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# Antinuclear antibodies☆

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ABSTRACT

Anti-nuclear antibodies (ANA) are immunoglobulin directed against autologous cell nuclear and cytoplasmic components. Besides the autoimmune ANA there are other ANA that can be detected in circulation, like natural and infectious ANA.

Because of its high sensibility, detection of the ANA must be done by indirect immunofluorescence (IIF) as screening test and all of those positive samples are convenient to confirm its specificity by ELISA, western blot or other techniques.

Positive ANA detected by IIF must be evaluated taking in to account the pattern and titer. The following recommended step is the specificity characterization (reactivity against extractable nuclear antigens [ENA], dsDNA, etc.) which is useful for the diagnosis and follow up of patients with autoimmune diseases, and by such reasoning, its detection must be performed in an orderly and reasonable way using guides or strategies focused to the good use and interpretation of the autoantibodies. The objective of this review is to present a compilation of the literature and our experience in the detection and study of the ANA.



## Anticuerpos antinucleares

*Palabras clave:*
Anticuerpos antinucleares
Inmunofluorescencia indirecta
Enfermedades autoinmunes

RESUMEN

Los anticuerpos antinucleares son inmunoglobulinas que reconocen componentes celulares autólogos (nucleares y citoplasmáticos). Además de los ANA autoinmunes, pueden estar en circulación ANA infecciosos y naturales.

La detección de ANA debe realizarse mediante inmunofluorescencia indirecta (IFI) en líneas celulares como prueba de tamizado inicial debido a su alta sensibilidad. Una muestra positiva para ANA, detectados mediante IFI, debe confirmarse mediante técnicas más sensibles y específicas, como ELISA, electroinmunotransferencia (Western blot) u otras.

Los ANA detectados por IFI deben ser evaluados según el patrón y el título. La detección específica de diversos autoanticuerpos (anti-ENA, ADNcd, etc.) resulta útil en el diagnóstico y seguimiento de pacientes con enfermedades autoinmunes. Por tal motivo, su detección debe realizarse de manera ordenada y razonable, empleando las guías o estrategias enfocadas al buen uso e interpretación de la presencia de autoanticuerpos. El objetivo de la revisión es presentar una recopilación de la literatura y nuestra experiencia en la detección y estudio de los ANA.



## Introduction

The study of antinuclear antibodies (ANA) began with the identification in patients with systemic lupus erythematosus (SLE) of the LE cell phenomenon, described by Hargraves in 1948.[1] The detection of LE cells has long been a test to confirm the diagnosis of SLE. However, years later it showed low specificity, as they may be present in patients with rheumatoid arthritis (25%), Sjögren syndrome (15%-20%), pancreatic cirrhosis (33%), chronic active hepatitis (50%-70%) and other diseases (1%-2% in myasthenia gravis and idiopathic thrombocytopenia purpura). In 1959, Holman showed that the LE cell phenomenon was due to the presence

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◆ In memoriam to our teacher and friend

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of antibodies that recognize nuclear antigens.[2] This led to the development of techniques such as immunodiffusion (double diffusion or radial Ouchterlony and counterimmunoelectrophoresis), hemagglutination, complement fixation, etc. and microscopy, using antibodies conjugated with fluorescent molecules (indirect immunofluorescence [IIF]) which increased the specificity and sensitivity for detection. Currently, the most used technique for the detection of ANA is IFI, which was developed in 1950 by Conns et al[3] and subsequently modified by Tan[4] in 1966, which employed as substrates slices of liver or kidneys of mice. Ten years later, the results of standardization for the detection of ANA in patients with rheumatic[5] disease wereublished.

Currently, the detection of ANA uses as substrates the HEp-2 and HeLa cell lines, the first for its ease of growth, the second one being the most common. ANA detection by IIF in these cell lines is considered the initial test laboratory supporting the diagnosis of autoimmune diseases due to its high sensitivity. However, given its relatively low specificity, it is necessary to use more sensitive and specific techniques such as radioimmunoassay (RIA), ELISA, electroimmunotransference (EIT) or Western blot, etc. to increase the sensitivity and specificity of ANA for diagnosis of autoimmune diseases.

## Definition of ANA and ENA

ANA are immunoglobulins that react against different autologous nuclear *(eg* dsDNA, SSA / Ro, centromere protein, etc.) and cytoplasmic components *(v. g.* Aminoacyl tRNA synthetase or Jo-1, mitochondria, etc.). The latter, however, are cytoplasmic antigens and antibodies that recognize them are also referred to as ANA.[6] While there is controversy regarding whether it is correct or not to call cytoplasmic patterns ANA, it is an easy way to describe autoantibodies that recognize ubiquitous antigens of the nucleus or cytoplasm, regardless of location, we refer to them as ANA. There is an interesting proposal which suggests that antibodies be called "anticellular" (Dr. Ignacio Garcia de la Torre, personal communication, Medical Conferences in Autoimmune Diseases Update, June 2009, Mexico DF), but this needs to be accepted by international consensus. From the perspective of the laboratory, antibodies that recognize antigens in both compartments are detected in a single antigen substrate (HEp-2 cells) and both are reported as ANA (nuclear and cytoplasmic pattern).

Moreover, the identification of antigens recognized by ANA were originally purified nuclear proteins studied by techniques of extraction with salt solutions, hence the name: extractable nuclear antigen, better known as ENA, of which there are over 100 known antigens. However, the most studied and best characterized are: SSA/SSB, RNP-U1/Sm, Sm, and Jo-1 Scl70.[6]

## Classification of ANA

Three types of ANA may be present in the circulation. One of them may be present in all individuals at relatively low titers and are part of the repertoire of *natural ANA*.[7] It is therefore important to establish reference values adjusted to ethnic populations that will be used as reference.[7-9] A study conducted in 2005 showed the importance of establishing normal values of ANA taking into account the ethnic group, the observed pattern and the antibodies titers.[9] A second type of ANA occur as a result of infectious processes. In this sense, the ANA whose origin are infectious processes are not associated with clinical manifestations of autoimmune disease and its titles are reduced when the infectious process that gave rise to them is resolved.[7] The third type is the *autoimmune ANA*,which reflect the loss of immune tolerance and its origin is multifactorial. Its production is dependent on genetic makeup, environment, hormonal changes, etc.[10] (Table).

## Techniques used for the detection of ANA

The substrate used for the detection of ANA is very important, since there are antigens whose concentration in tissue cells is very low in

**Table**
ANA classification

| ANA | Origin | Characteristics |
|---|---|---|
| **Natural** | The antigenic stimulus originating its synthesis is unknown<br>Children and older adults might have relatively high titers | 1. Produced by B $CD5^+$ cells<br>2. Los avidity<br>3. Coded by germinal line genes<br>4. Mainly IgM<br>5. Polyreactive<br>6. Not associated to clinical manifestations<br>Functions:<br>1. First line of defense vs pathogens<br>2. Clearance of damaged self molecules<br>3. Participate in the idiotype-anti-idiotype network |
| **Infectious** | Produced in response to external antigenic stimuli (infections) | 1. High avidity<br>2. IgG, IgA and IgM<br>3. Not associated to clinical manifestations of autoimmunity<br>4. Recognize ubiquitous components (DNA, phospholipids, etc.)<br>5. Titers lowered when antigenic stimuli disappear |
| **Autoimmune** | Stimulus originating their synthesis is endogenous or exogenous. They have a multifactorial origin (loss of immunologic tolerance, genetic component, interaction with the environment, others) | 1. High avidity<br>2. Mainly IgG but can also be IgA and/or IgM<br>3. Associated to clinical manifestations of autoimmunity<br>4. Recognize ubiquitous components (DNA, phospholipids, etc.)<br>5. Titers fluctuate throughout disease |

ANA indicates anti-nuclear antibodies.

contrast to what happens in HEp-2 cells, that because it is an epithelial cell line, has increased concentrations. Another characteristic of these cells is that they have more than 46 chromosomes, more than two nucleoli and, being very metabolically active, have a large number of mitochondria. Their nucleus is bigger than any normal epithelial cell, so the observation of nuclear and cytoplasmic patterns becomes a relatively easy task. However, it is important to confirm the specificity of the antibodies detected by ELISA, EIT or techniques with similar to or higher sensitivity and specificity.[11]

Another technique used for the detection of ANA is ELISA in which polystyrene plates are coated with macerated (mainly nuclei) of the HEp-2 or HeLa cell lines. In this case, specificity is minimal because if you get a positive result, one does not know the autoantigens that are shaping the positivity and reactivity. By contrast, the patterns obtained in HEp-2 cells allow the observer to suspect the specificity of ANA. For example, the homogeneous pattern suggests reactivity against chromatin antigens (nuclesomas, dsDNA, ssDNA and/or histones), the centromeric pattern suggests reactivity against centromere proteins CENP-A, CENP-B, CENP-C, etc.

It is important to stress that in third and fourth generation ELISA, the plates are coated with purified or recombinant antigens. The latter have a small number of epitopes.[12] This implies that one must be cautious in interpreting the results of initial screening and confirmation by other more sensitive and specific techniques. An example of this is when a patient's serum gives a centromeric pattern by IIF on HEp-2 cells and ELISA that detects anti-CENP-B activity is negative. This is because in HEp-2 cells all antigens that make up the centromeres (CENP-A, B, C, D, E and F) are present and in ELISA, the single antigen that is present is the CENP-B protein.

EIT, also known as Western blot, is a technique that has many uses, among them is the detection of activity against cellular components. It is highly sensitive and specific, and its use (because of cost, preparation time of the test, the test development time and number of samples that can be run at the same time) is focused mainly on research. However, recently some equipment companies have marketed it for use in diagnostic laboratories. The detection of ANA using EIT is useful to identify a large number of autoantigens. However, their interpretation is not straightforward. Results obtained in our laboratory show that there are antibodies that recognize only the native form of centromere proteins in HEp-2 cells and not denatured or modified forms often obtained in the EIT. This confirms that there are antibodies that recognize conformational epitopes of the antigens.[13,14]

Currently there are other techniques being used for the detection of ANA[11] such as enzymatic microimmunoassays and multiple luminometric detection techniques (xMAP technology), among others. The two techniques have the advantage of detecting multiple antigens in a single assay. However, one should also consider the above points: specific purified and/or recombinant antigens, sensitivity, specificity and modification of the native conformation of proteins.

## Patterns of ANA detected by IIF

The following describes the characteristics of the patterns that most often are detected by IIF on HEp-2 cells in the sera of patients with autoimmune disease.[14-19]

The *homogeneous* pattern is characterized by a homogeneous staining of the nucleus, whose intensity can vary depending on the

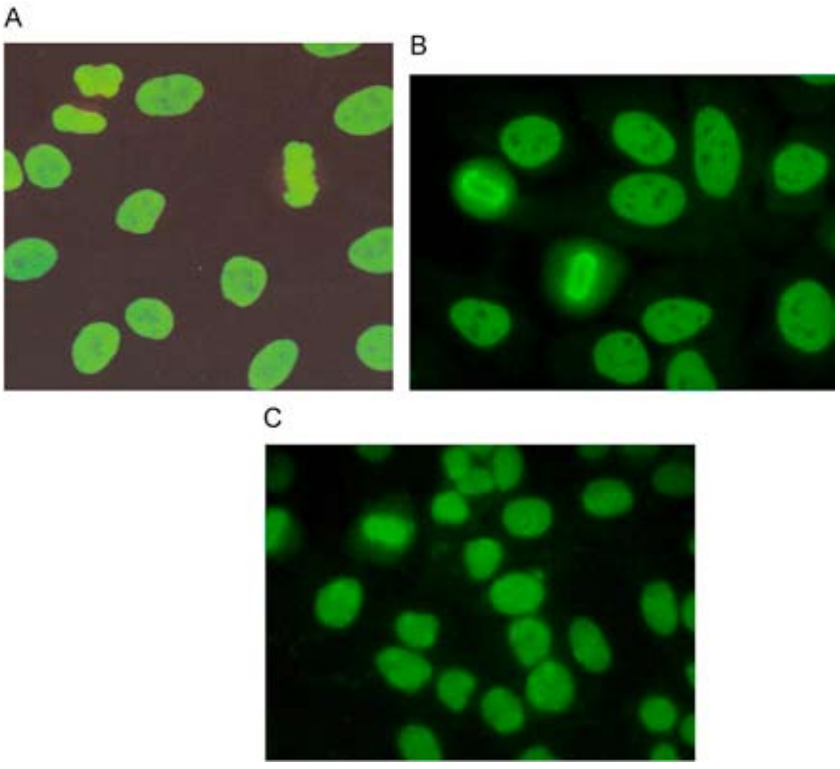


**Figure 1.** Homogeneous patterns: A) with compact metaphase and negative nucleoli staining, B) with well-limited metaphase and negative nucleoli staining, and C) with diffuse metaphase and positive nucleoli staining.

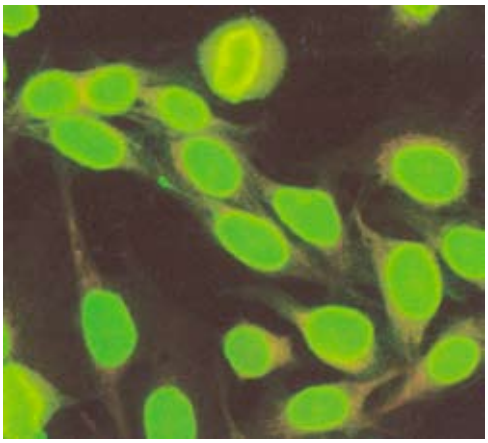

**Figure 2.** Peripheral pattern.

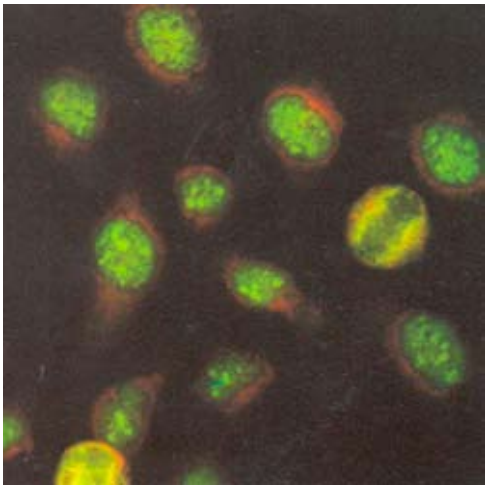

**Figure 3.** Coarse speckled pattern.

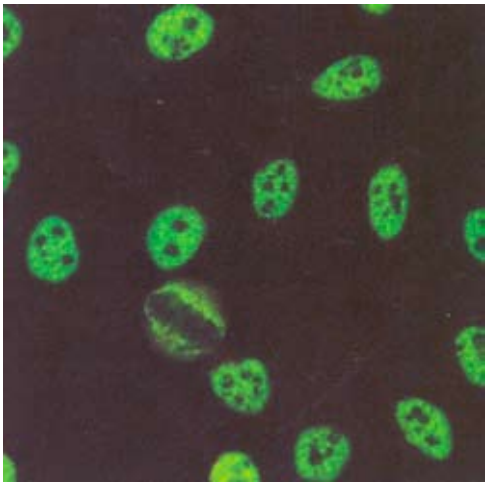

**Figure 4.** Fine speckled pattern.

concentration of antibodies in the serum. The plate of chromatin in dividing cells can be stained in a compact (Figure 1A), outlined (Figure 1B) or diffuse way (Figure 1C) and nucleoli may or may not be dyed.[20]

The *peripheral* pattern is characterized by regular staining around the nucleus, the center of this pattern shows less staining (Figure 2). The plate chromatin is stained in a compact and outlined manner.

The ANA patterns observed more frequently is spotted, both fine and coarse. The description of these patterns has generated confusion in the sense that various authors define the coarse speckled pattern as that in which the nucleus is observed to have stained coarse granules and fine speckled pattern is defined as fine nuclear granules. There is no problem when the pellets are too thick or too thin, at intermediate points interpretation is subjective. Our proposal is that the *coarse speckled pattern* must be defined as staining of the nucleus with coarse or fine granules, the nucleoli are stained and the chromatin plate in dividing cells does not stain, that is, there is no recognition of the components of chromatin (Figure 3).

As for the *fine speckled pattern*, this is characterized by the nucleus staining with coarse or fine granules, the nucleoli do not stain nor does the chromatin plaque in dividing cells (Figure 4). This definition of coarse and fine speckled pattern is easier to understand and interpret. It also has support in the difference of reactivity shown by the sera giving the patterns.[14]

*Centromeric* pattern has the characteristic that the nuclei are uniformly stained with fine speckles distributed in the nucleoplasm of interphase cells (Figure 5). The staining in dividing cells shows a fine speckle located on the chromatin plate.

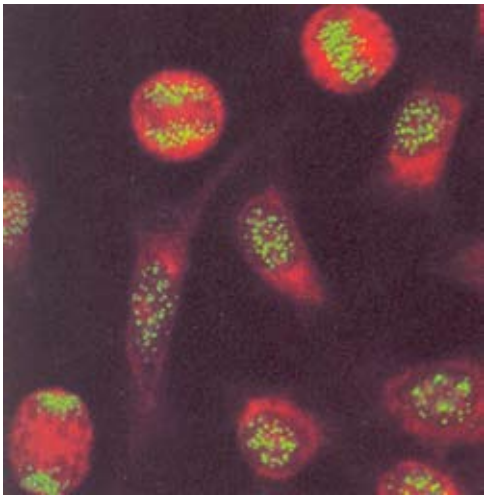

**Figure 5.** Centromeric pattern.

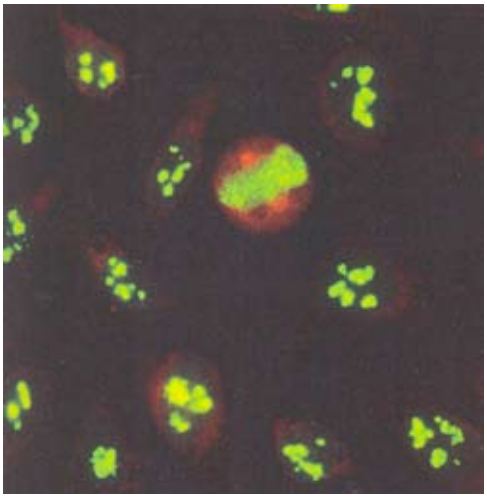

**Figure 6.** Nucleolar pattern.

The *nucleolar* pattern is distinguished by an intense staining of the nucleoli (Figure 6). The chromatin plate in dividing cells stains diffusely due to cross-reactivity of antibodies directed against nucleolar RNA with DNA in chromatin.

The pattern of the *nuclear lamina or laminar* is one in which staining is seen concentrated around the nucleus and does not extend into the cytoplasm (Figure 7). Unlike the peripheral pattern, the chromatin plate in dividing cells is negative.

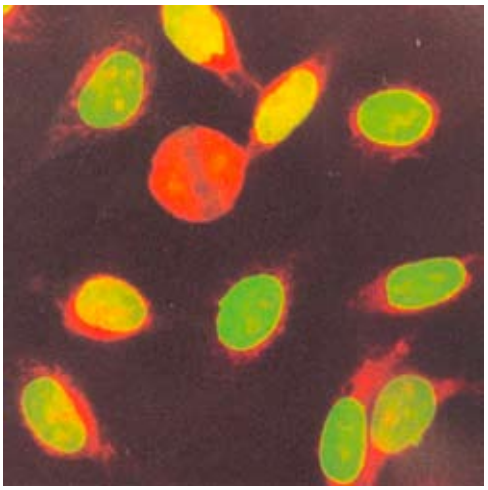

**Figure 7.** Nuclear lamina or laminar pattern.

*Centriolar* pattern is identified by intense staining of the centrioles in dividing cells. The stained structures can be identified in the G 2 phase, where two points are seen close together, and in metaphase, where they are located at the poles of the cell (Figure 8A). When the centrioles stain, spindle filaments and interphase cells have a fine speckled pattern. The pattern is defined as NuMA-1 (nuclear mitotic apparatus) (Figure 8B). The staining of centrioles and the mitotic spindle without staining the nucleoplasm in interphase cells is defined as NuMA-2 (Figure 8C).

In relation to patterns identified in cytoplasmic HEp-2 cells, we described below the most frequent. The *cytoplasmic* pattern is defined as homogeneous staining covering all of the cytoplasm (Figure 9).The *mitochondrial* pattern is characterized by a granular staining in dotted lines surrounding the nucleus and extending into the cytoplasm without covering it completely.

The recognition of components of the cytoskeleton (microtubules, microfilaments and intermediate filaments) gives a cytoplasmic pattern and is known as *intermediate filaments or smooth muscle* pattern and is characterized by threadlike staining of the cytoplasm[21] (Figure 10). A pattern of intermediate filaments with a titer greater than 1:160, according to the reference values previously reported in Mexican mestizos[9] should be interpreted as positive for-smooth muscle antibodies.

Other ANA patterns have been reported (*eg,* lysosomal, Na, PCNA, peroxisomes, etc.). That can be identified in HEp-2 cells, but are rare and their diagnostic value is under study.

Nuclear and cytoplasmic patterns described above are pure patterns. However, it is important to note that over 90% of ANA detected in HEp-2 cells present at least two different combined nuclear and/or cytoplasmic patterns.

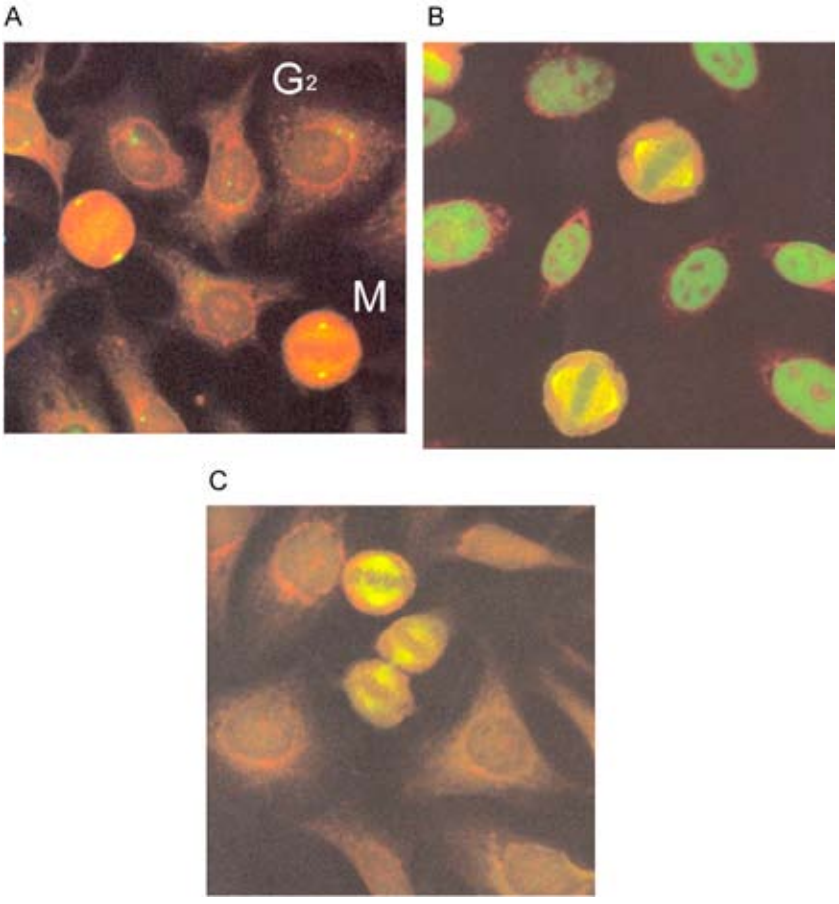


**Figure 7.** Pattern of antibodies that recognize antigens of the mitotic apparatus: A) anti-centrioli antibodies (the photograph shows HEp-2 cells in different stages of the cell cyle, $G_2$=$G_2$ phase; M=Metaphase); B) NuMA-1 pattern, and C) NuMA-2 pattern.

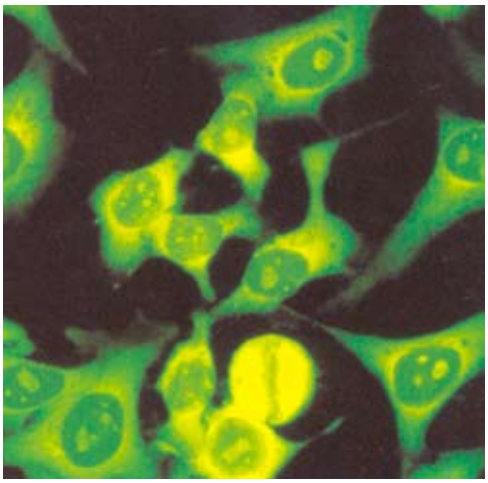

**Figure 9.** Cytoplasmic pattern.

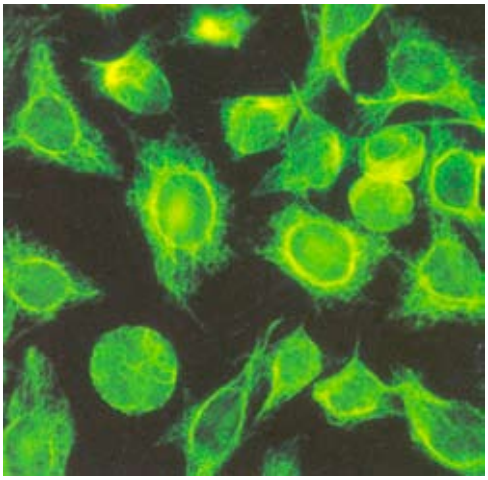

**Figure 10.** Intermediate filament or cytoskeletal pattern.

## Antinuclear antibodies and its clinical relevance

The clinical significance of ANA in autoimmune diseases has been widely studied. In this review it will be addressed in general. ANA are detected by IFI with a high frequency in autoimmune diseases such as SLE, Sjögren syndrome, mixed connective tissue disease, systemic sclerosis and others. However, their clinical relevance has not been well-established.[7]

ANA have a high prevalence at relatively low titers in healthy Mexican populations.[9,22] In children and older adults they are also detected, but at high titers.

The diagnostic patterns which are more meaningful for clinical significance are homogeneous and centromeric patterns. However, it is important to confirm the epitopes recognized by autoantibodies in the serum of patients, as mentioned below.

## Use and application of guidelines for the detection of ANA

The homogeneous pattern on HEp-2 cells suggests the presence of autoantibodies against components of chromatin. The chromatin consists of double-stranded DNA (dsDNA), histones (H1, H2A, H2B, H3, H4), nucleosomes and single stranded DNA (ssDNA). In a recent paper the specificity of these compounds in samples from patients with SLE who gave homogeneous pattern by IIF was evaluated.[23] The results showed reactivity against dsDNA (60%-80%), nucleosomes (95%), histones (20%-40%) and ssDNA (60%-80%). In our experience (work in progress), one often has samples of SLE patients with high titers of antibodies against a single component of chromatin, so we suggest confirming the homogeneous pattern by detecting reactivity against: 1) dsDNA, 2) nucleosomes, 3) histones and 4) ssDNA, taking into account the disease, patients progression and activity. If reactivity against chromatin components is negative, the sample may have activity against other antigens, the best studied of which is Scl-70.

On the other hand, studies in our laboratory, using immunochemical characterization through EIT of the sera giving a fine speckled pattern in the IFI, show reactivity against native SSA/Ro and 52 kDa SSA/Ro in 55% of the samples and SSB/La in 5%, followed by the recognition against the RNP/Sm molecule in 10%. As to the coarse speckled pattern, the reactivity is mainly against RNP/Sm in 65% of the samples, 36% reacted against SSA/Ro 52 kDa recombinant, 5% against SSB/La and 32% against CENP-B.[14] Based on our results and reports supported by other research groups, we suggest that the search for the specificities of the autoantibodies should be performed in an orderly and rational manner, based on the pattern and the title of ANA observed in HEp-2.

## Interpretation and specificity search

When a patient presents with clinical manifestations of autoimmune disease, the first test that must be applied is the detection of ANA by IFI technique using HEp-2 cells as substrate, due to its high sensitivity. Subsequently, the different patterns of ANA and the intensity (expressed in dilution titers) should be carefully evaluated to pass a second level of characterization by more sensitive and specific tests such as ELISA, radioimmunoassay, EIT, etc., to confirm the antigen recognized by the ANA present in the patient sample.

While there has not been a clear association between patterns and degrees of ANA with clinical manifestations of different autoimmune diseases, there is a clear association between patterns of ANA and the recognition of specific antigens. An example of this pattern is the association of homogeneous or peripheral patterms to high titers (>1:5120) and dsDNA and/or nucleosomes recognition. ANA do not characterize a particular autoimmune disease, but groups of autoantibodies are found more often in specific autoimmune diseases. Supported by the above, we suggest that the use and/or detection of autoantibodies should be done in an orderly manner, following the guidelines proposed by the American College of Pathologists.[11,19,24-27] With regard on how to proceed with requesting ANA, there are more questions than answers. While some groups have shown the importance of the implementation of guidelines designed for the proper use of ANA detected by IFI using HEp-2 cells as a substrate, the majority show a clear trend towards a decrease in the number of specific studies requested from the patients using such schemes or strategies. In this sense, Tampoia et al published a study in 2007 in 685 patients,[27] which revealed that the implementation of guidelines for the diagnosis using the previously validated detection of ANA[26] significantly reduces the number of immunoassays or the performance of “second level” tests made.

It is important to note that when there is a determination of ANA detected by negative IFI and the patient has clinical manifestations of autoimmune disease, one may pass to the second level of detection of antibodies by ELISA or EIT, because the sensitivity and specificity for the detection of autoantibodies is increasing.

Tampoia’s work also shows a significant decrease in the number of second level studies requested, studies whose cost have an impact on the economy of the patients. We therefore emphasize the

importance of the proper implementation of the guidelines and or algorithms developed taking into account: 1) ANA pattern, 2) cutoff points established in the ethnic group of interest, and 3) cutoff points adjusted or set by pattern, which will provide more results that help the clinician for the diagnosis and monitoring of patients.

## Conclusions

ANA are immunoglobulins that recognize autologous components of both the nucleus and the cytoplasm. However, in its original description this name was reserved only for those who were directed against components of the nucleus.

ANA determination by IIF is the main initial screening test for suspected autoimmune diseases. However, the presence of ANA may not necessarily be due to autoimmune origin, ie they may be natural or infectious.

The definition of the different ANA patterns determined by immunofluorescence in HEp-2 cells should include analysis of interphase cells and dividing cells, because there are antigens that are expressed only in certain phases of the cell cycle.

ANA must be interpreted based on values or cut-off points for staining patterns and the ethnic group with which it is to be compared.

The implementation of guidelines or algorithms that help identify the epitope or epitopes recognized by ANA have not only clinical, but also economic impact.

## Disclosures

The authors have no disclosures to make.

## References


1. Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements; “tart” cell and LE cell. Proc Mayo Clin. 1948;23:25-8.
2. Holman H, Deicher HR. The reaction of the lupus erythematosus (L.E.) cell factor with deoxyribonucleoprotein of the cell nucleus. J Clin Invest. 1959;38:2059-72.
3. Coons AH, Kaplan MH. Localization of antigen in tissue cells II: improvements in a method for the detection of antigen by means of fluorescent antibody. J Exp Med. 1950;91:1-13.
4. Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol. 1966;96:464-71.
5. Tan EM, Christian C, Holman HR, Homma M, Kunkel HG, Reichlin M, et al. Anti-tissue antibodies in rheumatic disease. Standardization and nomenclature. Arthritis Rheu. 1977;20:1419-20.
6. Tan EM. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine, in: advances in immunology. Pub Academic Press Inc. 1982;33:167-240.
7. Conrad K, SchöBler W, Hiepe F, Fritzler M. Autoantibodies in systemic autoimmune disease. A diagnostic reference. Autoantigens, autoantibodies, autoimmunity. Edi. PABST. 2002;2:21.
8. Tan EM, Feltkamp TEW, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in “healthy” individuals. Arthritis Rheu. 1997;40:1601-11.
9. 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.
10. Shoenfeld Y, Gershwin ME, Meroni PL. Autoantibodies. Elsevier. 2007;2:3-6.
11. Damoiseaux JGMC, Cohen Tervaert JW. From ANA to ENA: how to proceed? Autoimmunty Rev. 2006;5:10-7.
12. Shoenfeld Y, Gershwin ME, Meroni PL. Autoantibodies. Elsevier. 2007;2:37-46.
13. Torrico R. Prevalencia de autoanticuerpos en pacientes con diagnóstico de Esclerodermia. Tesina de Especialidad en Bioquímica Clínica. 2009. UNAM-INCMNSZ.
14. Pagola Santander L, Núñez Álvarez CA, Martínez Castillo A, Hernández-Ramírez DF, Cabral AR, García Hernández JL, et al. Caracterización inmunoquímica de los patrones de inmunofluorescencia (IFI) moteado fino y moteado grueso en pacientes con enfermedades autoinmunes. Reumatol Clin. 2009;5:55.
15. Bradwell AR, Hugues RG. Atlas of HEp-2 patterns. Third Ed. The Binding Site; 2007.
16. Mimori T. Atlas of antinuclear antibodies. Medical & Biological Laboratories CO, LTD. RhiGene Inc. 2nd Ed. 1999.
17. Sheldon J. Laboratory testing in autoimmune rheumatic diseases. Best Practice & and Research Clinical Rheumatology. 2004;18:249-69.
18. Keren DF. Antinuclear antibody testing. Clin Lab Med. 2002;22:447-74.
19. Tozzoli R, Bizarro N, Tonutti E. Guidelines for the laboratory use of autoantibody test in the diagnosis and monitoring of autoimmune rheumatic diseases. Am J Clin Pathol. 2002;117:316-24.
20. Martínez Bezies V. Asociación ente las características de las metafases con la actividad de los anticuerpos anticromatina en pacientes con enfermedades autoinmunes. Tesina de Especialidad en Bioquímica Clínica. 2007. UNAM-INCMNSZ.
21. Hugues RG, Surmacz MJ, Karim AR, Bradwell AR. Atlas of autoantibody patterns on Tissues. Third Ed. The Binding Site; 2008. p. 32-4.
22. Marin GG, Cardiel MH, Cornejo H, Viveros ME. Prevalencia de ANA en tres grupos de población mestizo-mexicana sana: donadores de sangre, personal médico-sanitario y familiares de pacientes con enfermedad autoinmune. Reumatol Clin. 2008;4:55.
23. Alvarado Hernández A, Cachafeiro Vilar A, Pascual Ramos V, Gabilondo Navarro F, Núñez Álvarez CA, Cabral AR, et al. Detección de anti-DNA de cadena doble por cuatro. Validación del ELISA caotrópico. Sent.
24. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antionuclear antibody test and test for specific autoantibodies to nuclear antigens. Arch Pathol Lab Med. 2000;124:71-81.
25. Solomon DH, Kavanaugh A, Schur PH. Evidence-based guidelines for the use of immunologic test: antinuclear antibody testing. Arthritis Rheu. 2002;47:434-44.
26. Tampoia M, Fontana A, Di Serio F, Maggiolini P, Pansini N. Application of a diagnostic algorithm in autoantibody testing: assessment of clinical effectiveness and economic efficiency. Clinic Chimica Acta. 2003;333:181-3.
27. Tampoia M, Brescia V, Fontana A, Zucano A, Morrone LF, Pansini N. Application of a combined protocol for rational request and utilization of antibody assays improve clinical diagnostic efficacy in autoimmune rheumatic disease. Arch Pathol Lab Med. 2007;131:112-6.