approval

The Human Research Ethics Committee of Khon Kaen University approved the study per the Helsinki Declaration and the Good Clinical Practice Guidelines (HE621025). All eligible patients signed informed consent before enrollment.

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