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ORIGINAL ARTICLE

The initial manifestations and final diagnosis of patients with high and low titers of antinuclear antibodies after 6 months of follow-up

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Background: The antinuclear antibody (ANA) test is the most commonly used test to screen for autoimmune diseases. However, only a limited numbers of studies have addressed the characteristics of patients positive for ANA. In this study, we aimed to clarify the relationship between initial presentations, ANA titer, and final diagnoses.
Methods: Patients who visited National Taiwan University Hospital and received a first ANA test were enrolled and then followed for a further 6 months. The symptoms and signs at the time of ANA testing, ANA titers, and the final diagnoses were recorded and analyzed.
Results: A total of 355 patients were positive for ANA. Joint pain was the most common initial presentation at the time of ANA testing. Compared with the patients with low ANA titers ($<$1:640), those with high ANA titers ($\geq$1:640) were more susceptible to autoimmune diseases. More importantly, of the patients with initial presentations of joint pain, fever, abnormal urinalysis, or skin rash/skin tightness, autoimmune diseases were more frequently diagnosed in those with high ANA titers than with low ANA titers ($p < 0.05$). In addition, both anti-double strand DNA antibodies and anti-extractable nuclear antibodies were more commonly detected in patients with high ANA titers.

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Conclusions: A high ANA titer seems to be a useful biomarker for the diagnosis of autoimmune diseases, especially for patients presenting with joint pain, fever, abnormal urinalysis, or skin rash/skin tightness.

Introduction

Autoantibodies directed against nuclear and cytoplasmic components of tissue cells have been known to play an important role in autoimmune diseases for several decades.[1,2] The methods used to detect antinuclear antibodies (ANAs) evolved from the lupus erythematosus cell phenomenon into indirect immunofluorescence assay and enzyme-linked immunosorbent assay. At present, the ANA test is the most commonly used autoantibody test and also one of the most over ordered tests in the clinical laboratory.[3] Many clinicians use the ANA test to screen for autoimmune diseases, including systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis (SSc), and mixed connective tissue disease. However, ANA can be detected not only in autoimmune diseases but also in other medical conditions, such as liver disease, malignancy, chronic infections, and thyroid disease.[3]

Although many studies have reported the distribution of various ANA titers, patterns, and associated diseases, there are only limited data available on the relationship between initial presentations, different ANA titers, and final diagnoses. In addition, Vaile et al.[4] concluded that setting a higher ANA titer cutoff point (1:640) is of limited benefit to predict autoimmune disease. The purpose of our study is to clarify the relationship between initial presentations, ANA titers, and final diagnoses and combined with initial presentations, to determine whether the patients with high ANA titers are more susceptible to autoimmune diseases.

Material and methods

From September 2007 to March 2008, the patients who visited National Taiwan University Hospital and received a first ANA test were enrolled. ANA were detected by immunofluorescence assay techniques using human epithelial tumor cell lines, HEp-2 cells as substrate, and an immunoglobulin G-specific conjugate to reveal ANA binding. Because more than 30% of normal individuals have been found to have low ANA titers,[5] the patients with negative or positive results at a titer of 1:40 were excluded. In our laboratory, an ANA titer of 1:640 is defined as a "high titer" because of a 0.5% prevalence of positives in normal individuals. Therefore, we divided the patients into a high titer group ($\geq$1:640) and low titer group ($<$1:640). The initial symptoms and signs on presentation were recorded and divided into 14 categories (shown in Table 1). Tests for anti-extractable nuclear antigen (anti-ENA) or anti-double strand DNA (anti-dsDNA) were also performed subsequently in some patients. The patients positive for ANA were followed for a further 6 months. The final diagnoses were then classified into three major categories (shown in Table 2); autoimmune diseases, nonautoimmune diseases, and not confirmed. Systemic autoimmune diseases and organ-specific autoimmune diseases were categorized together as autoimmune diseases. The nonautoimmune diseases were subdivided into seven categories.

All statistical analyses were performed using SPSS software version 15.0. (SPSS Inc., Chicago, IL, USA). Differences between groups in categorical variables were examined

Table 1 The initial presentations of patients positive for ANA test

Initial presentations	Total (n = 355), n (%)	Adult (n = 320), n (%)	Child (n = 35), n (%)
Hematologic problems	35 (9.9)	32 (10)	3 (8.6)
Abnormal urinalysis findings	12 (3.4)	10 (3.1)	2 (5.7)
Liver function impairment	36 (10.1)	34 (10.6)	2 (5.7)
Joint pain	132 (37.2)	116 (36.4)	16 (45.7)
Muscle weakness/myalgia	7 (2.0)	5 (1.6)	2 (5.7)
Oral lesions	23 (6.5)	22 (6.9)	1 (2.9)
Raynaud's phenomenon	14 (3.9)	13 (4.1)	1 (2.9)
Skin presentations[a]	51 (14.4)	38 (11.9)	13 (37.1)
Sicca syndrome	44 (12.4)	44 (13.8)	0 (0)
Lymphadenopathy	7 (2.0)	6 (1.9)	1 (2.9)
Cardiopulmonary s/s	17 (4.8)	17 (5.3)	0 (0)
Neuropsychologic problems	21 (5.9)	20 (6.3)	1 (2.9)
Fever	28 (7.9)	24 (7.5)	4 (11.4)
Others	9 (2.5)	9 (2.8)	0 (0)

[a] Skin presentations included skin rash or skin tightness.
ANA = antinuclear antibody; s/s = symptoms/signs.

Table 2 Comparison of the disease categories between the high- and low-titer groups

Disease categories	ANA ≥ 1:640 ($n = 118$), (%)	ANA < 1:640 ($n = 237$), (%)
Autoimmune diseases	84 (71.2)[a]	83 (35.0)
Nonautoimmune disease	19 (16.1)	62 (26.2)
1. Hepatic diseases	4	6
2. Malignancy	3	6
3. Dermatologic diseases	1	14
4. Musculoskeletal diseases	3	10
5. Hematologic diseases	1	0
6. Infectious diseases	4	14
7. Others	3	12
Not confirmed	15 (12.7)	92 (38.8)[a]

[a] A p value less than 0.05 was considered to be statistically significant.
Hepatic diseases did not include autoimmune hepatitis and viral hepatitis. The diagnosis of skin rash related to systemic autoimmune disease was excluded from the dermatologic diseases. Similarly, the diagnosis of musculoskeletal symptoms (arthralgia, muscle pain, and so on) associated with other autoimmune diseases were excluded from musculoskeletal disease. Hematologic abnormalities unrelated to autoimmune diseases were categorized as hematologic diseases. Infectious diseases included chronic viral hepatitis and other microorganisms confirmed by serologic evidence or culture.
ANA = antinuclear antibody.

using Fisher's exact test or the Chi-square test. A p value less than 0.05 was considered statistically significant.

Results

Characteristics of the patient population

A total of 355 patients (84 males and 271 females) whose first ANA tests were positive and higher than 1:40 were enrolled and followed for a further 6 months. Three hundred twenty of them were adults, ranging in age from 19.7 to 84.6 years with the mean age being 49.8 years. The remaining patients were children, ranging in age from 0.6 to 17.9 years with the mean age being 11.1 years.

The initial presentations for ANA tests

As shown in Table 1, the most common initial presentations in the 355 patients were joint pain (37.2%), followed by skin presentations (14.4%) and sicca syndrome (12.4%). The adult patients were more likely to present with sicca syndrome but less likely to have skin involvement.

Distribution of the ANA patterns and associated final diagnoses

Table 3 shows the ANA immunofluorescent patterns and the final diagnoses. The most common pattern was "homogeneous" (42%), followed by "mixed" (23.9%), "speckled" (16.9%), "centromere" (9.3%), and "nucleolar" (7.9%). In contrast to the other four patterns, the "nucleolar" pattern was less associated with autoimmune diseases (45—55% *vs.* 10.7%).

Table 3 Frequency of ANA patterns and final diagnoses ($n = 55$)

Diagnosis	Homogeneous ($n = 149$), n (%)	Speckled ($n = 60$), n (%)	Centromere ($n = 33$), n (%)	Nucleolar ($n = 28$), n (%)	Mixed ($n = 85$), n (%)[a]
No conclusion	45 (29.5)	18 (30.0)	12 (36.3)	12 (42.9)	20 (23.5)
Nonautoimmune disease	31 (24.8)	13 (21.6)	6 (18.2)	13 (46.4)	18 (21.2)
Autoimmune disease	73 (45.7)	29 (48.4)	15 (45.5)	3 (10.7)	47 (55.3)
SLE	21	10	3	1	14
SCLE	1	2	0	0	0
Rheumatoid arthritis	9	4	0	0	3
Systemic sclerosis	1	1	2	0	3
Sjögren's syndrome	11	3	7	0	12
Dermatomyositis	2	0	0	0	1
Vasculitis	3	1	1	0	1
MCTD	0	4	0	0	0
Ankylosing spondylitis	0	1	1	0	2
Raynaud's syndrome	1	0	0	0	1
ITP	4	0	0	0	2
Autoimmune hepatitis	1	2	1	1	5
Autoimmune pancreatitis	1	0	0	0	0
Autoimmune thyroiditis	6	0	0	0	0
Lichen planus	6	0	0	0	3
Others	6	1	0	1	0

[a] Mixed type: two or more of the other four patterns (homogeneous, speckled, centromere, and nucleolar).
ANA = antinuclear antibody; ITP = idiopathic thrombocytopenic purpura; MCTD = mixed connective tissue disease; SLE = systemic lupus erythematosus; SCLE = subacute cutaneous lupus erythematosus.

The distribution of various ANA titers

Figure 1 demonstrates that among the 355 patients positive for ANA, 118 (33.2%) had an ANA titer $\geq$ 1:640 and 237 (66.8%) had an ANA titer $<$ 1:640. Compared with the male patients (12/84, 14.2%), a higher proportion of the female patients (77/271, 28.4%) were found to have an ANA titer $\geq$ 1:1,280.

The disease categories of patients with positive ANA test

The final diagnoses of the patients with positive ANA tests after 6 months of follow-up are shown in Table 2. One hundred sixty-seven patients (47.0%) had autoimmune diseases, and 81 (22.8%) had nonautoimmune diseases. However, no confirmed diagnoses could be made in the remaining 107 (30.1%) patients. Compared with the patients with low ANA titers, those with high ANA titers were highly associated with autoimmune diseases (71.2% *vs.* 35.0%). The detailed diagnoses of the 167 patients classified as autoimmune disease are shown in Table 4. The most common autoimmune disease in the high titer group was SLE, found in 40 of the 90 patients (44.4%), followed by SS (16.7%), SSc (6.7%), autoimmune hepatitis (6.7%), and autoimmune thyroiditis (6.7%). However, SS (23.4%) was the most common autoimmune disease in the 77 patients with low ANA titers, followed by rheumatoid arthritis (16.9%), SLE (11.7%), lichen planus (10.4%), and idiopathic thrombocytopenic purpura (6.5%). Most of the patients with SLE, SSc, mixed connective tissue disease, autoimmune thyroiditis, and autoimmune hepatitis had high ANA titers (81.6%, 85.7%, 100%, 100%, and 66.7%, respectively). In contrast, most of the patients with rheumatoid arthritis, lichen planus, and idiopathic thrombocytopenic purpura had low ANA titers (81.3%, 88.9%, and 83.3%, respectively).

In addition, Table 2 demonstrates the categories of nonautoimmune diseases. In contrast to the patients who had autoimmune diseases, most of the patients with nonautoimmune diseases (62/81, 76.5%) had ANA titers lower than 1:640. Among the nonautoimmune diseases, infectious diseases accounted for the highest proportion (22.2%), followed by dermatologic diseases (18.5%) and musculoskeletal diseases (16.0%). Hepatic diseases included five patients with chronic hepatitis of unknown origin, one patient with Dubin-Johnson syndrome, one patient with acute nonviral hepatitis, and three patients with nonviral liver cirrhosis. Nine adult patients (three in the high titer group and six in the low titer group) had malignancy, but none of the children had malignancies at the end of our study. The diagnosis of these nine patients included myelodysplastic syndrome, lymphoma, leukemia, pancreatic cancer, colon cancer, cholangiocarcinoma, and multiple myeloma (not shown in the table). Infectious diseases included chronic hepatitis B or C infection, infectious

Table 4 The final diagnoses of 167 patients who had autoimmune diseases after follow-up for 6 months

Diagnoses	ANA $\geq$ 1:640 (n = 90), n (%)	ANA $<$ 1:640 (n = 77), n (%)
Systemic lupus erythematosus	40 (44.4)	9 (11.7)
Subacute cutaneous lupus erythematosus	1 (1.1)	2 (2.6)
Rheumatoid arthritis	3 (3.3)	13 (16.9)
Sjögren's syndrome	15 (16.7)	18 (23.4)
Systemic sclerosis	6 (6.7)	1 (1.3)
Raynaud's syndrome	1 (1.1)	1 (1.3)
Vasculitis	3 (3.3)	3 (3.9)
Idiopathic thrombocytopenic purpura	1 (1.1)	5 (6.5)
Mixed connective tissue disease	4 (4.5)	0 (0)
Ankylosing spondylitis	1 (1.1)	3 (3.9)
Dermatomyositis	1 (1.1)	2 (2.6)
Lichen planus	1 (1.1)	8 (10.4)
Autoimmune hepatitis	6 (6.7)	4 (5.2)
Autoimmune pancreatitis	0 (0)	1 (1.3)
Autoimmune thyroiditis	6 (6.7)	0 (0)
Others	1 (1.1)	7 (9.0)
Behcet's disease	0	2
Antiphospholipid syndrome	0	1
Evans syndrome	1	0
Multiple sclerosis	0	1
Palindromic rheumatism	0	1
Dry eye syndrome	0	2

ANA = antinuclear antibody.

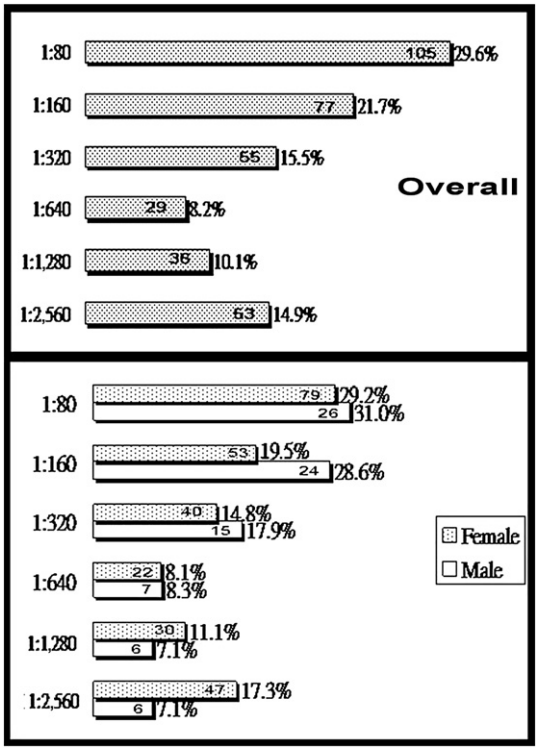

Figure 1. Frequency of ANA titer in all 355 patients. Among the 355 patients positive for ANA, 118 (33.2%) had an ANA titer $\geq$ 1:640 and 237 (66.8%) had an ANA titer < 1:640. Compared with the male patients (blank bar), a higher proportion of the female patients (77/271, 28.4%) were found to have an ANA titer $\geq$ 1:1,280. ANA = antinuclear antibody.

mononucleosis, and tuberculosis. Nevertheless, it must be noted that 92 (38.8%) of the 237 patients with low ANA titers and 15 (12.7%) of the 118 patients with high ANA titers had no confirmed diagnoses during the 6 months of observation.

The association among initial presentations, ANA titers, and autoimmune diseases

As can be seen in Table 5, 72.5% of the patients with high ANA titers and joint pain and 37% of the patients with low ANA titer and joint pain; 100% of the patients with high ANA titers and fever and 12.5% of the patients with low ANA titers and fever; 100% of the patients with high ANA titers and abnormal urinalysis findings and 14.3% of the patients with low ANA titers and abnormal urinalysis findings; and 100% of the patients with high ANA titer and skin presentations and 14.3% of the patients with low ANA titer and skin presentations were finally confirmed with autoimmune diseases. Combined, for the patients with initial presentations of joint pain, fever, abnormal urinalysis findings, or skin presentations, those who had high ANA titers were significantly more susceptible to autoimmune diseases than those with low ANA titers.

The frequency of ANA profiles and specific ENA

Some patients received anti-dsDNA tests and anti-ENA tests after their first ANA test. Anti-dsDNA tests were performed in 127 patients and 27 (21.2%) were positive. As we expected, 26 (96.2%) of the 27 patients with positive anti-dsDNA tests were finally diagnosed as SLE (not shown in the table). A total of 154 patients received anti-ENA tests and 55 (35.7%) of them showed positive results (shown in Table 6). Among the 55 patients with positive anti-ENA tests, 49 (87.5%) were found to have autoimmune diseases. Table 7 shows the components of anti-ENA antibodies in these 49 patients.

Discussion

An ANA test is initially suggested if the clinician feels there is a reasonable suspicion of SLE based on historical information, physical findings, and the results of other laboratory tests. However, the ANA test has also become a common screening method for other autoimmune diseases.[3,6] Many studies have confirmed the importance of the ANA test in the diagnosis of autoimmune diseases and demonstrated the trend that patients with higher ANA titers are more susceptible to autoimmune diseases. In addition, ANA can also be

Table 5 The prevalence of autoimmune diseases among initial presentations and ANA titers

Initial presentations	ANA titer	*n*	Autoimmune disease		*p*
			Yes, *n* (%)	No, *n* (%)	
Hematologic problems	<1:640	19	7 (36.8)	12 (63.2)	0.06
	≥1:640	16	11 (68.7)	5 (31.3)	
Joint pain	<1:640	92	35 (37.0)	57 (63.0)	<0.001
	≥1:640	40	29 (72.5)	11 (27.5)	
Fever	<1:640	16	2 (12.5)	14 (87.5)	<0.001
	≥1:640	12	12 (100)	0 (0)	
Oral lesion	<1:640	19	13 (68.4)	6 (31.6)	NS
	≥1:640	4	4 (100)	0 (0)	
Raynaud's phenomenon	<1:640	3	1 (33.3)	2 (66.7)	0.093
	≥1:640	11	10 (90.9)	1 (9.1)	
Lymphadenopathy	<1:640	3	2 (66.6)	1 (33.4)	NS
	≥1:640	3	3 (100)	0 (0)	
Liver function impairment	<1:640	22	5 (22.7)	17 (77.3)	NS
	≥1:640	14	5 (35.7)	9 (64.3)	
Abnormal urinalysis finding	<1:640	7	1 (14.3)	6 (85.7)	<0.05
	≥1:640	5	5 (100)	0 (0)	
Skin presentations	<1:640	29	10 (34.5)	19 (65.5)	<0.05
	≥1:640	22	15 (68.2)	7 (31.8)	
Muscle weakness/myalgia	<1:640	4	1 (25.0)	3 (75.0)	NS
	≥1:640	3	3 (100)	0 (0)	
Sicca syndrome	<1:640	26	20 (76.9)	6 (23.1)	NS
	≥1:640	18	14 (77.8)	4 (22.2)	
Cardiopulmonary s/s	<1:640	10	2 (20.0)	8 (80.0)	NS
	≥1:640	7	4 (57.1)	3 (42.9)	
Neuropsychiatric s/s	<1:640	15	2 (13.3)	13 (86.7)	NS
	≥1:640	6	3 (50)	3 (50)	

The comparison of percentage of autoimmune diseases between high- and low-ANA titers is presented as *p* values.
A *p* value less than 0.05 was considered to be statistically significant.
ANA = antinuclear antibody; NS = not significant; s/s = symptoms/signs.

Table 6 The frequency of anti-ENA antibodies and anti-dsDNA antibodies between high titer and low titer ANA

Autoantibodies tests	ANA $\geq$ 1:640, *n* (%)	ANA $<$ 1:640, *n* (%)
Anti-dsDNA test	$n = 66$	$n = 61$
Positive	22 (31.8)	5 (8.2)
Autoimmune diseases	Yes = 22 No = 0	Yes = 4 No = 1
Anti-ENA test	$n = 76$	$n = 78$
Positive	41 (53.9)	14 (17.9)
Autoimmune diseases	Yes = 38	Yes = 11
SLE	21	3
Sjögren's syndrome	8	70
MCTD	4	0
Systemic sclerosis	2	0
SCLE	1	0
Raynaud's syndrome	2	0
Antiphospholipid syndrome	0	1
Autoimmune diseases	No = 3	No = 3

ANA = antinuclear antibody; dsDNA = double-stranded DNA; ENA = extractable nuclear antibodies; MCTD = mixed connective tissue disease; SLE = systemic lupus erythematosus.

detected in nonautoimmune diseases, such as hepatic disease, malignancy, chronic infections, or thyroid disease.[3] In this study, we collected the patients whose first ANA test was positive and focused on the relationships between their initial presentations, immunofluorescence patterns, ANA titers, and the final diagnoses.

Table 1 summarizes the initial presentations and shows that joint pain was the most common presentation in patients with positive ANA tests, both in adults and children. This is not surprising because most of the common autoimmune diseases present with arthritis or arthralgia during the disease course. For example, about 60% of SLE patients,[7] 54–84% of SS,[8,9] and 12–66% of SSc[10,11] patients have joint involvement. In our study, only the adult patients presented with sicca syndrome. This could be explained by the fact that most of the cases of SS occur in midlife[12] but that it is a rare disease in childhood.[13] Because many autoimmune diseases have various and broad-ranging cutaneous manifestations,[14] skin presentations, including skin rash or skin tightness are also commonly seen in patients with positive ANA tests.

Roberts-Thomson et al.[15] reported 5,718 patients who were positive for ANA with the most common immunofluorescent patterns being homogeneous (39%), speckled (20%), mixed (17%), nucleolar (8%), Ro (7%), and centromere (4%). Our results were similar with a slight difference. The most common patterns were homogeneous (42%), followed by mixed (23.9%), speckled (16.9%), centromere (9.3%), and nucleolar (7.9%). Generally, the homogeneous pattern is linked to SLE and the speckled pattern to scleroderma or SS.[16] However, the homogeneous pattern can be found in many autoimmune diseases and, in contrast, various ANA patterns in the same autoimmune disease. Although, the ANA pattern once played an important role in the prediction of various autoimmune diseases, it has been replaced by more specific ANA tests, such as the anti-dsDNA test or anti-ENA test.

Chronic hepatitis C virus and hepatitis B virus infection were the most common infectious diseases associated with ANA in our study. Some studies have reported that ANA can be detected in 21–34% of hepatitis C virus-infected individuals[17–19] and 18.2% of hepatitis B virus-infected patients.[20] Although the mechanism remains unclear, hepatitis C infection plays an important role in the pathogenesis of immunologic derangement, which may result in ANA production.[21] Previous studies have reported positive ANA in up to 27% of patients with malignancies.[22–24] Therefore, the possibility of malignant diseases should be kept in mind despite the strong association between ANA and autoimmune diseases.

The presence of ANA in cancer may reflect an autoimmune response to nuclear antigens, which are perturbed in cellular transformation[25] or because of the higher prevalence of both cancer and ANA among the elderly.[26] This may also explain why malignant diseases were only found in the adults in our study.

Dinser et al.[27] reported that elevated ANA titers have a low positive predictive value of 4% for developing ANA-associated autoimmune diseases in the absence of clinical suspicion. In our study, the patients with ANA titers $\geq$ 1:640 and initially presenting with joint pain, fever, abnormal

Table 7 The components of anti-ENA antibodies in the patients with autoimmune diseases

Autoantibodies tests	SLE	SCLE	RA	Systemic sclerosis	MCTD	Sjögren's syndrome	Raynaud's syndrome
Anti-ENA test	37	2	8	7	4	31	2
Anti-ENA (+)	25	1	0	2	4	15	2
Anti-RNP (+)	11	0	0	0	4	1	2
Anti-SSA (+)	15	1	0	2	3	15	1
Anti-SSB (+)	10	1	0	1	0	10	1
Anti-SCL70 (+)	0	0	0	0	0	0	0
Anti-SM (+)	10	0	0	0	2	1	0
Anti-histone (+)	0	0	0	0	0	0	0
Anti-Jo1 (+)	0	0	0	0	0	0	0

ENA = extractable nuclear antibodies; MCTD = mixed connective tissue disease; RA = rheumatoid arthritis; SCLE = subacute cutaneous lupus erythematosus; SLE = systemic lupus erythematosus; RNP = ribo-nucleo-protein; SSA = Sjogren's Syndrome A; SSB = Sjogren's Syndrome B; SCL = scleroderma; SM = Smith.

urinalysis findings, or skin presentations were highly associated with autoimmune diseases. Therefore, these symptoms may increase the positive predictive value of elevated ANA titers for autoimmune diseases. In addition, long-term follow-up may be needed for the patients without definite diagnosis, especially in those with high ANA titers and presenting with joint pain, fever, abnormal urinalysis finding, or skin presentations.

The limitation of our study includes the duration of follow-up and the relatively small case numbers. All the patients receiving an ANA test were only followed for 6 months, which might not be long enough for an autoimmune disease to become established. The definition of immunofluorescent pattern depended on the judgment of medical laboratory technologists; thus, the ANA test reports of the same patients may have been different in various laboratories. In addition, the diagnosis of autoimmune diseases depended on the judgment of physicians whose background in training and experience were different. This may have affected the distribution and classification of the final diagnoses. However, at the least our study showed the overall distribution of ANA titers, patterns, and the final results in a medical center.

We found a trend that patients with high ANA titers were more likely to be associated with autoimmune diseases. However, there were still a lot of exceptions. An ANA test should not be used alone to exclude nonautoimmune diseases without other information. When patients have ANA titers $\geq$ 1:640 and present with joint pain, fever, abnormal urinalysis, or skin presentations, long-term follow-up and other laboratory tests (C3/C4, rheumatoid factor, and so on) are needed for the final diagnosis.

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