Comparison of indirect immunofluorescence and line immunoassay for autoantibody detection

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Abstract

Objectives

The aim of the present paper is to evaluate the diagnostic performance of indirect immunofluorescence (IIF) and line immunoassay (LIA) for autoantibody (autoAb) detection and provide sufficient information to interpret the results of autoAb tests.

Methods

The study included 1,052 patients for whom IIF and LIA tests had been performed simultaneously for a systemic autoimmune disease work-up. All patients were divided into either the systemic autoimmune group or non-autoimmune group, and the systemic autoimmune group was further divided into systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome, systemic sclerosis (SSc), and dermatomyositis/polymyositis (DM/PM). The diagnostic performance of IIF and LIA was analysed according to the distribution of IIF patterns and autoAbs identified by LIA.

Results

The overall sensitivity/specificity of IIF and LIA for systemic autoimmune disease was 63.5%/80.3% and 66.1%/83.2%, respectively. IIF showed higher sensitivity for SLE than LIA, but the sensitivity of LIA was higher for Sjögren's syndrome and DM/PM. The speckled pattern was the most commonly observed pattern in systemic autoimmune diseases with the exception of SSc. In the majority of systemic autoimmune diseases and their various IIF patterns, both anti-Ro-52 and anti-SS-A were the most prevalent autoAbs. In addition, a majority of the systemic autoimmune diseases showed specific dominant positive patterns or a combination of IIF and LIA results that were disease specific.

Conclusion

Utilising both methods together not only increased the sensitivity in most cases but also provided more information from the combination of results, augmenting their interpretation with the advantage of simultaneous identification of autoAbs.

Key words

systemic autoimmune disease, autoantibody, indirect immunofluorescence, line immunoassay, sensitivity

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Introduction

Antinuclear antibodies (ANA) are consistently found in the serum of patients with systemic autoimmune diseases or related symptoms. Identification of ANA can be a critical step in diagnosing systemic autoimmune disease (1). Although indirect immunofluorescence (IIF), enzyme immunoassay (EIA), and multiplex bead flow cytometry are available for ANA detection, IIF is the most commonly used screening method and is regarded as the gold standard (2-4). ANA testing using IIF alone provides basic information regarding the antigens involved, but does not allow for the identification of specific antigens (5). Therefore, if the result of an ANA screening test using IIF is positive, further identification tests for specific antigens such as anti-extractable nuclear antigen (ENA) and anti-double stranded deoxyribonucleic acid (anti-dsDNA) tests, are needed. Immunodiffusion, EIA, western blotting, line immunoassay (LIA), multiplex immunoassay, or flow cytometry can be used for further identification of ANA specificity (6, 7).

LIA with purified or recombinant antigen-absorbed membrane strips is widely utilised because of its easy use and shorter processing time. Additionally, LIA provides an opportunity to identify several autoantibodies (autoAbs) simultaneously, although the sensitivity for anti-Sm and anti-Scl-70 is lower than for other autoAbs (8). In many laboratories in Korea, LIA is used as a confirmatory test for IIF to identify the specificity of autoAbs in samples that are IIF positive, or to rule out the possibility of false negative IIF results.

To date, various studies have been conducted regarding different patterns of IIF and autoAbs detected in various ways and their relevance to systemic autoimmune diseases. However, there are no reports focusing on the comparison of results from both IIF and LIA in relation to systemic autoimmune diseases at a single medical centre. The aim of the present study was to evaluate the diagnostic sensitivity and specificity of both IIF and LIA in order to compare the IIF pattern with the autoAbs identified by LIA with respect to systemic autoimmune diseases and, finally, to provide the information needed to interpret the results of autoAbs tests.

Materials and methods

Patients

Between January 2009 and December 2010, 1,052 samples were referred for simultaneous ANA testing using IIF and LIA as part of a work-up for systemic autoimmune diseases at Kyung Hee University Hospital at Gangdong. Among 1,052 serum samples, 189 samples comprised the systemic autoimmune disease group and 829 samples comprised the non-autoimmune disease group. The remaining 34 samples, which were obtained from patients with ankylosing spondylitis, Behçet's disease, and strongly suspected but not yet diagnosed SLE or Sjögren's syndrome, were excluded from the study. The systemic autoimmune disease group included patients with systemic lupus erythematosus (SLE, n=38), rheumatoid arthritis (RA, n=88), Sjögren's syndrome (n=33), systemic sclerosis (SSc, n=7), overlap syndrome (n=14), and dermatomyositis/polymyositis (DM/PM, n=9). Rheumatologists made the diagnoses according to clinical presentations and laboratory results. The non-autoimmune disease group included rheumatology patients who did not present with any evidence of systemic autoimmune disease aside from arthralgia or fibromyalgia and patients from other clinical departments who were not diagnosed with a systemic autoimmune disease.

Indirect immunofluorescence (IIF) method

Serum samples were diluted (1:40) with phosphate buffer solution using HEp-2 cell slide (Kallestad, Bio-Rad, Redmond, USA). Tests were performed using the PhD System (Bio-Rad Laboratories, Hercules, USA) according to the manufacturer's instructions. Two experts at the Department of Laboratory Medicine interpreted the IIF results using a Nikon fluorescence microscope. Fluorescence intensity was scored semi-quantitatively from negative to 4+ relative to the intensity

of the positive (4+ and 1+) and negative controls at x200.

The IIF pattern was classified as speckled, dense fine speckled, homogeneous, cytoplasmic, nucleolar, discrete speckled (DS), and other pattern by contemporary nomenclature using HEp-2 cells (2). The dense fine speckled pattern was defined as interphase nucleoplasmic staining accompanied by positivity on a majority of chromatin plates of metaphase cells (2, 9). The cytoplasmic pattern included various cytoplasmic staining patterns such as diffuse or fine speckled, mitochondrial-like, lysosomal-like, golgi-like, and cytoskeletal cytoplasmic patterns. Patterns classified as other included nuclear dots, centriole, and mitotic apparatus patterns (2).

LIA

A Euroimmun ANA Profile 3 Euroline kit (EUROIMMUN, Luebeck, Germany) was used for the LIA. In brief, we used nylon strips covered with recombinant and purified antigens in distinct lines with a plastic backing coated with the following antigens: nuclear ribonucleoprotein (nRNP)/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, proliferating cell nuclear antigen (PCNA), Jo-1, centromere protein B (CENP-B), dsDNA, nucleosomes, histones, ribosomal protein-p, and anti-mitochondrial antibodies (AMA-M2). The nylon strip was incubated with serum at a 1:101 dilution. After the specific autoAbs in the patient sera were bound to the corresponding antigen sites, the strip was incubated with enzyme conjugate to facilitate the colour reaction. EUROLineScan software was used to evaluate the signal intensity, and the results were graded from negative to 3+.

Data analysis

We analysed the overall sensitivity and specificity of the IIF and LIA for all patient samples along with the sensitivity for individual systemic autoimmune diseases. The distribution of IIF patterns and autoAbs identified by LIA were investigated for each systemic autoimmune disease. We also analysed autoAbs identified by LIA according to certain IIF patterns.

Table I. Comparison of sensitivity and specificity between the IIF and LIA methods.

	Number (n=1018)	Systemic autoimmune diseases (n=189)	Non-autoimmune diseases (n=829)
IIF+/ LIA+	158 (15.5%)	94 (59.5%*, 49.7%†)	64 (40.5%*, 7.7%†)
IIF+/ LIA-	125 (12.3%)	26 (20.8%, 13.8%)	99 (79.2%, 11.9%)
IIF- / LIA+	106 (10.4%)	31 (29.2%, 16.4%)	75 (70.8%, 9.0%)
IIF- / LIA-	629 (61.8%)	38 (6.0%, 20.1%)	591 (94.0%, 71.3%)
		Sensitivity	Specificity
IIF only		63.5%	80.3%
LIA only		66.1%	83.2%
IIF or LIA		79.9%	71.3%

*The percentages of patients with non-autoimmune and systemic autoimmune diseases among patients for whom both test methods were used.
†The percentage of patients with each combination of results among patients with non-autoimmune and systemic autoimmune diseases.
IIF: indirect immunofluorescence; LIA: line immunoassa.

Table II. Sensitivities of the IIF and LIA methods for each systemic autoimmune disease.

	RA (n=88)	SLE (n=38)	Sjögren's syndrome (n=33)	Overlap syndrome (n=14)	DM/PM (n=9)	SSc (n=7)
IIF+/ LIA+ (n=94)	18 (20.5%)	32 (84.2%)	23 (69.7%)	12 (85.7%)	2 (22.2%)	7 (100%)
IIF+/ LIA- (n=26)	20 (22.7%)	5 (13.2%)	0	0	1 (11.1%)	0
IIF-/ LIA+ (n=31)	18 (20.5%)	0	7 (21.2%)	2 (14.3%)	4 (44.4%)	0
IIF-/ LIA- (n=38)	32 (36.4%)	1 (2.6%)	3 (9.1%)	0	2 (22.2%)	0
Sensitivity (overall)						
IIF only (63.5%)	43.2%	97.4%	69.7%	85.7%	33.3%	100%
LIA only (66.1%)	40.9%	84.2%	90.9%	100%	66.7%	100%
IIF or LIA (79.9%)	63.6%	97.4%	90.9%	100%	77.8%	100%

RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; DM: dermatomyositis; PM: polymyositis, SSc: systemic sclerosis. See Table I for additional abbreviations.

Results

Comparison of sensitivity and specificity between the IIF and LIA (Table I)

Among 1,018 samples, 283 (27.8%) and 264 (25.9%) were positive according to IIF and LIA, respectively. The overall sensitivity of IIF and LIA for systemic autoimmune disease was 63.5% and 66.1%, respectively, indicating that approximately 35% of systemic autoimmune disease patients were negative with IIF or LIA. The overall specificity of IIF and LIA was 80.3% and 83.2%, respectively.

The sensitivity for each specific systemic autoimmune disease is summarised in Table II. The sensitivity of IIF was higher than LIA only in SLE. The remaining diseases showed similar sensitivities between the two methods or higher sensitivities for LIA than for IIF. Specifically, in cases of Sjögren's syndrome and DM/PM, the sensitivity of LIA was significantly higher than the IIF method (greater than 90% and 66.7%, respectively).

Analysis of the results according to IIF and LIA

Among the 1,018 samples, 158 (15.5%) were IIF+/LIA+ and approximately one third or more (40.5%) were obtained from the non-autoimmune disease group (Table I). IIF-/LIA- was observed in 629 (61.8%) samples and most (94.0%) were from the non-autoimmune disease group. There were 231 (22.7%) samples with a discrepancy between IIF and LIA. There were 125 (12.3%) and 106 (10.4%) IIF+/LIA- and IIF-/LIA+ samples, respectively, and the majority of the samples were obtained from the non-autoimmune disease group (79.2% and 70.8%, respectively).

Among 189 samples from patients

in the systemic autoimmune disease group, the frequency of concurrent positivity for both methods was 49.7%, but only 30.2% of samples were positive by one method only (IIF+/LIA- 13.8%, IIF-/LIA+ 16.4%). Additionally, 20.1% of patients with systemic autoimmune disease were negative by both methods (IIF-/LIA-).

Among 38 SLE patients, 32 (84.2%) were positive by both IIF and LIA. Although no patient was positive with LIA only, five patients (13.2%) were positive with the IIF method only. Out of 33 Sjögren's syndrome patients, 23 (69.7%) were positive by both IIF and LIA. While there were no patients that were positive by the IIF method only, seven patients (21.2%) were positive by LIA only with identification of autoAbs (anti-SS-A, anti-Ro-52, and anti-SS-B). All patients with SSc were positive by both IIF and LIA with various IIF patterns and autoAbs. Characteristically, the positivity of IIF and LIA appeared to have variable patterns in samples from RA patients.

Analysis of IIF patterns according to systemic autoimmune diseases (Table III)

The speckled pattern was the most common IIF pattern (97, 34.3%) among the 283 samples that were positive by the IIF method regardless of the LIA results. The cytoplasmic pattern (66, 23.3%), homogeneous pattern (45, 15.9%), nucleolar pattern (42, 14.8%), and dense fine speckled pattern (38, 13.4%) were also observed. When the data were analysed according to systemic autoimmune diseases, the speckled pattern was also the most common pattern observed in each systemic autoimmune disease except SSc. The frequency of the speckled pattern was 64.3% in overlap syndrome, 55.3% in SLE, 42.4% in Sjögren's syndrome, 22.2% in DM/PM, 14.8% in RA, and 14.3% in SSc.

In RA patients, the speckled pattern was the most often observed (14.8%), but it was not the predominant pattern, unlike the other systemic autoimmune diseases. Other patterns, each in less than 10% of samples, were also observed. In SSc patients, the DS (42.9%) and cytoplasmic (42.9%) patterns were the most common, unlike the other systemic autoimmune diseases. In addition, the speckled pattern (55.3%), the homogeneous pattern (34.2%) and the cytoplasmic pattern (23.7%) were dominant in SLE patients. In the non-autoimmune disease group, the observed patterns varied and the prevalence of each pattern was approximately 5% or less. Commonly found IIF patterns in the non-autoimmune disease group were cytoplasmic (5.3%), speckled (4.5%), nucleolar (4%), and dense fine speckled (3%).

Table III. Distribution of IIF patterns and autoAbs identified according to systemic autoimmune disease.

	Sjögren's syndrome (n=33)	SLE (n=38)	RA (n=88)	Overlap syndrome (n=14)	SSc (n=7)	DM/PM (n=9)	Non-autoimmune (n=829)
IIF+ (n of patients=283)							
Speckled (n=97, 34.3%)	**14 (42.4%)**	**21 (55.3%)**	**13 (14.8%)**	**9 (64.3%)**	1 (14.3%)	**2 (22.2%)**	37 (4.5%)
DFS (n=38, 13.4%)	2 (6.1%)	3 (7.9%)	8 (9.1%)	0 (0%)	0 (0%)	0 (0%)	25 (3.0%)
Homogeneous (n=45, 15.9%)	3 (9.1%)	**13 (34.2%)**	8 (9.1%)	2 (14.3%)	2 (28.6%)	0 (0%)	17 (2.1%)
Cytoplasmic (n=66, 23.3%)	2 (6.1%)	9 (23.7%)	6 (6.8%)	1 (7.1%)	**3 (42.9%)**	**1 (11.1%)**	44 (5.3%)
Nucleolar (n=42, 14.8%)	2 (6.1%)	2 (5.3%)	3 (3.4%)	1 (7.1%)	1 (14.3%)	0 (0%)	33 (4.0%)
DS (n=21, 7.4%)	3 (9.1%)	1 (2.6%)	2 (2.3%)	2 (14.3%)	**3 (42.9%)**	0 (0%)	10 (1.2%)
Other patterns (n=14, 4.9%)	0 (0%)	2 (5.3%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)	11 (1.3%)
LIA+ (n of patients=264)							
Sm (n=9, 3.4%)	0 (0%)	7 (18.4%)	0 (0%)	1 (7.1%)	1 (14.3%)	0 (0%)	0 (0%)
nRNP/Sm (n=24, 9.1%)	0 (0%)	**10 (26.3%)**	0 (0%)	**10 (71.4%)**	1 (14.3%)	0 (0%)	3 (0.4%)
SS-A (n=112, 42.4%)	**26 (78.8%)**	**18 (47.4%)**	**22 (25.0%)**	**6 (42.9%)**	0 (0%)	0 (0%)	**40 (4.8%)**
Ro-52 (n=117, 44.3%)	**22 (66.7%)**	**16 (42.1%)**	**18 (20.5%)**	**6 (42.9%)**	1 (14.3%)	**5 (55.6%)**	**49 (5.9%)**
SS-B (n=45, 17%)	**14 (42.4%)**	**10 (26.3%)**	4 (4.5%)	**4 (28.6%)**	1 (14.3%)	0 (0%)	12 (1.4%)
Scl-70 (n=16, 6.1%)	0 (0%)	1 (2.6%)	4 (4.5%)	2 (14.3%)	2 (28.6%)	0 (0%)	7 (0.8%)
Jo-1 (n=5, 1.9%)	0 (0%)	0 (0%)	2 (2.3%)	0 (0%)	1 (14.3%)	1 (11.1%)	1 (0.1%)
PM-Scl (n=2, 0.8%)	0 (0%)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)	0 (0%)	1 (0.1%)
dsDNA (n=32, 12.1%)	0 (0%)	**15 (39.5%)**	1 (1.1%)	3 (21.4%)	0 (0%)	0 (0%)	13 (1.6%)
Histone (n=36, 13.6%)	2 (6.1%)	**10 (26.3%)**	5 (5.7%)	1 (7.1%)	0 (0%)	0 (0%)	18 (2.2%)
CENP-B (n=20, 7.6%)	3 (9.1%)	1 (2.6%)	2 (2.3%)	2 (14.3%)	**4 (57.1%)**	0 (0%)	8 (1.0%)
Nucleosome (n=20, 7.6%)	1 (3.0%)	**11 (28.9%)**	0 (0%)	4 (28.6%)	0 (0%)	0 (0%)	4 (0.5%)
PCNA (N=3, 1.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (0.4%)
AMA-M2 (n=21, 8%)	1 (3.0%)	4 (10.5%)	1 (1.1%)	0 (0%)	1 (14.3%)	0 (0%)	14 (1.7%)
Ribosomal P (n=22, 8.3%)	1 (3.0%)	**11 (28.9%)**	2 (2.3%)	2 (14.3%)	0 (0%)	0 (0%)	6 (0.7%)

autoAbs: autoantibodies; DFS: dense fine speckled; DS: discrete speckled; nRNP: nuclear ribonucleoprotein; CENP-B: centromere protein B; AMA-M2: anti-mitochondrial antibodies. See Tables I and II for additional abbreviations.

The total sum of IIF patterns and identified autoAbs exceeded the number of patients because of concomitant positives. The number of patients with multiple positivity on IIF was 43. Among them, 41 samples showed double patterns and 2 showed triple patterns. On LIA, multiple positivity was shown in 123 patients and their distribution was as follows: 8 autoAbs were detected in 2 samples, 7 autoAbs in 2 samples, 6 autoAbs in 1 samples, 5 autoAbs in 2 samples, 4 autoAbs in 14 samples, 3 autoAbs in 37 samples and 2 autoAbs in 65 samples.

Analysis of autoAbs identified by LIA according to systemic autoimmune diseases (Table III)

The most prevalent autoAbs identified in 264 LIA+ samples regardless of the results of IIF were anti-Ro-52 (117, 44.3%), anti-SS-A (112, 42.4%), anti-SS-B (45, 17%), anti-histone (36, 13.6%), and anti-dsDNA (32, 12.1%). Generally, both anti-Ro-52 and anti-SS-A were the most prevalent autoAbs in the systemic autoimmune disease group, except for a few specific autoimmune diseases. Even in the non-autoimmune disease group, anti-Ro-52 (5.9%) and anti-SS-A (4.8%) were the most frequently observed autoAbs.

In the samples from SLE patients, a variety of autoAbs such as anti-dsDNA (39.5%), anti-nucleosome (28.9%), anti-ribosomal P (28.9%), anti-nRNP/Sm (26.3%), anti-histone (26.3%), anti-SS-B (26.3%), anti-SS-A (47.4%), and anti-Ro-52 (42.1%), were identified more than 20% of the time, which was unlike the other systemic autoimmune diseases. The majority of Sjögren's syndrome patients were positive for anti-SS-A (78.8%) and anti-Ro-52 (66.7%), and about a half had anti-SS-B (42.4%). RA patients had a higher frequency of anti-SS-A (25%) and anti-Ro-52 (20.5%), while other autoAbs appeared at a low frequency (less than 5%). In overlap syndrome, the most frequently observed autoAb was anti-nRNP/Sm (71.4%), followed by anti-SS-A (42.9%) and anti-Ro-52 (42.9%). Unlike the other systemic autoimmune diseases showing a high frequency of anti-SS-A and anti-Ro-52, DM/PM showed a characteristic finding of isolated anti-Ro-52 (55.6%) in the absence of anti-SS-A (0%).

AutoAbs identified by LIA according to IIF pattern (Table IV)

According to the analysis of the distribution of autoAbs, anti-SS-A and anti-Ro-52 were identified at a high frequency (30%~) regardless of the IIF pattern, even in IIF-negative samples. Among the 72 sera with the speckled pattern, anti-SS-A (66.7%) and anti-Ro-52 (65.3%) were the most common autoAbs, followed by anti-SS-B (40.3%) and anti-nRNP (26.4%). Anti-Ro-52 (45.7%), anti-AMA M2 (37.1%), and anti-SS-A (34.3%) were frequently detected in the 35 sera with the cytoplasmic pattern. In the 29 sera exhibiting the homogeneous pattern, the LIA results revealed anti-SS-A (44.8%), anti-nucleosome (44.8%), anti-histone (34.5%), anti-dsDNA (34.5%), and anti-Ro-52 (24.1%). In the 21 samples with the DS pattern, anti-CENP-B (95.2%), anti-Ro-52 (38.1%), and anti-SS-A (28.6%) were detected with LIA.

Discussion

The results from the present study reveal differences in the sensitivity of the IIF method according to disease. The sensitivities for SLE (97.4%) and SSc (100%) were the highest. In another study that included 238 patients with SSc, more than 90% showed positivity on IIF (10) and it is assumed that IIF could be the best screening method to detect SSc because of its high sensitivity. However, the IIF assay showed low sensitivity and specificity for PM/DM, RA, and Sjögren's syndrome while the sensitivity and specificity to detect systemic autoimmune diseases in general were 63.5% and 80.3%, respectively, which is consistent with the results from previous studies (11). The sensitivity (66.1%) and specificity (83.2%) of LIA for systemic autoimmune disease in general also proved to be similar to the IIF method, although it had a higher sensitivity than IIF for Sjögren's syndrome and DM/PM. The discrepancy rate between the two methods was approximately 22.7% (231/1018). A higher sensitivity (80%) could potentially be achieved when both methods are utilised together although the specificity could decrease to 71%. Therefore, utilising both ANA screening and LIA, which increases the sensitivity while making simultaneous identification of major autoAbs possible, could be more

Table IV. Distributions of specific autoAbs by LIA according to IIF pattern.

IIF pattern	Sm	RNP/Sm	SS-A	Ro-52	SS-B	Scl-70	Jo-1	PM-Scl	dsDNA	Histone	CENP-B	Nucleosome	PCNA	AMA-M2	Ribosomal P
Negative (n=106)	0	1	**36**	**40**	8	4	4	1	12	15	0	1	2	2	4
	0%	0.9%	**34%**	**37.7%**	7.5%	3.8%	3.8%	0.9%	11.3%	14.2%	0%	0.9%	1.9%	1.9%	3.8%
Speckled (n=72)	8	**19**	**48**	**47**	**29**	4	0	0	9	8	0	9	1	2	11
	11.1%	**26.4%**	**66.7%**	**65.3%**	**40.3%**	5.6%	0%	0%	12.5%	11.1%	0%	12.5%	1.4%	2.8%	15.3%
DFS (n=8)	0	0	**4**	**3**	1	1	0	0	2	**4**	0	1	0	0	1
	0%	0%	**50%**	**37.5%**	12.5%	12.5%	0%	0%	25%	**50%**	0%	12.5%	0%	0%	12.5%
Homogeneous (n=29)	3	4	**13**	**7**	5	5	1	0	**10**	**10**	2	**13**	0	2	5
	10.3%	13.8%	**44.8%**	**24.1%**	17.2%	17.2%	3.4%	0%	**34.5%**	**34.5%**	6.9%	**44.8%**	0%	6.9%	17.2%
Cytoplasmic (n=35)	3	5	**12**	**16**	5	5	1	1	4	5	2	4	0	**13**	5
	8.6%	14.3%	**34.3%**	**45.7%**	14.3%	14.3%	2.9%	2.9%	11.4%	14.3%	5.7%	11.4%	0%	**37.1%**	14.3%
Nucleolar (n=15)	0	1	**5**	**6**	2	3	0	0	1	3	0	2	0	1	3
	0%	6.7%	**33.3%**	**40%**	13.3%	20%	0%	0%	6.7%	20%	0%	13.3%	0%	6.7%	20%
DS (n=21)	0	3	**6**	**8**	2	0	0	0	1	1	**20**	2	0	2	1
	0%	14.3%	**28.6%**	**38.1%**	9.5%	0%	0%	0%	4.8%	4.8%	**95.2%**	9.5%	0%	9.5%	4.8%

The total sum of identified autoAbs exceeded the number of samples because of concomitant positives. See Tables I, II and III for abbreviations.

clinically useful than the conventional two-step ANA screening method (IIF followed by identification of specific autoAbs). Although performing both tests simultaneously may not increase the sensitivity in SLE, identifying multiple autoAbs is still an advantage. In addition, we do not recommend using additional tests to identify autoAbs only in cases of IIF positivity because false negative results could occur due to the loss of antigenicity during cell fixation or test procedures (8). In cases of Sjögren's syndrome or DM/PM, the LIA detection rate for anti-SS-A and anti-Ro-52, although not characterised by specific IIF patterns, was high, and the sensitivity was increased when both the IIF and LIA were used together as opposed to IIF alone.

Notably, anti-SS-A, anti-Ro-52, or both were the most frequently detected autoAbs in any IIF pattern except for anti-CENP-B in the DS pattern. Even in the cases of IIF-/LIA+, anti-Ro-52 and anti-SS-A were frequently found with the LIA method (37.7% and 34%, respectively). Moreover, when the data were analysed according to each systemic autoimmune disease, anti-SS-A and anti-Ro-52 were the most commonly identified autoAbs among all of the systemic autoimmune diseases except for SSc and overlap syndrome. In addition, the detection rates for anti-Ro-52 and anti-SS-A in the non-autoimmune disease group were 5.9% and 4.8%, respectively, while other autoAbs were detected at frequencies below 2%.

Anti-Ro-52 alone identified without other autoAbs by LIA appeared in 42 cases (data not shown), and 67% of them were IIF negative. Similarly, there were 25 cases of anti-SS-A alone by LIA, and 76% of them were IIF negative; therefore, the presence of anti-Ro-52 or anti-SS-A is probably not related to any specific IIF pattern. The detection of anti-Ro-52 is notable because of its outstanding ability to provoke immunity; it is recognised as the most antigenic protein known in humans (12). Anti-Ro-52 is detected at a higher frequency in Sjögren's syndrome, SSc, and myositis (13) and is associated with non-autoimmune diseases such as viral infections and neoplastic diseases (14). In a single study on the association between anti-SS-A and anti-Ro-52, isolated detection of anti-Ro-52 or measurement of a relatively higher reactivity in comparison with anti-SS-A implied a slight association with myositis and even less with SSc. The presence of reactivity for either antigen or a higher reactivity of SS-A compared to Ro-52 was associated with Sjögren's syndrome or SLE in terms of connective tissue diseases (13). Such tendencies were also apparent in the present study with both anti-Ro-52 and anti-SS-A detected simultaneously in most cases. On the other hand, anti-SS-A was detected at a slightly higher frequency than anti-Ro-52 in various autoimmune diseases such as Sjögren's syndrome, RA, SLE, and overlap syndrome. Unlike other systemic autoimmune diseases, 55.6% of DM/PM patients were positive for anti-Ro-52 in the absence of anti-SS-A. In the non-autoimmune disease group, the rate of anti-Ro-52 (5.8%) detection was higher than that of anti-SS-A (4.7%). Reportedly, anti-SS-A is detected in 15.5% out of 1,400 people in the general population (15). However, the prevalence differs according to testing methods and subject selection.

While the majority of systemic autoimmune diseases included in the present study showed specific dominant positive patterns or a combination of IIF and LIA results, RA patients were somewhat different in that the speckled pattern was most commonly observed (14.8%) on IIF, but was not significantly dominant. Additionally, the combination of IIF and LIA results was evenly distributed; therefore, the diagnostic sensitivity was not increased when the tests were performed together. Despite RA being the most prevalent systemic autoimmune disease in this study, the sensitivity of IIF or LIA for RA was the lowest. In another study that investigated the performance of IIF in patients with connective tissue diseases, more than 70% IIF positivity was shown for SLE and SSc, whereas less than 10% IIF positivity was observed for RA (16).

The present study had several limitations. Although there are no reference methods or reference materials for the identification of autoAbs, the cross-validation against another method to confirm the autoAbs identified by LIA was not performed. Specifically, since anti-dsDNA was not detected by LIA in several cases (data not shown) compared to the ELISA method, an anti-dsDNA analysis was not performed. Additionally, the identification of autoAbs by LIA used in the present study was limited to only 15 different autoAbs, including six anti-ENAs. Also, the number of subjects with autoimmune diseases was not evenly distributed.

In summary, in this comparison of two commonly performed methods for the detection of autoAbs in Korean laboratories, neither IIF nor LIA were found to be very sensitive or specific for systemic autoimmune diseases when performed separately. Considering that specific dominant positive patterns or a combination of IIF and LIA results were observed for certain diseases, these two methods together could be useful. Utilising both methods together not only increases the sensitivity in most cases but also provides more information from the combination of results in addition to the advantage of simultaneous autoAbs identification. Clinical signs, symptoms, patient history, and other laboratory results also need to be considered.

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