Original article

Sensitivity and specificity of ANA and anti-dsDNA in the diagnosis of systemic lupus erythematosus: A comparison using control sera obtained from healthy individuals and patients with multiple medical problems

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Summary

Background: **Antinuclear antibodies (ANA) and anti-double stranded DNA (anti-dsDNA) are often tested as a screening tool in patients with suspected systemic lupus erythematosus or connective tissue diseases. ANA can be seen in healthy controls (HC) and patients with multiple medical problems (MMP).**

Objective: **To determine the sensitivity and specificity of ANA and anti-dsDNA in SLE patients, using sera from HC and MMP patients.**

Methods: **Serum samples from HC, MMP and SLE patients, 100 in each group, were analyzed for the presence of ANA and anti-dsDNA, by indirect immunofluorescent assay, using a HEp-2 cell and *Crithidia luciliae* as substrates, respectively.**

Results: **The prevalence of ANA at a titer of ≥1:80 and ≥ 1:160 was 8% and 4%, respectively, in HC; and it was 12% and 6% respectively, in MMP patients. The prevalence of anti-dsDNA was 0% in HC and 3% in MMP patients. When using HC sera for the diagnosis of SLE, the sensitivity of ANA at a titer of ≥ 1:80 and ≥ 1:160 was 98% and 90%, respectively, with specificity of 92% and 96%, respectively. The specificity decreased to 88% and 94%, respectively, when using sera from MMP patients. The specificity of anti-dsDNA was 100% and 97%, when using sera from HC and MMP patients, respectively.**

Conclusion: **ANA and anti-dsDNA gave high sensitivity and high specificity in patients with SLE, even when using MMP patient's sera as controls. Physicians should take care in interpreting ANA and anti-dsDNA results in MMP patients who do not have signs or symptoms of SLE or connective tissue diseases. *(Asian Pac J Allergy Immunol 2013;31:292-8)***

Key words: *ANA, anti-dsDNA, lupus, multi-system disease, sensitivity, specificity*

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Submitted date: 13/7/2012

Accepted date: 25/9/2012

Introduction

Anti-nuclear antibodies (ANA), a heterogeneous group of autoantibodies against nuclear antigens, are often tested as a screening tool in patients with suspected systemic lupus erythematosus (SLE) or other connective tissue diseases.[1-3] The prevalence of positive ANA tests in various autoimmune rheumatic diseases varies greatly, e.g., 90-100% in systemic lupus erythematosus (SLE), 60-80% in systemic sclerosis (SSc), 40-70% in Sjogren's syndrome, 30-80% in polymyositis/dermatomyositis, and 30-50% in rheumatoid arthritis (RA), and the prevalence of autoantibodies has been shown to differ between different races.[2,4] A study performed in Thailand in 1987 found that the prevalence of ANA in patients with SLE, RA, SSc and a healthy control (HC) was 97%, 31.7%, 90.4% and 11.6%, respectively.[5] Despite the high sensitivity of the ANA test for screening autoimmune rheumatic diseases, particularly SLE, its specificity is low, as these antibodies can be present also in the general population, elderly individuals, malignancies, infections, thyroid disease, etc.[2,6,7] The interpretation of ANA

should be accompanied by a titer and pattern. ANA at a titer of ≥ 1:160 is accepted generally as significant, because it is not seen commonly in healthy individuals.[2,3,8] Although the speckled pattern (fine and coarse speckled) is seen most commonly in patients with connective tissue disease, only the fine speckled pattern presents in healthy individuals.[9] Anti-double stranded DNA antibodies (anti-dsDNA) are a part of the antibodies detected by ANA, and are tested commonly in SLE. They are more specific to SLE (97% specificity), but much lower in sensitivity (60%).[1] ANA and anti-dsDNA are two of the 11 criteria for the classification of SLE.[10]

The prevalence of ANA and anti-dsDNA varies among autoimmune diseases, depending on the substrates (rat liver or HEp-2 cell) and assay methods [e.g. indirect immunofluorescent (IIF), enzyme-link immunosorbant assay (ELISA), immunodiffusion, or radioimmunoassay (RIA)],[3,11,12] as well as the experience of the technicians.[13-15] Although the ELISA method is used widely at present and has higher sensitivity, the IIF method using *Crithidia luciliae* as a substrate gives higher specificity results.[16-18] Therefore, the ELISA method should be used as a screening test for anti-dsDNA and positive results should be confirmed using the *Crithidia luciliae* IIF technique.[17] Currently, determination by the IIF technique of ANA and anti-dsDNA using HEp-2 cells and *Crithidia luciliae* as a substrate is accepted generally as a standard assay method.[3,12]

Patients in a tertiary care medical center with multiple medical problems (MMP) are often tested for ANA and anti-dsDNA in order to rule out possible autoimmune rheumatic diseases. When the ANA result is positive (or to a lesser extent anti-DNA positive), the physician becomes confused by the possibility for the diagnosis of SLE or other autoimmune diseases, despite the absence of signs or symptoms suggesting these diseases in the MMP patients. Furthermore, other specific autoantibody tests (e.g., anti-cardiolipin (ACL), anti-Sm, anti-RNP, etc.) are often ordered to complete the autoantibody profile. This not only leads to more confusion in the diagnosis, but also increases in the cost of the investigation.

Therefore, this study aimed to determine the sensitivity and specificity of the ANA and anti-dsDNA criteria for the classification of SLE, and compare the use of sera from HC and MMP patients in the Thai population.

Methods

This study was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University and the work conformed to the provisions of the World Medical Association's Declaration of Helsinki. One hundred subjects in each group of HC, MMP and SLE patients were enrolled in the study. All of them gave their written informed consent prior to enrollment. The HC group comprised medical personnel or healthy individuals, with no medical problems who were not taking regular medication. The term 'MMP patients' referred to subjects with more than one current medical problem who were admitted to the Internal Medicine Ward of Chiang Mai University Hospital. MMP patients and HC with signs and symptoms suggesting connective tissue disease were excluded. The diagnosis of medical problems and current medications were recorded. SLE patients were followed up at the Rheumatology Clinic, Chiang Mai University Hospital; and they met the 1997 American College of Rheumatology revised criteria for the classification of SLE.[10] The cumulative clinical manifestations of SLE patients were recorded according to the diagnostic criteria.

The serum samples from these subjects were kept at -20°C and analyzed simultaneously by RW, who had been performing IIF assay for over ten years and was blinded to the clinical data. The determination of ANA and anti-dsDNA was performed by IIF assay using HEp-2 cells [IIFT Mosaic HEp-20-1/Liver (Monkey), EUROIMMUN, Madizinische Labordiagnostika AG, Seekamp, Lübeck, Germany] and *Crithidia luciliae* (nDNA Fluro-Kit™, Diasorin Inc., Stillwater, Minnesota, USA) as substrates. The patterns of ANA were classified as fine speckle (FS), coarse speckle (CS), homogeneous (Ho), peripheral (P), nucleolar (Nu), and centromere (C). The ANA was determined at an initial titer of 1:80. A titer of ANA and anti-dsDNA at ≥ 1:160 and ≥ 1:10, respectively, was considered clinically significant. In this study, the maximum titer of ANA and anti-dsDNA was determined to be 1:1280. A titer of more than 1:1280 was considered as 1:1280 for analysis.

Statistical analysis

The SPSS program version 15.0 (Chicago, Illinois, U.S.A) was used for statistical analysis. Continuous data were reported as mean ± standard deviation (SD) and categorical data as frequency or percentages. The sensitivity and specificity of ANA and anti-dsDNA, with a 95% confidence interval (95% CI), was determined in SLE subjects by using sera from HC and MMP patients.

Results

This study comprised 100 HC patients (51 females, 49 males) with a mean ± SD age of 45.59 ± 15.06 years. The SLE patients consisted of 98 females and two males, with a mean ± SD age and duration of disease of 36.20 ± 11.69, and 7.32 ± 5.65 years, respectively. According to the diagnostic criteria, the cumulative clinical manifestations of the SLE patients are shown in Table 1. Mucocutaneous lesions, arthritis and renal disorders were among the common manifestations. Among 100 MMP patients (30 females, 70 males, mean ± SD age of 54.36 ± 16.50 years), the mean number of medical problems was 3.03 ± 1.08 (range 2-8). A significant difference in age also existed between the three groups ($p < 0.001$).

Details of the disease and the medication used in patients with MMP are shown in Table 2. Hypertension, diabetes mellitus, dyslipidemia and renal insufficiency were among the common diseases. Six patients had mycobacterium infection, of which five were caused by *Mycobacterium tuberculosis*. Each of four patients had a carcinoma of the larynx, hepatocellular carcinoma, non-Hodgkin's lymphoma, and T-cell lymphoma, respectively. Antihypertensive medicine, including angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), calcium channel blockers and β-blockers, were used commonly in these MMP patients, with a mean number of anti-hypertensive drugs of 1.54±0.76 items (range 1-3). Other commonly used medications were diuretics, statins and aspirin.

The prevalence of ANA and anti-dsDNA in HC, SLE and MMP patients is shown in Table 3. The details of age, sex, ANA titer and pattern, and occupations in HC; and underlying diseases, and current medications used in MMP patients are shown in Table 4 and 5, respectively. None of the HC group tested positive for anti-dsDNA. However, case 9, 12 and 7 in the MMP group tested positive for anti-dsDNA, with a titer of 1:10, 1:20 and 1:40, respectively, as shown in Table 5.

The sensitivity and specificity of ANA and anti-dsDNA are shown in Table 6. When using HC sera, the ANA titer of ≥ 1:80 and ≥ 1:160 gave a sensitivity of diagnosis of 98% and 90% in SLE, respectively, and specificity of diagnosis of 92% and 96% SLE, respectively. The specificity declined slightly to 88% and 94%, respectively, when using MMP patients' sera as controls. The specificity of anti-dsDNA was 100% when using HC sera, but declined

Table 1. Cumulative clinical manifestations in SLE patients

Clinical manifestations	% (n=100)
Malar rash	45
Discoid rash	33
Photosensitivity	54
Oral ulcers	36
Arthritis	66
Renal disorder	66
Serositis	
Pleuritis	10
Pericarditis	0
Neurological disorder	
Seizures	14
Psychosis	3
Hematologic disorder	
AIHA	34
Leukopenia	50
Thrombocytopenia	20

slightly to 97% when using MMP patients' sera as controls. However, the sensitivity of anti-dsDNA in this SLE population was 37%.

Discussion

This study found that HC patients had a positive test for ANA at a titer of ≥1:80 in 8% of cases, of which 4% had a titer of ≥1:160. One HC was positive for ANA at a titer of 1:1280 (FS pattern). None of these HCs had any signs or symptoms that suggested connective tissue diseases and none took regular medicine that could cause a positive ANA test. None of the HCs had a positive anti-dsDNA.

The importance of the high ANA titer (≥ 1:160) present in the HC group was not clear. No relationship between age and occupations and the presence of ANA in the HC group could be found. The presence of ANA in healthy individuals might be related to the features of the assay system, or to true intrinsic immunological disturbances.[19] The presence of ANA is not uncommon in healthy individuals, in particular elderly women.[7] A recent study in Mexico found that ANA was not uncommon in healthy individuals, particularly among medical personnel like those in this study and that a titer of up to 1:320 also could be seen.[6] A slight increase in the prevalence of positive ANA among medical personnel handling blood samples or having direct contact with the patients supports the hypothesis of a transmissible agent capable of

Table 2. Diseases and common medications used by MMP patients

Diseases	% (n = 100)	Medications	% (n = 100)
Hypertension	31	Anti-hypertensive	39
Chronic renal failure	21	- Calcium channel blocker	11
Diabetes Mellitus	20	- ACEI or ARB	17
Dyslipidemia	15	- β-blocker	16
Congestive heart failure	12	- Hydralazine	4
HIV infection	9	Furosemide	24
Mycobacterial infection	6	Spironolactone	10
Cirrhosis	6	Hydrochlorothiazide	5
Gout	6	Aspirin	16
Chronic HBV infection	5	Statin	15
Chronic HCV infection	4	Allopurinol	12
Malignancies	4	Omeprazole	12

HIV = human immunodeficiency virus, HBV = hepatitis B virus, HCV = hepatitis C virus, ACEI = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker

Table 3. Prevalence of ANA and anti-dsDNA among SLE, HC, and MMP patients

	SLE % (n = 100)	HC % (n = 100)	MMP patients % (n = 100)
ANA positive			
Mode (min, max)	1280 (0,1280)	0 (0,1280)	0 (0, 1280)
ANA positive ≥ 1:80			
Positive ≥ 1:80	98	8	12
Positive ≥ 1:160	90	4	6
Anti-ds DNA positive			
Mode (min, max)	80 (10, 1280)	0	[10,20,40]*
Positive	37	0	3

* Three MMP patients had positive anti-dsDNA at a titer of 1:10, 1:20 and 1:40.

producing autoantibodies possibly existing in SLE patients.[6,20] ANA and anti-dsDNA can be positive for years before clinical symptoms of SLE develop.[21] Therefore, long term follow up should be made in those HCs who had a significant titer of ANA, in order to determine the possibility of connective tissue disease development in the future. In one study, up to 18.5% of healthy individuals, with a high titer of ANA, were found to develop some form of connective tissue disease in a mean follow up of 11 years.[22]

Twelve percent of the MMP patients in this study had a positive ANA test at a titer of ≥ 1:80, and 6% had a titer of ≥ 1:160 (four of them had a titer of ≥ 1:320, in which one had a titer of 1:1280). The presence of ANA in patients with medical problems, particularly in the elderly, has been well recognized.[2,7] MMP patients in this study also had a significantly higher age than that of SLE patients and HC subjects. The presence of a rather high ANA titer in MMP patients could cause diagnostic problems, especially when physicians are unaware of ANA limitations and do not follow the definition of SLE diagnostic criteria strictly. These issues could cause over diagnosis of SLE. In contrast, anti-dsDNA was found in three cases of MMP patients, giving specificity of 97% for the diagnosis of SLE. These findings confirmed that anti-dsDNA is highly specific to the diagnosis of SLE, even in the MMP population. Therefore, the presence of anti-dsDNA in MMP patients should alert physicians to possible SLE and they should try to search for other criteria for a definite diagnosis.

In this study, the prevalence of positive anti-dsDNA in SLE patients was 37%, which was lower than previous reports (60-80%).[15,23,24] Anti-dsDNA had been shown to correlate with active renal involvement and exacerbation of the disease, particularly early in the course of SLE.[25] An increase in the level of anti-dsDNA is often associated with renal flare; and the level of antibody declines during clinical inactivity or remission. The reasons for the rather low prevalence of anti-dsDNA in this study might be because all of the population had a long disease duration (mean 7.3 years), and the majority did not have active nephritis at the time of the study.

Most MMP patients received much medication for treating their underlying diseases. Many drugs are reported to produce ANA and a lupus-like syndrome, and have been reviewed extensively.[26,27] Hydralazine, procainamide, chlorpromazine and quinidine are among the medicines with a commonly strong association with drug-induced lupus like syndrome. Patients with a drug-induced lupus syndrome have positive ANA and anti-histone antibodies in 90-95% of cases. However, only 6-10% of the patients who received hydralazine developed clinical lupus syndrome.[28,29] Higher daily doses of hydralazine, slow acetylator type, and the

Table 4. Details of ANA titer and pattern and occupations of HC who were positive for ANA

Case	Age/Sex	ANA titer	ANA pattern	Occupations
1	47/F	1:80	FS	Nurse
2	23/F	1:80	Nu	Laboratory technician
3	30/M	1:80	Nu	Worker
4	68/M	1:80	CS	Healthy elderly
5	29/F	1:160	FS + Nu	Nurse
6	72/F	1:320	Ho	Healthy elderly
7	73/F	1:640	Ho	Healthy elderly
8	34/F	1:1280	Fs	Secretary

FS = fine speckle, CS = coarse speckle, Ho = homogeneous, P = peripheral, Nu = nucleolar.

presence of the HLADRw4 phenotype are risk factors for the development of hydralazine induced lupus like syndrome.[28] Three of four (75%) patients in this study, who received hydralazine, were positive for ANA. All had been taking hydralazine for a long duration, but none of them had clinical signs or symptoms that were suggestive of SLE. Unfortunately, there was no opportunity to follow these patients in order to see if they developed lupus syndrome later.

The presence of ANA in patients with malignancies has been well described, with a prevalence of 5-55%, depending on the malignancy type.[2,30,31] The presence of ANA in these patients might reflect an increase in incidence with aging, or a dysregulation of the immune system.[31] One in four

Table 5. Details of ANA titer and pattern, underlying diseases and current medication used in MMP patients who were positive for ANA

Case	Age/Sex	ANA titer	ANA pattern	Underlying disease	Current medication
1	65/M	1:80	FS	Gout, rheumatic heart disease (post valvular replacement), CKD	Metropolol, furosemide, warfarin, hydralazine, isosorbide dinitrate, erythropoietin
2	56/M	1:80	CS	Cirrhosis, hepatoma, HCV	Omeprazole, spironolactone, furosemide, propanolol
3	72/M	1:80	NA	HT, CAD, CHF, COPD, gout	Isosorbide dinitrate, aspirin, simvastatin, furosemide, theophylline, enalapril, allopurinol
4	64/M	1:80	CS	Gout, rheumatic heart disease, CKD, CVD	Warfarin, atenolol, furosemide
5	88/M	1:80	FS + Nu	MDS, HT, BPH, CAD, CKD	Esomeprazole, carvedilol, manidipine, isosorbide dinitrate, hydralazine, artorvastatin, erythropoietin
6	48/F	1:80	FS	Cirrhosis, HBV, CKD	Vitamin B1-6-12, folic acid
7	48/F	1:160*	P	MDS, CKD	Folic acid, lorazepam, ferous sulfate, vitamin B complex
8	66/M	1:160	FS	DCM, loculated pleural effusion, hemoptysis, atrial fibrillation	Digoxin, furosemide, losartan, isosorbide dinitrate
9	54/M	1:320*	Ho + Nu	Steven Johnson's syndrome, CVD, HT, dyslipidemia	Simvastatin, sodium valproate, amlodipine
10	65/F	1:320	CS	HT, DM, dyslipidemia, gout, CAD	Aspirin, furosemide, bisoprolol, spironolactone, isosorbide dinitrate, hydralazine
11	59/M	1:640	FS	Hepatoma, cirrhosis, HBV	Lamivudine, silymarin
12	72/F	1:1280*	Ho	HT, CAD, CKD, CHF	Carvedilol, simvastatin, hydralazine, warfarin, furosemide, manidipine, isosorbide dinitrate, allopurinol

* = also had positive anti-dsDNA test. FS = fine speckle, CS = coarse speckle, Ho = homogeneous, P = peripheral, Nu = nucleolar. NA = not available in the report.

ARF = acute renal failure, BPH = benign prostatic hypertrophy, CAD = coronary artery disease, CHF = congestive heart failure, CKD = chronic kidney disease, COPD = chronic obstructive pulmonary disease, CVD = cerebrovascular disease, DCM = dilated cardiomyopathy, DM = diabetes mellitus, HBV = hepatitis B virus infection, HCV = hepatitis C virus infection, HT = hypertension, MDS = myelodysplastic syndrome

Table 6. Sensitivity and specificity of ANA, anti-dsDNA and their 95% CIs in patients with SLE using sera from HC and MMP patients

	ANA ≥ 1:80	ANA ≥ 1:160	anti-dsDNA
Sensitivity	98	90	37
	(92.3-99.7)*	(82.0-94.8)	(27.7-47.3)
Specificity			
HC	92	96	100
	(84.4-96.2)	(89.5-98.7)	(95.4-100.0)
MMP patients	88	94	97
	(79.6-93.4)	(86.9-97.5)	(90.9-99.2)

Data are expressed as %, *(-) = 95% CI

(25%) of the patients with malignancies in this study had a positive ANA test at a titer of 1:640. As patients with malignancies can have signs and symptoms that suggest rheumatic or connective tissue diseases (or paraneoplastic syndrome), the presence of ANA, and to a lesser extent anti-dsDNA, also might lead to physicians diagnosing SLE or connective tissue disease and overlooking the possibility of underlying malignancies, particularly in elderly patients.

There were some limitations in this study. We did not determine the presence of specific autoantibodies in the HC and MMP patients who were positive for ANA at a high titer (≥ 1:160). Therefore, the presence of uncommon autoantibodies (e.g., anti-Ro, anti-La) could be missed. Also, we did not have the chance to follow HC and MMP patients who had positive ANA and anti-dsDNA tests to see if they developed certain connective tissue diseases.

In addition, the question of using MMP patients infected with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus, or *Mycobacterium tuberculosis,* as well as those who have chronic inflammatory disease with clinical symptoms suggesting SLE, as a control group, might be raised; as it might give more information in clinical practice; instead of using MMP patients from general medical services, who are without signs and symptoms of connective tissue diseases. The presence of ANA, and perhaps anti-dsDNA, in these patients has been well recognized.[32-35] Thus, the presence of ANA or anti-dsDNA in those who had clinical signs and symptoms suggesting SLE would create the question of whether the patients actually had SLE or some other form of connective tissue disease. Therefore, in this study we decided to use MMP patients selected randomly from the general medical service wards, as they might have taken drugs that could give a positive ANA and anti-sDNA test in addition to the presence of the antibody from the disease itself.

In conclusion, this study confirmed that ANA was found more commonly in MMP patients than HC ones. ANA testing was sensitive for screening SLE. Anti-dsDNA was rare in HC, and may be seen occasionally in patients with MMP. The specificity of anti-dsDNA in the diagnosis of SLE was high, even in the population of MMP subjects. However, physicians should be aware that anti-dsDNA could be positive in patients with MMP. They should be cautioned about the use of anti-dsDNA in support of SLE diagnosis in patients with MMP who did not have clinical signs or symptoms suggestive of SLE or connective tissue diseases.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgement

The study was supported in part by the Faculty of Medicine, Chiang Mai University Endowment Fund.

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