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Predictive value of antinuclear antibodies in autoimmune diseases classified by clinical criteria: Analytical study in a specialized health institute, one year follow-up

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ABSTRACT

Introduction: Determination of antinuclear antibodies (ANA) by indirect immunofluorescence (IIF) is usually the initial test for the diagnosis of systemic rheumatic diseases (SRD). Assigning predictive values to positive and negative results of the test is vital because lack of knowledge about ANAs and their usefulness in classification criteria of SRD leads to inappropriate use. *Methods*: Retrospective study, ANA tests requested by different specialties, correlation to patients' final diagnosis. *Results*: The prevalence of autoimmune disease was relatively low in our population yielding a low PPV and a high NPV for the ANA test. 40% of the patients had no clinical criteria applied prior to test. Coexistence of two or more autoimmune disorders affects prevalence and predictive values. *Conclusion*: Application of the test after careful evaluation for clinical criteria remarkably improves the positive likelihood ratio for the diagnosis.

1. Introduction

Immunological assays for the detection of antinuclear antibodies (ANA) are useful and important complementary tools for the diagnosis and follow-up of patients with autoimmune diseases [1]. The identification of the antigen–antibody coupling is the common end-point for all techniques; however, several differences exist as for the utility, sensitivity, specificity, and predictive values of each test [1,2].

In general, if a patient presents clinical manifestations of an autoimmune disease, the first test to be requested is ANA detection by indirect immunofluorescence using HEp-2 cells, due to its great sensitivity [1,3]. The different possible patterns, the intensity, and the titers obtained by consecutive dilutions must be carefully examined. Identification of the antigens recognized by the ANA is further evaluated by more specific tests such as ELISA, radioimmunoanalysis (RIA) or electroimmunotransference (EIT) [2,4].

The use of these tests requires knowledge of their fundamental aspects and also of the clinical classification criteria of each disorder in order to contribute to an appropriate diagnosis [5,6].

The usefulness of this testing has been evaluated in retrospective studies of patients with systemic rheumatic disease (SRD), and it has been proven that its positive predictive value is low due to the relatively large amount of false positive results. For specific rheumatic diseases, the ANA test yields a positive predictive value of 11%, a negative predictive value of 97%, and a sensitivity and specificity of 42% and 85% respectively [7].

Several physiological and pathological factors might favor the development of ANA in the non-rheumatic population, such as pregnancy, advanced age, family history of autoimmune disease, as well as infectious, cardiovascular or oncological diseases [8–12]. This situation conveys challenges such as interpretative standardization [13].

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Table 1
Percentage of positive ANA requested by several medical specialties and correlation with the use of clinical classification criteria.

Department	Total of tests requested (%)	Used clinical criteria	Antibodies in 1:40 dilution	Did not use clinical criteria	With specific antigen	With confirmed AD 213	With autoimmune disease (AD) 213			Without AD 160	Without autoimmune disease 160			
							UCCBR and Sp (+)	UCCBR and Sp (−)	NUCBR and Sp (+)	NUCBR and Sp (−)	UCCBR and Sp (+)	UCCBR and Sp (-)	NUCBR and Sp (+)	NUCBR and (Sp (−)
Rheumatology	278 (75)	170 (61)	274	108 (39)	156	194/278 (70)	111 (57)	44 (23)	18 (9)	21 (11)	84 (30)	4 (5)	11 (13)	25 (30)	44 (52)
Immunology	6 (2)	3 (50)	34	3 (50)	3	5 /6 (83)	2 (40)	1 (20)	1 (20)	1 (20)	1 (17)	0	0	0	1 (100)
Nephrology	34 (9)	9 (27)	18	25 (73)	17	11/34 (33)	7 (67)	1 (9)	2 (18)	1 (9)	23 (68)	0	1 (4)	7 (30)	15 (65)
Adult cardiology	27 (7)	4 (15)	26	23 (85)	8	1/27 (4)	0 (0)	0	1 (100)	0	26 (96)	2 (8)	2 (8)	7 (27)	15 (58)
Cardiopneumology	20 (5)	1 (5)	3	19 (95)	3	2/20 (5)	0	1 (1)	0	1 (50)	18 (90)	0	0	4 (22)	14 (77)
Emergency department	3 (0.8)	0 (0)	4	3 (100)	3	0 (0)	0 (0)	0	0	0	3 (100)	0	0	3 (100)	0
Pediatric cardiology	5 (1)	0 (0)	5	5 (100)	3	0 (0)	0 (0)	0	0	0	5 (100)	0	0	3 (60)	2 (40)
Total	373 (100)	187 (50)	364	186 (50)	193	213/373 (57)	120/213 (56)	47/213 (22)	22/213 (10)	24/213 (11)	160/373 (43)	6/160 (4)	14/160 (8)	49/160 (31)	91/160 (57)

AD: autoimmune disease,UCCBR: utilized clinical criteria before the request of test, NUCBR: non-utilized clinical criteria before request of test, Sp(+): positive antibody specificity Sp(−): negative antibody specificity.

A high percentage of patients with high autoantibodies titers do not manifest any clinical signs of autoimmune disease. This may be due to the existence of circulating antigens that are not routinely tested for, such as those resulting from infectious stimuli, from multifactorial synthesis or those naturally produced by CD5+ cells [14]. For this reason, clinicians should pay close attention to the titers in which the ANAs are reported, taking into account that in healthy individuals, antibodies should be negative or can be present in low titers, and that intermediate titers may be present in non-affected relatives of patients with autoimmune diseases or in elders with chronic infections or neoplasms [8,11,12,15].

In Mexico, ANA prevalence has been studied in healthy individuals and consensus has been reached as to consider positive a gross mottled pattern in dilutions over 1:160, while homogeneous, centromeric, peripheral or centriolar patterns should be considered positive even in dilutions as low as 1:40 [16]. Their presence can be, nevertheless, due to natural antigens [14,17,18].

In some instances the recognition of antibodies directed to known antigens cannot be achieved. This complicates the accurate measurement of the antibody's predictive value [19,20].

The objective of the present study was to determine the predictive values (PPV, NPV) of ANA testing for suspected SDR by analyzing the pre-test assessment of rheumatologic clinical criteria as well as post-test diagnosis.

2. Methods

We analyzed samples for ANA studies requested to our lab during a twelve-month period. The tests were selected if they were performed by IIF in HEp-2 cells (INOVA Diagnostics INC San Diego USA) and if an initial positive result at a 1:40 dilution led to successive dilutions. An informed consent was obtained for each test form each patient.

Furthermore, the presence of specific auto-antibodies was evaluated by ELISA (ORGENTEC Diagnostica GmBh Carl-Seiss Mainz,Germany) using purified extractable nuclear antigens (ENA) for Sm, RNP/Sm, SSA/Ro, SSB/LA, Anti-Scl-70, and anti-centromere as well as crithidia luciliae substrate.

An ANA test was considered to be positive when titers were superior to the following dilutions: Nuclear pattern: homogeneous > 1:40, coarse speckled and fine speckled > 1:160, laminar and peripheral > 1:40. Cell cycle: nucleolar, centromeric, and centriolar > 1:40. Cytoplasmic > 1:80 and micotocondrial > 1:160.

Each patient's clinical file was reviewed by a qualified rheumatologist to acknowledge, if the suspected diagnosis was confirmed or if there was an alternative final diagnosis. We confirmed form the records the evaluation made for the presence of diagnostic clinical criteria in each patient. Clinical criteria considered for each disease the following the guidelines for diagnosis.

2.1. Statistical analysis

Sample size was calculated by correlation as follows:

$$n = \frac{n'}{1 + n' \cdot N}$$

$$n' = \frac{S^2}{\sigma^2}$$

where $N=1374$, standard error, Se=0.025, $p=0.18$, S^2 the sample variance $p(1-p)=(018)(1-p)=(0.18)\,(0.82)=0.1476\}$, σ^2 is