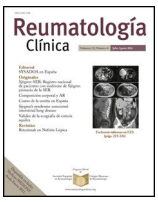
Reumatol Clin. 2017;13(3):150–155

Sociedad Española de Reumatología - Colegio Mexicano de Reumatología

Reumatología Clínica

www.reumatologiaclinica.org



Original article

Association Between Antinuclear Antibodies Titers and Connective Tissue Diseases in a Rheumatology Department☆

Raúl Menor Almagro,[a,*] Juan Francisco Rodríguez Gutiérrez,[b] María Auxiliadora Martín-Martínez,[c] María José Rodríguez Valls,[a] Concepción Aranda Valera,[a] José Luís de la Iglesia Salgado[a]

[a] *Sección de Reumatología, Hospital General de Jerez, Jerez de la Frontera, Spain*
[b] *Servicio de Hematología, Hospital General de Jerez, Jerez de la Frontera, Spain*
[c] *Unidad de Investigación, Sociedad Española de Reumatología, Madrid, Spain*

ARTICLE INFO

Article history:
Received 12 June 2015
Accepted 30 March 2016
Available online 7 April 2017

Keywords:
Antinuclear antibodies
Indirect immunofluorescence
Dilution titers
Systemic connective

ABSTRACT

Objective: To determine the dilution titles at antinuclear antibodies (ANA) by indirect immunofluorescence observed in cell substrate HEp-2 and its association with the diagnosis of systemic connective tissue disease in ANA test requested by a Rheumatology Unit.
Method: Samples of patients attended for the first time in the rheumatology unit, without prior ANA test, between January 2010 and December 2012 were selected. The dilution titers, immunofluorescence patterns and antigen specificity were recorded. In January 2015 the diagnosis of the patients were evaluated and classified in systemic disease connective tissue (systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, undifferentiated connective, antiphospholipid syndrome, mixed connective tissue and inflammatory myophaty) or not systemic disease connective tissue.
Result: A total of 1282 ANA tests requested by the Rheumatology Unit in subjects without previous study, 293 were positive, predominance of women (81.9%). Patients with systemic connective tissue disease were recorded 105, and 188 without systemic connective tissue disease. For 1/640 dilutions the positive predictive value in the connective was 73.3% compared to 26.6% of non-connective, and for values ≥1/1280 85% versus 15% respectively. When performing the multivariate analysis we observed a positive association between 1/320 dilution OR 3.069 (95% CI: 1.237–7.614; $P = .016$), 1/640 OR 12.570 (95% CI: 3.659–43.187; $P = .000$) and ≥ 1/1280 OR 42.136 (95% CI: 8.604–206.345; $P = .000$).
Conclusion: These results show association titles dilution ≥1/320 in ANA's first test requested by a Rheumatology Unit with patients with systemic connective tissue disease. The VPP in these patients was higher than previous studies requested by other medical specialties. This may indicate the importance of application of the test in a targeted way.

Asociación entre títulos de anticuerpos antinucleares y conectivopatías sistémicas en una Unidad de Reumatología

Palabras clave:
Anticuerpos antinucleares
Inmunofluorescencia indirecta
Títulos de dilución
Conectivopatías

RESUMEN

Objetivo: Determinar los niveles en los títulos de anticuerpos antinucleares (ANA) observados por inmunofluorescencia indirecta en sustrato de célula HEp-2, y su asociación con el diagnóstico de enfermedad del tejido conectivo sistémica en las pruebas solicitadas por una Unidad de Reumatología.
Método: Se seleccionaron muestras de pacientes que acudían por primera vez a consulta de reumatología, sin prueba de ANA previa, durante el periodo comprendido entre enero de 2010 y diciembre de 2012. Se registró el título de dilución, patrón y especificidad antigénica. En enero de 2015 se valoraron los

☆ Please cite this article as: Menor Almagro R, Rodríguez Gutiérrez JF, Martín-Martínez MA, Rodríguez Valls MJ, Aranda Valera C, de la Iglesia Salgado JL. Asociación entre títulos de anticuerpos antinucleares y conectivopatías sistémicas en una Unidad de Reumatología. Reumatol Clin. 2017;13:150–155.

* Corresponding author.
E-mail address: menoralmagro@hotmail.com (R. Menor Almagro).

diagnósticos de los pacientes y se clasificaron en conectivopatías sistémicas (lupus eritematoso sistémico, síndrome de Sjögren, esclerosis sistémica, conectivopatía indiferenciada, síndrome antifosfolípido, enfermedad mixta del tejido conectivo y miopatía inflamatoria) o no conectivopatía sistémica.
Resultado: De un total de 1.282 pruebas solicitadas por la Unidad de Reumatología en sujetos sin estudio previo 293 resultaron positivas, predominando las mujeres (81,9%). Con conectivopatía sistémica se registraron 105 pacientes y 188 sin conectivopatía. En diluciones 1/640 el valor predictivo positivo en las conectivopatías fue de 73,3% frente al 26,6% de las no conectivopatías, y para valores ≥1/1.280, 85% frente al 15% respectivamente. Al realizar el análisis multivariante se observó una asociación positiva entre las diluciones 1/320 OR 3,069 (IC 95%: 1,237-7,614; p = 0,016), 1/640 OR 12,570 (IC 95%: 3,659-43,187; p = 0,000) y ≥ 1/1.280 OR 42,136 (IC 95%: 8,604-206,345; p = 0,000).
Conclusión: Estos resultados muestran asociación de títulos de dilución ≥ 1/320 para la primera prueba de ANA realizada en una Unidad de Reumatología con pacientes con conectivopatía sistémica. El VPP en estos pacientes resultó superior a estudios previos desarrollados por otras especialidades médicas. Esto puede indicar la importancia de una solicitud de la prueba de forma dirigida.

Introduction

Antinuclear antibodies (ANA) are immunoglobulins directed against autologous components of the cell nucleus and cytoplasm.[1] Testing for ANA is highly important in the diagnosis of systemic and organ-specific autoimmune diseases. The results provide information on the clinical course and complications of the disease. In fact, ANA may be present years before any symptoms develop.[2]

The gold standard for the detection of ANA is indirect immunofluorescence (IIF).[3,4] Its usefulness has increased progressively since 1957, when fluorochrome-tagged antibodies were used to demonstrate that the serum of patients with systemic lupus erythematosus (SLE) contained antibodies that produced homogeneous nuclear fluorescence in human tissues.[5] At the present time, HEp-2 cells (from a human epithelial cell line obtained from laryngeal carcinoma) are used as a substrate, as they offer advantages over the substrates classically used (from rodent liver and kidney) as they express antigens present in all the phases of the cell cycle.[6]

Approximately 100 different autoantibodies have been detected, corresponding to more than 35 immunofluorescence patterns, some of which are specific antigens. The most common patterns are homogeneous, fine speckled, coarse speckled, nucleolar, cytoplasmic and centromere. The less frequent patterns include pleomorphic, multiple nuclear dots (MND), centrosomal, nuclear mitotic apparatus (NuMA) and nuclear membrane, although it is not uncommon to identify different autoantibodies in the serum of certain patients, which results in a mixed pattern.[1] The titer of ANA is obtained after serial dilutions, with a phosphate regulator, of patient serum that binds to the HEp-2 cell substrate, to which we added a polyclonal antibody against fluorescein-labeled human immunoglobulins.[7] Although each laboratory should establish its own cutoff point, it is recommended that that the screening test be done with a dilution >1:160.[8,9]

One of the great limitations to IIF is the lack of specificity, with a low positive predictive value (PPV). In the general population, the ANA test is positive in up to 25%–30% of those who undergo it, according to different studies, mostly at low titers, although 5% of those tested may have titers >1:160.[10,11] The results are more likely to be positive among individuals over 65 years (with female predominance) and in patients with infections, paraneoplastic neurological syndrome, liver diseases, chronic fatigue syndrome or neoplasms.[1,12] The negative predictive value of the test is high for certain connective tissue diseases, which include SLE, Sjögren's syndrome, systemic sclerosis and inflammatory myopathy. At the present time, it is a standard technique in immunological laboratories and enables us to predict, diagnose and determine the activity of a large number of diseases.[2,8–10]

For this reason, we proposed to study the ANA titer levels established by IIF in the first sample collected from patients in the rheumatology department of our hospital, and to determine their association with the diagnosis of systemic connective tissue diseases, as well as the different patterns observed and the antigenic specificities.

Material and Methods

Design

We describe a prospective study of blood samples from patients referred to a secondary level hospital to determine the association between ANA titers and the development of systemic connective tissue diseases.

Patients and Samples

Our report was based on the results of samples received in the immunology unit of the hematology service of the Hospital de Jerez, located in southern Spain. The samples, which had been requested by the rheumatology department, were collected between January 2010 and December 2012 from individuals with no clinical diagnosis who had never undergone an ANA test. Our hospital is a secondary care center with 550 beds. The rheumatology department, staffed by 6 rheumatologists, covers a population of 450,000 persons, with an average outpatient workload of around 1550 visits each month.

Laboratory Tests

The first samples were collected to determine the positive and negative results in the test. The analysis consisted of IIF using the HEp-2 cell line (Euroimmun, Germany) as substrate, and the titer level and pattern were recorded. For those patients with a titer higher than 1:40, dilutions of 1:80, 1:160, 1:320, 1:640 and 1:1280 were performed. When the sample had positive mitoses in HEp-2 cells, a screening test was carried out to check for the presence of anti-double-stranded (ds) DNA antibodies, using IIF on *Crithidia luciliae* (Euroimmun, Germany). If the latter was positive, the titer was determined by chemoluminescence (Menarini, Italy). On the other hand, in all the cases of positive ANA, we used an extractable nuclear antigen (ENA) immunoblot test, the EUROLINE ANA Profile 5 (IgG) commercial kit, (Euroimmun, Germany). This kit provides a qualitative in vitro assay for human immunoglobulin (Ig) G antibodies against 18 different antigens: ribonucleoprotein (RNP)-70, RNP-A, RNP-C, Ro52, Ro60 La/SSB, Scl-70, Smith (Sm), U1-nRNP complex, nucleosome, Jo-1, Pm-Scl, centromere protein

Table 1
Sensitivity and Specificity of the Major Antibodies Determined by Immunoblotting Using the EUROLINE ANA Kit From Euroimmun (Germany).

Antibody	Sensitivity (%)	Specificity (%)
Anti-RNP	100	98
Anti-Sm	93	99
Anti-SSA	90	99
Anti-Ro52	97	92
Anti-La	68	100
Anti-Scl70	99	99
Anti-Jo1	77	100
Anticentromere	91	99
Antiribosomal P protein	95	98
Anti-dsDNA	89.3	98.2

ANA, antinuclear antibodies; ds, double-stranded; RNP, ribonucleoprotein; Sm, Smith.

B (CENP B), proliferating cell nuclear antigen (PCNA), dsDNA, histones, ribosomal P protein and Mi-2 in serum or plasma (sensitivity and specificity of the major antigens shown can be seen in Table 1). The immunological tests were analyzed by a single immunologist trained in IIF and in enzyme immunoassay tests.

Variables and Operational Definitions

We recorded the sociodemographic variables (age and sex) of patients with a positive result in their first test, as well as the diagnosis, the dilution titer, IIF pattern and antigenic specificities.

The clinical diagnosis of the patients was reviewed in February 2015, at least 2 years after the analytical evidence of the disease. This was done using Diraya, the clinical health record software program utilized in the health system of Andalusia, the Spanish autonomous community in which our hospital is located. The patients were classified into two groups depending on whether or not they had developed a systemic connective tissue disease. The classification of connective tissue diseases was based on an update of the standard approach of the American College of Rheumatology, which is used in many of the guidebooks regarding our specialty.[13] We considered SLE, Sjögren's syndrome, systemic sclerosis, undifferentiated connective tissue disease, antiphospholipid syndrome, mixed connective tissue disease and inflammatory myopathy to be systemic connective tissue diseases. This information was recorded retrospectively by 4 rheumatologists of the rheumatology department of the Hospital de Jerez, who utilized the same definitions and were trained in the collection of data from health records.

Statistical Analysis

We used a descriptive analysis of the categorical variables, expressed in absolute and relative frequencies, and of the continuous variables with a normal distribution in terms of the mean and standard deviation, whereas those that did not have a normal distribution are shown as medians and interquartile range (IQR: 25th–75th percentile). Age was analyzed as a continuous variable, and as a categorical variable, using 65 years as the cutoff point. The Kolmogorov–Smirnov test was used to check for normality.

The analysis was stratified according to the presence or absence of a clinical diagnosis of systemic connective tissue disease. The chi-square test was utilized to compare the groups, using 2×2 tables, in terms of the categorical variables, like the types of IIF patterns and the different antibodies. The mean age in each group was calculated with Student's *t* test.

To analyze the association between ANA titers and the development of a systemic connective tissue disease, we constructed a logistic regression model to determine the odds ratio (OR), together with the 95% confidence interval (95% CI) adjusted for potential confounding factors. The dependent variable was connective tissue disease (yes/no) and the independent variables were selected depending on the clinical and statistical criteria from those with a $P < .20$ in the bivariable analysis. The final model was adjusted for all the potential confounding variables, after the multicollinearity among them had been explored. The accuracy of the model was analyzed using the area under the receiver operating characteristic (ROC) curve.

All of the tests were performed using the SPSS statistical software package (version 16). A $P < .05$ was considered to indicate statistical significance.

Results

The immunology unit received a total 9478 samples during that period. In all, 25.5% (n = 2426) were requested by the rheumatology department; positive results were obtained in 39.7% (n = 965), which corresponded to 576 patients (85% women).

The samples included those of 1282 patients who were being examined in the rheumatology department for the first time, and had not undergone a previous ANA test. There were 293 (22.8%) in which the result was positive, with a predominance of women (81.9%) and a mean age of 47.02 ± 16.7 years; 247 patients (84.8%) were under 65 years.

In the distribution according to the disorders corresponding to these first positive samples in our patients, 105 (35.9%) were classified in the group of systemic connective tissue diseases and 188 individuals (64.1%) did not have these conditions. In the group of systemic connective tissue diseases, the most common finding was SLE, diagnosed in 38 patients, with a PPV of 12.9%, followed by Sjögren's syndrome detected in 29 (PPV 9.8%), systemic sclerosis in 17 (PPV 5.8%) and undifferentiated connective tissue disease in 14 (PPV 4.7%). In the group of patients without systemic connective tissue diseases, the most frequent diagnoses were 26 cases of rheumatoid arthritis (associated with Sjögren's syndrome in 2), 14 patients with spondyloarthropathies (10 were psoriatic arthropathies), 13 with fibromyalgia, 10 with osteoarthritis, 9 with discoid lupus and 5 with microcrystalline arthritis. When these findings were reviewed, 10 patients were being studied and 30 had no rheumatic disease.

The pattern most frequently observed in patients in the group of connective tissue diseases without systematic involvement was homogeneous (44.6%), followed by fine speckled (34.6%), and in patients with systemic connective tissue diseases, they were fine speckled (44.7%) and homogeneous (28.5%). We had no patients with coarse speckled or PCNA. The most common antigenic specificities were anti-Ro52, anti-Ro60, anti-dsDNA and anti-La, without positivity for topoisomerase, anti-Jo1 or smooth muscle (Table 2).

In the analysis of the PPV of the different disorders according to their titration, we observed a higher value in patients with a diagnosis of systemic connective tissue diseases. Thus, for titers of 1:640, the group of connective tissue diseases had a PPV of 73.3%, versus 26.6% for those that did not connective tissue diseases, and for a titer $\geq$1:1280, a PPV of 85% and 15%, respectively, differences that persisted when the patients were classified according to age (Table 3).

In the multivariate analysis, we observed a positive association between the systemic connective tissue diseases and the dilutions of 1:320, OR 3.069 (95% CI: 1.237–7.614; $P = .016$); 1:640, OR 12.570 (95% CI: 3.659–43.187; $P = .000$); and $\geq$1:1280 OR 42.136 (95% CI: 8.604–206.345; $P = .000$) (Table 4). The discriminatory power of the diagnostic test using the area under the ROC curve was 0.749.

Discussion

The findings obtained in this study demonstrate the association between the presence of rheumatic disorders classified as systemic

Table 2
Demographic and Immunological Characteristics of the Patients Included in the Study.

	Connective tissue diseases without systemic involvement		Systemic connective tissue diseases		
	n	PPV (%)	n	PPV (%)	*P*
Patients	188	64.10	105	35.80	
Mean age, years	46.5 (SD ± 18.2)		47.9 (SD ± 13.8)		.455
<65	154	62.30	93	37.60	
≥65	32	72.70	12	27.20	
Sex					
Women	146	60.80	94	39.10	.011
Men	42	79.20	11	20.70	
IIF pattern					
Homogeneous	84	73.6	30	26.4	.007
Fine speckled	65	58.0	47	42	.85
Cytoplasmic	9	75.0	3	25	.424
Nucleolar	18	69.2	8	30.8	.572
Centromere	6	37.5	10	62.5	.022
Nuclear dots	3	50.0	3	50	.465
Nuclear membrane	1	25.0	3	75	.1
Mixed	1	50.0	1	50	.675
Cell cycle	1	100	0	0	.454
Antibodies					
Anti-Ro52	4	10	40	90	0
Anti-Ro60	1	2.8	34	23.7	0
Anti-La	0	0	14	100	0
Anti-DNA	3	17.6	14	82.4	0
Anti-Sm B	0	0	5	100	.002
Anti-Sm D	0	0	3	100	.19
Anti-RNP 70	0	0	6	100	.001
Anti-RNP-A	0	0	6	100	.001
Anti-RNP-C	0	0	6	100	.001
Anti-ribosomal P protein	0	0	1	100	.456
Anticentromere	4	50	8	50	.022
Anti-histone	1	50	2	50	.259
Anti-AMA	1	33.3	3	66.6	.098
Anti-Mi-2	0	0	1	100	.18

AMA, antimitochondrial antibodies; IIF, indirect immunofluorescence; PPV, positive predictive value; RNP, ribonucleoprotein; SD, standard deviation; Sm, Smith.

connective tissue diseases and higher titers in a positive ANA test from the time of the first analysis when compared with those of patients who were free of these diseases.

The utility of this test in connective tissue diseases and the healthy population has been extensively studied, and was established in recent guidelines for its use.[14,15] However, there are few studies carried out exclusively with patients evaluated in a rheumatology department who had not undergone this immunological test.[16] Thus, in our cohort, we present the clinical and immunological data associated with a positive ANA test using the

Table 3
Number of Patients and Positive Predictive Value of the Dilution Titers of the Major Diseases Dealt With in the Study.

Dilution using IIF	Systemic connective tissue diseases						Connective tissue diseases without systemic involvement						Total
	>65 years		<65 years		Total for connective tissue diseases		>65 years		<65 years		Total for diseases without systemic involvement		*n*
	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)	
1:80	3	4	8	10.8	11	14.8	13	17.5	50	67.5	63	85.1	74
1:160	1	0.97	25	24.2	26	25.2	15	14.5	62	60.1	77	74.7	103
1:320	4	6.2	25	39	29	45.3	3	4.6	32	50	35	54.6	64
1:640	4	13.3	18	60	22	73.3	1	3.3	7	23.3	8	26.6	30
≥1:1280	0	0	17	85	17	85	0	0	3	15	3	15	20

	SLE		Sjögren's syndrome		Systemic sclerosis		Undifferentiated connective tissue disease		RA		Fibromyalgia		Osteoarthritis	
	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)	n	PPV (%)
1:80	3	4	0	0	2	2.7	4	5.4	9	12.1	4	5.4	5	6.7
1:160	9	8.7	8	7.7	4	3.8	3	2.9	11	10.6	4	3.8	4	3.8
1:320	12	18.7	10	15.6	2	3.1	4	6.2	3	4.6	5	7.8	1	1.5
1:640	8	26.6	6	20	7	23.3	0	0	1	3.3	0	0	0	0
≥1:1280	6	30	5	25	2	10	3	15	2	10	0	0	0	0

IIF, indirect immunofluorescence; PPV, positive predictive value; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Table 4
Multivariate Analysis of the Dilution Titers Established in Indirect Immunofluorescence for Patients With and without Systemic Connective Tissue Disease, Adjusted for Potential Confounding Variables.

Variables	Crude OR	Adjusted OR	*P*
Age	1.005 (0.991–1.020)	0.998 (0.979–1.018)	.872
Sex (using men as reference)	2.458 (1.205–5.013)	4.124 (1.555–10.932)	.004
ANA titer (using 1:80 as reference)			
ANA titer 1:160	1.96 (0.90–4.28)	1.460 (0.621–3.443)	.386
ANA titer 1:320	4.68 (2.09–10.48)	3.069 (1.237–7.614)	.016
ANA titer 1:640	16.00 (5.70–44.88)	12.570 (3.659–43.187)	.000
ANA titer ≥1:1280	32.97 (8.26–131.58)	42.136 (8.604–206.345)	.000
Fine speckled pattern	1.533 (0.941–2.498)	0.936 (0.460–1.907)	.856
Nucleolar pattern	0.779 (0.327–1.858)	1.121 (0.386–3.260)	.834
Centromere pattern	3.193 (1.126–9.052)	0.657 (0.141–3.068)	.594
Anti-Ro52	28.750 (9.897–83.520)	27.380 (8.250–90.869)	.000

ANA, antinuclear antibodies; OR: odds ratio.

first samples requested by a rheumatology department of a specialty hospital in Andalusia.

Among the diagnosis observed, we found that 158 patients (56.6%) with no systemic connective tissue disorders had a positive result on this first ANA test; they included 30 persons with no rheumatic disease. A number of reports involving populations other than that dealt with in our study have shown a considerable proportion of positive results in healthy individuals.[8,17,18] This could be due to two major reasons: the large number of persons presenting with these conditions who are seen in the rheumatology departments in Spain, where connective tissue disorders represent only 19% of the cases reviewed in the outpatient clinic,[19] and the insidious onset of rheumatic diseases, which can, at the start, mimic connective tissue diseases, with the known positive test result in conditions like psoriatic arthropathy, rheumatoid arthritis and fibromyalgia.[2,20–22] Of the patients being seen in the rheumatology department for the first time, as many as 989 (77.1%) of those who underwent the ANA test had a negative result. This outcome may be surprising for a series of tests requested by rheumatology specialists. However, in a review of the literature, our finding (a positive result for 28.1% of the samples) is even slightly greater than that of studies performed in rheumatology departments of secondary and tertiary hospitals, which show data on positive ANA tests of nearly 15% and 20%, respectively, and even higher if they are compared with other medical specialties.[23]

Concerning the titers of the dilutions for the ANA test that correspond to rheumatic diseases not classified as systemic connective tissue disorders, and those observed in healthy individuals, they are lower than in those patients with systemic connective tissue diseases.[8,18,24] In the different studies carried out on the utility of the ANA test in the general population, a dilution >1:160 is the value at which there begins to be a greater frequency in the association with these connective tissue diseases with multiorgan involvement.[2,25,26] In the analysis of our results, we found an association between dilutions >1:320 and the systemic connective tissue disorders in our patient population, values that increased markedly with higher dilutions (1:640 and >1:1280). Thus, for our series, we established, a greater probability of developing a systemic connective tissue disease in those patients with higher dilutions from the moment of their first visit. These values constitute a difference with rheumatoid arthritis, an autoimmune disease in which the greatest number of patients are diagnosed with positive samples at titers <1:160.

In a comparison of this level of titers for the ANA test in our rheumatology department with previous studies done for other medical specialties, a notably higher PPV is shown for connective tissue diseases, both for lower dilutions (>1:80 = 36% vs 10%) and higher dilutions (>1:1280 = 85% vs 38.9%). These differences are even greater in a substudy on SLE, in which the PPV is 12.9% in a dilution >1:80 and 30% for >1:1280 (vs 2.2% and 5.6%, respectively, in a study undertaken by professionals of other specialties).[25]

Age is also an important factor in the positive result of the test. In our work, among the group of systemic connective tissue diseases, we found a higher PPV among individuals of less than 65 years, since the presentation of these conditions is most frequent during the fourth and fifth decades of life. However, this is not the case in connective tissue disorders without systemic involvement, and if we classify the patients according to age group, we do not observe a higher rate of positive test results for those over 65 years, as would be expected due to the well-known increase in prevalence of a positive test in individuals with an advanced age who do not have a connective tissue disease.[2,27] Moreover, it is in patients over 65 years of age without systemic involvement where the highest dilutions are recorded,[1] a finding that is not reported in our study, in which for that age group, only 3 patients had dilutions of 1:320 (PPV 4.6%) and 1 with 1:640 (PPV 3.3%), and none of them had higher dilutions.

Although previous studies have associated a greater clinical significance with homogeneous, centromere and cytoplasmic patterns, in our patients with connective tissue diseases the most widespread pattern was fine speckled. The PPV of individuals with the homogeneous and cytoplasmic pattern was considerably lower than in the group without connective tissue disorders.[20] There was a predominance of the centromere pattern with a PPV of 62.5%, which corresponded to 7 cases of systemic sclerosis, 1 of SLE and 2 of undifferentiated connective tissue disorders. Moreover, in contrast to recent reports that show a higher prevalence of the fine speckled pattern among persons who do not have diseases of this type, our data show a higher number of individuals with a homogeneous pattern in connective tissue diseases without systemic involvement, with a PPV of 58% versus 41.9% for systemic connective tissue disorders.[1,28,29]

In reference to autoantibodies, the data do correspond to previous studies.[1,16,23] The highest frequency is associated with anti-Ro52, anti-Ro60, anti-dsDNA and anti-La, with positive samples in 44, 35, 17 and 15 patients, respectively. They all predominantly expressed the fine speckled pattern, and in systemic connective tissue diseases, with a markedly significant difference between the two groups. We should point out that a positive result for this pattern when linked to anti-Ro52 may be influenced by its association with anti-Ro60 and/or anti-La, as when detected in isolation its pattern is usually cytoplasmic.[30] Finally, anti-dsDNA antibodies can sometimes be found in patients with other autoimmune or infectious diseases or in individuals with no clinical symptoms, although 85% of the latter group may develop SLE during the 5 years after being found positive for dsDNA.[31] In our series, we found 3 patients with positive results (1 with a titer of 1:320 and 2 with 1:640), who were still being studied and no

disease had been detected to date. Their course should be the subject of a thorough study.

In conclusion, all of these findings show an increment in the frequency of ANA at high titers observed in IIF from the time of the first test sample, indicating a systemic connective tissue disease with, as opposed to connective tissue diseases without systemic involvement, establishing this association for dilutions >1:320. The PPV of our patients with systemic involvement of the connective tissue is higher than in previous studies requested by professionals from other medical specialties, since a targeted examination and the support of other ancillary examinations requested by rheumatology departments lead to a more focalized analysis of the test. Although in previous studies, 3 years after a positive test result are sufficient for defining the disease in terms of whether or not it is a systemic disorder involving the connective tissue, our work will be easier in a later analysis of the diagnoses in those individuals who, classified as free of a systemic connective tissue disease, present a high dilution in the ANA titer. Studies with a larger number of participants would increase the reliability of the analysis according to age group and specific disorder.

Ethical Disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of Interest

The authors declare they have no conflicts of interest.

References

1. Cabiedes J, Núñez-Álvarez CA. Anticuerpos antinucleares. Reumatol Clin. 2010;6:224–30.
2. O'Sullivan M, McLean-Tooke A, Loh RK. Antinuclear antibody test. Aust Fam Physician. 2013;42:718–21.
3. Agmon-Levin N1, Damoiseaux J, Kallenberg C, Sack U, Witte T, Herold M, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis. 2014;73:17–23.
4. Krause C, Ens K, Fechner K, Voigt J, Fraune J, Rohwäder E, et al. EUROPattern Suite technology for computer-aided immunofluorescence microscopy in autoantibody diagnostics. Lupus. 2015;24:516–29.
5. Holborow EJ, Weir DM, Johnson GD. Aserum factor in lupus erythematosus with affinity for tissue nuclei. Br Med J. 1957;2:732–4.
6. Op de Beeck K, Vermeersch P, Verschueren P, Westhovens R, Mariën G, Blockmans D, et al. Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. Autoimmun Rev. 2011;10:801–8.
7. Campos-González ID, Viveros ME, Cardiel MH. Utilidad clínica de las pruebas inmunológicas especializadas en reumatología en un hospital de segundo nivel de atención en México. Reumatol Clin. 2007;3:110–6.
8. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in healthy individuals. Arthritis Rheum. 1997;40:1601–11.
9. Keren DF. Antinuclear antibody testing. Clin Lab Med. 2002;22:447–74.
10. Volkmann ER, Taylor M, Ben-Artzi A. Using the antinuclear antibody test to diagnose rheumatic diseases: when does a positive test warrant further investigation? South Med J. 2012;105:100–4.
11. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis. 2010;69:1420–2.
12. Wanchu A. Antinuclear antibodies: clinical applications. J Postgrad Med. 2000;46:144–8.
13. Manual SER de las enfermedades reumáticas. 6.ª ed. España: Elsevier SL; 2014.
14. Solomon DH, Kavanaugh AJ, Schur PH. Evidence based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum. 2002;47:434–44.
15. Benito-Garcia E, Schur PH, Lahita R. Guidelines for immunologic laboratory testing in rheumatic diseases: anti-Sm and anti-RNP antibodies test. Arthritis Rheum. 2004;51:1030–44.
16. Fitch-Rogalsky C, Steber W, Mahler M, Lupton T, Martin L, Barr SG, et al. Clinical and serological features of patients referred through a rheumatology triage system because of positive antinuclear antibodies. PLoS One. 2014;9:e93812.
17. Rosas I, Gómez EI, Núñez Álvarez CA, Huerta MT, Alvarado A, Cabiedes J. Prevalencia de anticuerpos antinucleares (ANA) en donadores sanos del Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Rev Mex Reumatol. 2005;20:72.
18. Marin GG, Cardiel MH, Cornejo H, Viveros ME. Prevalence of antinuclear antibodies in 3 groups of healthy individuals: blood donors, hospital personnel and relatives of patients with autoinmune diseases. J Clin Rheumatol. 2009;15:325–9.
19. Rodríguez Gómez M, Gómez-Reino J, Galdo Fernández F, González-Gay M, Hernández Rodríguez I, Ibáñez Ruán JJ, Grupo Gallego de Estudio Epidemiológico de Enfermedades Reumáticas. Actividad asistencial en las consultas externas de las unidades de reumatología de Galicia. Reumatol Clin. 2006;2:239–46.
20. Nishimura S, Nishiya K, Hisakawa N, Chikazawa H, Ookubo S, Nakatani K, et al. Positivity for antinuclear antibody in patients with advanced rheumatoid arthritis. Acta Med Okayama. 1996;50:261–5.
21. Kötter I, Neuscheler D, Günaydin I, Wernet D, Klein R. Is there a predisposition for the development of autoimmune diseases in patients with fibromyalgia? Retrospective analysis with long term follow-up. Rheumatol Int. 2007;27:1031–9.
22. Calzavara PG, Cattaneo R, Franceschini F, Tosoni C, Martinelli M, Carlino A. Antinuclear antibodies in psoriatic arthritis and its subgroups. Acta Derm Venereol Suppl (Stockh). 1989;146:31–2.
23. Avery TY, van de Cruys M, Austen J, Stals F, Damoiseaux JG. Anti-nuclear antibodies in daily clinical practice: prevalence in primary, secondary, and tertiary care. J Immunol Res. 2014;2014:401739.
24. Giannouli E, Chatzidimitriou D, Gerou S, Gavriilaki E, Settas L, Diza E. Frequency and specificity of antibodies against nuclear and cytoplasmic antigens in healthy individuals by classic and new methods. Clin Rheumatol. 2013;32:1541–6.
25. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. Am J Med. 2013;126:342–8.
26. Satoh M, Vázquez-del Mercado M, Chan EK. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. Mod Rheumatol. 2009;19:219–28.
27. Ocaña Medina C, García Hernández F, del Castillo Palma MJ, Wichmann I, Respaldiza N, Sánchez Román J. Frecuencia de anticuerpos antinucleares en ancianos sanos. Rev Esp Reumatol. 2004;31:368–71.
28. Mahler M, Fritzler MJ. The clinical significance of the dense fine speckled immunofluorescence pattern on HEp-2 cells for the diagnosis of systemic autoimmune diseases. Clin Dev Immunol. 2012;2012:494356.
29. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum. 2011;63:191–200.
30. Keech CL, Gordon TP, McClustey J. Cytoplasmic acumulation of the 52 kDa Ro/SSA-A nuclear autoantigen in transfected cell lines. J Autoimmun. 1995;8:699–712.
31. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. Ann Rheum Dis. 2004;63:386–94.