Antinuclear factor in dermatology[*]
Fator antinúcleo na dermatologia[*]

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Abstract: This is a review and update article on antinuclear antibodies assays, in particular the antinuclear factor, in which are approached histological and epidemiological aspects, physiopathogenesis, identification methods, their specificities and interpretation, correlating them to their applicability in the dermatologist's and general clinician's clinical practice.
Keywords: Antinuclear antibodies; Autoimmunity; Collagen diseases; Fluorescent antibody technique

Resumo: *Trata-se de artigo de revisão e atualização sobre a pesquisa dos anticorpos antinucleares, em especial do fator antinúcleo, em que são abordados os aspectos históricos, epidemiológicos, fisiopatogenia, métodos de identificação, suas especificidades e interpretação, correlacionando-os com sua aplicabilidade na prática clínica do dermatologista e do clínico geral.*
Palavras-chave: Anticorpos antinucleares; Autoimunidade; Doenças do colágeno; Imunofluorescência

INTRODUCTION

The significance of antinuclear antibodies (ANA) assays nowadays is vast and far-reaching, owing to new substrates and more accurate techniques which are used for their detection. Today, their positivity is interpreted as presence of antibodies not only against elements of the cell nucleus - as the name suggests - but it also signifies the presence of auto-antibodies against elements of the cytoplasm and nucleolus. Thus, it is likely that there will soon be a change in denomination to a more comprehensive expression such as "antinuclear, anticytoplasmatic and antinucleolar assay".[1-3] Its interpretation should always be made under the light of clinical correlation, patterns of fluorescence deposits observed in the target cell and maximal positivity of the dilution.

The antinuclear factor (FANA) assay is an indispensable element in instances of clinical suspicion of auto-immune diseases, especially those belonging to the group of collagen diseases - lupus erythematosus (LE), rheumatoid arthritis (RA), sclerodermia, dermatomyositis (DM), Sjögren's Syndrome (SS), mixed syndrome and overlap. A positive FANA does not necessarily mean the presence of a collagen disea-

se, it can mean from its presence a familial feature with the likelihood or not of the bearer developing a certain collagen disease when faced with various stimuli throughout the years to the real presence of an auto-immune disease, or even its prognosis. Still, it its possible that it occurs in a share of the normal world population and in some chronic diseases, e.g., billiary fibrosis, as well as in acute viral infections. All this interpretation variability is due to the variable specificity that occurs because of the multiplicity of intranuclear, nucleolar and intracytoplasmatic elements which are potentially capable of behaving like antigens facing diverse conditions, even though this antigenicity does not always translate into disease. Hence, the historic conception of positive FANA as a synonym for collagen disease must be reevaluated more strictly and always with regard to clinical correlation, besides family history.[4]

HISTORICAL ASPECTS

Investigations of the presence of auto-antibodies began with the findings of LE cells in the sera of lupic patients (1948), which were used for years as a

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diagnostic instrument mainly for lupus erythematosus and rheumatoid arthritis.[5-7] Years later (1957), the physiopathogenic mechanism for the formation of LE cells was unravelled,[3,8-11] namely, they were the result of anti-deoxyribonucleoprotein antibodies-induced apoptosis. Therefore, the formation of the LE cell is a late event in relation to FANA production. From there, new techniques allowed the use of antinuclear antibody assays, widening the efficacy is the diagnosis of plasmatic auto-immune events directed against cellular elements, unlike LE cell assay, because it is much more accurate and early.

ANA detection, at first, was made possible thanks to indirect immunofluorescence techniques. Thus, the ANA assay became the method of choice for diagnostic screening of auto-immune diseases. With the advent of other techniques, among them immunodiffusion, ELISA, immunoprecipitation, immunoblot, the refinement of ANA detection techniques was made possible, hence revealing, in a specific fashion, against which cellular element the antibody would be formed,[12-16] i.e., ANA directed against DNA proteins (anti-DNA), against ribonucleoprotein (anti-RNP) and so forth. Even today novel techniques are constantly being introduced with the aim to further improve ANA detection.

EPIDEMIOLOGICAL ASPECTS

There are no universal data regarding FANA positivity in the general population,[16] but its positivity in dilution titles that do not go over 1/160 in the healthy population, mainly above 60 years of age, is well known, with no pathological meaning, and which occurs regardless of race, in undefined ratios, albeit more often in women over 14 years-old. It can also be present in members of the family of lupus erythematosus, rheumatoid arthritis and other collagen diseases bearers.[2,7,16,17]

In lupus erythematosus, in which its expression is most frequent, it is present in up to 20% of chronic cutaneous forms; in up to 50% of patients with subacute cutaneous form; and in almost 100% of systemic disease bearers. In approximately 5% of patients in whom the systemic form of the disease is diagnosed, FANA positivity may be absent. This is probably due to the employment of inappropriate detection techniques or sample collection, or to the presence of other auto-antibodies, such as anti-Ro/SSA, or of antibodies directed against plasmatic elements, such as antiphospholipid, antiplatelet, not to mention those still unknown.[15-18]

Positivity in the other collagen diseases is extremely variable. It can be positive in up to 50% of patients with rheumatoid arthritis; 20% in sclerodermia with no systemic manifestations and over 80% in the systemic forms. In dermatomyositis/poliomyositis positivity can reach 40-80%. In Sjögren's Syndrome, it can be positive in over 50% of patients. Still, it can be positive in rheumatic disease, fibromyalgia, thyroid disease, auto-immune hepatitis, and silicon implant bearers,[16] and sometimes in the presence of viral or bacterial infection, in which case they are transient.[16,17,19] They can also be associated with malignancies, and its occurrence has already been reported in Virchowian leprosy, not to mention sporadic reports in numerous chronic diseases, degenerative or not.[2,20]

PHYSIOPATHOGENESIS

The reason for FANA presence in some individuals when they are faced with certain stimuli could be explained, in part, by the characteristics of histocompatibility antigens that are present (HLA-B8, -DR2 e -DQ3), along with genetic familial aspects. A positive FANA can also occur after stimuli such as drugs and viruses, for instance, which also depends on family and histocompatibility antigen characteristics, among other still unknown factors.[10,16,21,22]

In principle, the initial event should be cell apoptosis. One of the observed experimental models occurs mainly in keratinocytes, when the disease is expressed in the skin, or in circulating and endothelial cells, among others, before specific stimuli.[5,6,23]

Besides genetic predisposition, several factors can trigger cell apoptosis (e.g., viral and bacterial infections, and drugs). Ultraviolet cytotoxic irradiation[7,10,23] is one of the main causes which, because of incidence in the skin, induce keratinocyte apoptosis. From there, exposure of both nuclear and cytoplasmatic elements to predisposed immunocompetent cells initiates the formation of antibodies against certain nuclear or cytoplasmatic proteins, which receive various denominations, according to their antigenic reactive structure: anti-ribonucleoprotein antibodies (RNP), antibodies against DNA, among others. These auto-antibodies, when circulating, deposit in different organs and tissues, trigger fixation of the complement system, thus leading to inflammation and dysfunction of the target organ.[2,16,17,22,24]

The formation of the "antinuclear factor" complex can have a specific meaning, as it is a marker for various diseases. It can have a prognostic meaning or even no important meaning at all regarding the presence of auto-immune diseases. This is why its interpretation should always be related to the site of fluorescence, deposit pattern, maximal observed dilution and clinical correlation.[2,16,17,25-27]

IDENTIFICATION METHODS

Auto-antibodies identification methods include indirect immunofluorescence - the quickest, easi-

est and cheapest method - ELISA, radioimmunoassay, immunodiffusion, counterimmunoelectroforesis, immunoprecipitation and immunoblot. All these methods are subject to false-positive results, mainly when the evaluated patient is in the age range over 60.[28-32]

Substrates used for ANA detection by means of indirect immunofluorescence - FANA - can be from rat liver cells or human larynx tumor cells (Hep-2 cells). Hep-2 cells are better due to their large nucleus and to the easy exposure of their components. Rat liver cells might not express all identifiable proteic components for humans. FANA expression sensitivity of Hep-2 cells approaches 100%; albeit with a higher risk of false-positives, for it can express antigenic intracellular elements with no pathogenic meaning. Rat liver cells express FANA in approximately 90% of instances and are highly specific, with a lower false-positive possibility than Hep-2 substrates. However, they are not able to express certain proteins, such as the so-called RO/SSA, thus being subject to false-negative. Hence, the use of Hep-2 substrate is preferable due to its high sensitivity and virtual lack of false-negative possibility. When using Hep-2 substrate, the likelihood of finding FANA-negative lupus erythematosus, for instance, is extremely low.[1,2,16,32-35]

An FANA positive by means of Hep-2 substrate can be confirmed by the detection through rat liver substrate, which validates its value by practically excluding the possibility of false-positive, hence having a great diagnostic value for a given collagen disease.[1,2]

Anyway, a positive FANA should be evaluated and correlated with clinical suspicion, deposit pattern and dilution. Different possible deposit patterns correlate with likely cellular elements that behave as antigens. Thus, depending on FANA deposit pattern, it is possible to infer against which intracellular element the autoantigen/autoantibody reaction is happening, hence knowing the real meaning of FANA. From there, the specificity of the ANA marker of the suspected disease should be assessed.[2,16,17,35]

Regarding the dilution of its positivity, titles over 1/160 are considerable, when initial standard dilutions used are 1/40, 1/80, 1/160 and so forth. Titles inferior to 1/160 should be evaluated with restrictions and always with regard to FANA specificity, correlated with clinical suspicion.

Observed morphologic criteria must refer to deposit patterns: nuclear, nucleolar, cytoplasmatic, of the mitotic machinery and in metaphasic plaque, and the reading should be made in the last dilution in which the pattern is observed. The Brazilian Guidelines propose that, even if reactivity is positive only in the cytoplasm, FANA should be considered positive; thus giving origin to new FANA interpretations, that now include not only antinuclear but also anticytoplasmatic and other antibodies.[1,2,16,35,36] Since there is no worldwide consensus, it is possible to find in foreign publications the designation of negative FANA in situations where anti-cytoplasmatic antibodies are positive, e.g. anti-Ro/SSA, and others.[36]

The manner by which FANA deposits is called FANA pattern. Thus, it can be observed forming a line around the nucleus - peripheral FANA; covering the entire nucleus - homogenous FANA (Figure 1); non-uniform varied speckled - speckled FANA (Figure 2); deposited on the centromere - centromeric FANA (Figure 3), besides other patterns (Figures 4 and 5) listed on chart 1.

Therefore, by associating FANA localization, deposit pattern and maximal observed dilution title, one may suppose which is the specificity of the FANA and then consider it as a marker or not of a keratin disease. Albeit inespecific, its positivity is an indication for specfic search for the suspected disease. From then on, the specificity of the obtained FANA is investigated (Chart 1).[24,25,28-30]

In short, a positive FANA can signify auto-antibodies present in the nucleus, nucleolus and also cell cytoplasm, and must interpreted according to the last postive dilution observed, as to fluorescence location (nucleus, nucleolus, cytoplasm or mitotic machinery) and to deposit morphology, whether homogenous, diffuse, speckled etc.[1,2,16,26,36-38]

FANA must be presented as follows: a) positive or reactant, maximal dilution in multiples of 1/40, reaction location and observed deposit pattern - in this way, a correlation of its specificity can be made,[1,16,24] as shown in chart 1; b) Negative or non-reactant FANA.
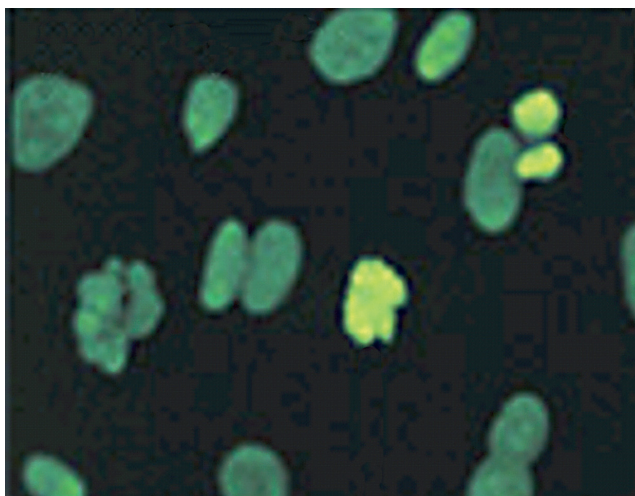

FIGURE 1: Homogenous nuclear FANA - can represent anti-nDNA antibody, marker of SLE
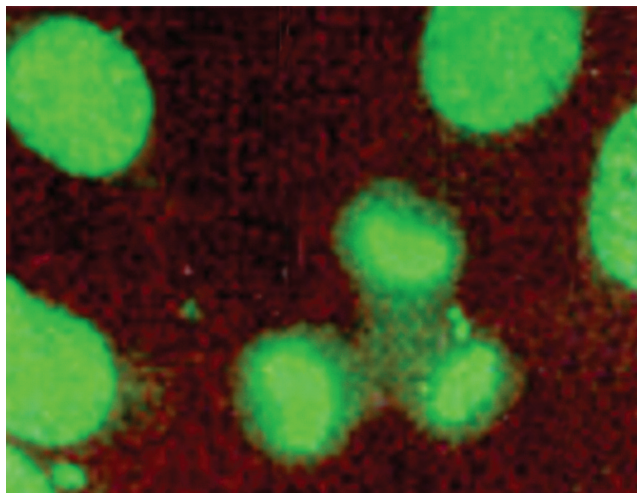
FIGURE 2: Dense fine speckled nuclear FANA - it is the most frequent pattern in healthy population or in inflammatory processes, with no established clinical correlation

FIGURE 4: Fine speckled nuclear FANA - corresponds to anti-Ro/SSA and anti-La/SSB antibodies, markers of Sjögren's syndrome, subacute cutaneous LE and neonatal LE

AUTO-ANTIBODY CHARACTERISTICS AND SPECIFICITY

Anti-DNA: it is subdivided into nDNA (native or double helix) and sDNA (simple or single helix) and histones. The diagnostic importance resides in the presence of antigenicity of nDNA, present in a percentage that ranges from 70 to 80% of SLE-bearing patients, but that can also occur in the presence of rheumatoid arthritis and Sjögren's Syndrome, in lesser proportions, besides other chronic auto-immune diseases. It is very often associated to a high index of renal lupic disease. High titles indicate disease activity, and a correlation exists between title decrease and improvement of activity, and vice-versa.[16,25,31,39-42]
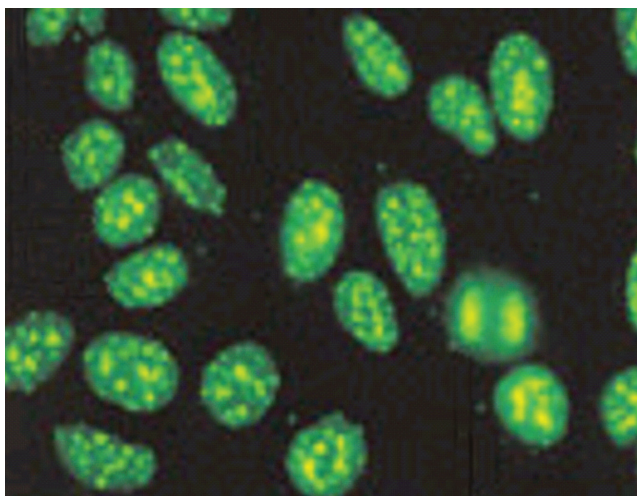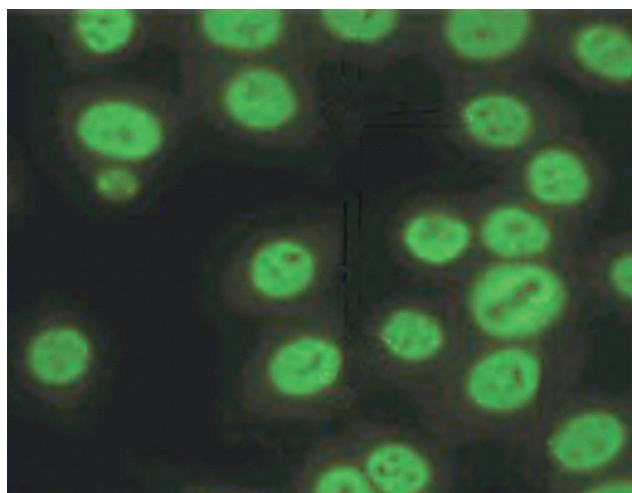
sDNA is found in other collagen diseases, in addition to other chronic diseases and drug-induced lupus.

Antinucleosomes: these are antibodies directed against proteins that compose nucleosomes - histone-codified DNA-proteins (H1, H2A, H2B, H3 e H4) - anti-DNA-histones. An isolated positivity is a marker for drug-induced lupus erythematosus, and, when associated to nDNA positivity, is a marker for systemic lupus disease. It is positive in 70% of SLE patients.[16,43]

AntiPCNA (anti-proliferating cell nuclear antigen antibody): it is marked by a 36 kD protein, which is involved in replication and DNA repairing. It is positive in up to 5% of SLE patients and has not yet

FIGURE 3: Centromeric FANA - can represent anticentromeric antibody, found in CREST syndrome and primary billiary cirrhosis
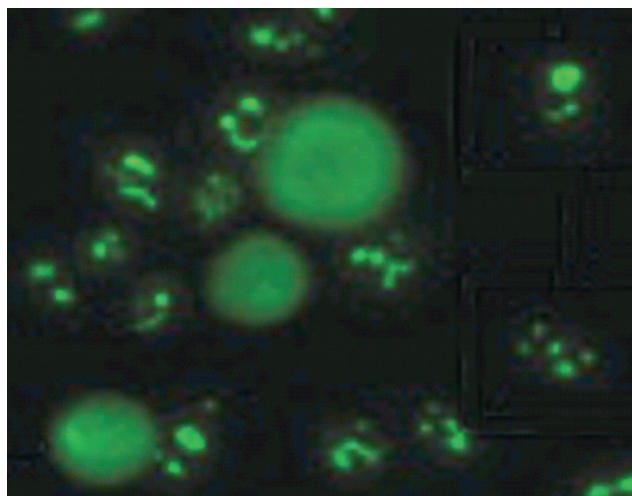

FIGURE 5: Nucleolar FANA - can correspond to anti-RNA polimerase antibody, which can indicate diffuse sclerodermia with a tendency to systematization, and to anti-PM-Scl antibody, which occurs with the overlapping of collagen diseases, mainly sclerodermia and poliomyositis

CHART 1: Correlation between patterns of ANF deposit and specificity and possible clinical association

Deposit pattern	Specificity	Clinical correlation
Dense fine stippled nuclear (Most common pattern)	Inespecific	Healthy population Diverse inflammatory diseases (auto-immune or not)
Centromeric nuclear	Anticentromere	CREST
Homogenous nuclear	Anti-nDNA antibody DNA-histones	SLE Drug-induced LE
Diffuse stippled nuclear	Anti-Sm Anti-RNP	SLE Mixed Syndrome, RA Systemic sclerosis, SLE Neonatal LE
Fine speckled nuclear	Anti-Ro/SSA and La/SSB	SLE Subacute cutaneous LE Neonatal LE, Sjögren's Syndrome
Nucleolar	Antinucleolus	Systemic sclerosis
Homogenous nucleolar	AntiPM/Scl	Overlap syndrome (SScl-DM)
Fine stippled cytoplasmatic	AntiJo-1 (anti-RNA-sinthetase)	Poliomyositis/DM
Mixed deposit patterns	Anti-Scl-70	Systemic Sclerosis SLE Overlap syndrome
Other patterns		

been described in other collagen diseases; however, its presence has been demonstrated in viral hepatites. It is generally represented by nucleolar speckled FANA.[44,45]

AntiKU: This auto-antibody is directed against a subunit of chromatin-protein that is involved in replication and DNA repairing. It is represented by a positive nuclear or nucleolar diffuse FANA. Its positivity means immunobiological activity. It can be present in proportions that go up to 40% in sclerodermia, poliomyositis, SLE and rheumatoid arthritis.[16,45]

Anti-RNA-Polimerase: represented by nucleolar FANA in isolated spots. These are auto-antibodies directed against enzymatic fractions of polipeptides that compose RNA and that are subdivided into three classes - RNA I, II and III.Its positivity is described in SLE, rheumatoid arthritis, sclerodermia, mixed syndrome and overlap syndrome. It has no diagnostic importance yet, and its positivity varies up to 14% in SLE.[4,16,46]

Anti-Sm and Anti-RNP: they are auto-antibodies directed against RNP complex proteins (U1, U2, U4, U5) - anti-RNP, and against Sm polipeptides (D1, D2, D3, E, F e G) - anti-Sm, which are involved in RNA synthesis. They are represented as gross nuclear speckled. Anti-RNP is present in SLE in 30 to 40% of

patients and can be associated to pictures of Raynaud's phenomenon or disease, myosites, esophageal diseases, arthralgias and arthritis, sclerodactily and neonatal LE; even though it is an important marker of possible lupic disease, it has been described in practically all collagen diseases. Anti-Sm is considered to be a specific auto-antibody and a marker of systemic lupus erythematosus, although it is only positive in a percentage that ranges from 20 to 30% of these patients.[2,16,22,31,32,47]

Anti-Ro/SSA and antiLa/SSB: both are antibodies directed against proteins that are part of RNA composition - they are ribonucleoproteins of distinct molecular weights, 52 to 60 kD and 43 to 52 kD, respectively.

The anti-Ro/SSA antibody, when described, was difficult to detect owing to a low concentration in tissue substrates and this is why it has been related to patients with negative FANA; however, it currently has a more accurate detection as a consequence of more accurate techniques. Although it is more commonly related to Sjögren's Syndrome, it is found in around 40% in SLE and subacute cutaneous lupus erythematosus (SCLE), in the latter meaning an increased chance of the disease becoming systemic. In addition,

it is a marker of neonatal lupus erythematosus (NLE), associated with photosensitization, lymphopenia and systemic disease with possible pulmonary affection. It is commonly associated with the presence of anti-La/SSB. Its presence can be related to deficiency of fractions C2 and C4 of the complement.[2,16,31,32,43,48,49]

Anti-La/SSB antibody is present in SLE in percentages that go from 10 to 15%. It is also found in neonatal LE, albeit less frequently than anti-Ro/SSA. Its simultaneous presence with anti-Ro/SSA, in patients with SLE, seems to provide protection against renal aggression that occurs in lupic disease. The isolated presence of anti-La/SSB antibody is not habitual, and, when it occurs, there seems to be no systemic disease, neither in adults nor in newborns.[2,16,31,32,43,48,49]

Both antibodies have been described in rheumatoid arthritis, primary billiary cirrhosis, multiple mieloma, sclerodermia and dermatopoliomyositis.

AntiP-ribosomal: anti-ribosomal antibodies are directed against ribonucleoprotein complexes involved with mRNA and are calles antiP-ribosomal antibodies. They are represented by positive FANA both in the nucleus and in cytoplasm and nucleolus, and for that they are better visualized in dividing cells, in metaphasic plaques. They are highly specific for the diagnosis of SLE, ocurring in a proportion ranging from 10 to 20% of patients, even though they are also encountered in the normal population in undefined proportions. Its presence seems to be related to lupic psychiatric disorders, including psychosis. Its isolated occurrence is rare and generally associated with anti-nDNA,anti-Sm and anti-phospholipid antibodies.[4,16,31,46]

Anticentromere: antibody turned against a portion of the chromosome that undergoes a constriction during mitosis - centromere. It is demonstrated by a discontinuous punctiform fluorescence. It occurs in up to 30% of patients who suffer from systemic sclerodermia of the CREST type (calcinosis, Raynaud, esophageal disease, sclerodactily and teleangectasies). It can also be present in generalized cutaneous sclerodermia in a proportion that varies between 30 and 40% of patients, in Hashimoto's thyroiditis, Raynaud's phenomenon and primary biliary cirrhosis.[16,26,31,37,50,51]

Anti-Scl-70: antitopoisomerase I. Antibody that recogniazes the carboxi-end portion of DNA-topoisomerase I. It can be represented by nuclear and nucleolar FANA of mixed patterns. It is present in a percentage ranging from 22 to 40% of patients with sclerodermia with a tendency of generalizing or with systemic affection, in particular lung compromising with fibrosis and cardiac compromising, not to mention hemorrhagic spots in the extremities of the body. It is a marker of long-term evolution disease. It may be found in systemic lupus erythematosus and more seldom in dermatomyositis and rheumatoid arthritis.[31,35,50-52]

Anti Pm-Scl: antibodies directed against degrading ribosomal proteins. They are represented by homogenous nucleolar FANA. They are more associated with the presence of overlap syndrome, in special the association between sclerodermia and myositis. They also occur in sclerodermia and SLE or myositis and SLE, and are also associated with the presence of arthritis, dermatomyositis cutaneous lesions and calcinosis. They are found in 3% of patients with systemic sclerodermia, 5% of patients with poliomyositis, 5% in cutaneous sclerodermia and in 25% of patients with overlap syndrome.[31,50,52]

Anti-RNA-synthetase: specific antibodies directed against products of cytoplasmatic reactivity, i.e., specific aminoacids related to RNA-synthetase. They are represented by cytoplasmatic FANA. Five different antibodies are identified: antiJo-1, antiPL-7, antiPL-12, antiEJ and antiOJ, each of them turned against a different aminoacid, histidine, treonin, alanine, glycine and isoleucine, respectively. The clinical representation of these auto-antibodies is similar. AntiJo-1 is the most commom and it is present in up to 20% of poliomyositis bearers and, in a smaller scale, in dermatomyositis bearers. Poliomyositis is seemingly most commonly associated to anti-Jo-1, and dermatomyosite to other anti-synthetase antibodies. Generally speaking, the presence of these auto-antibodies indicates anti-synthetase syndrome, characterized by arthritis, Raynaud, sclerodactily, intersticial pulmonary disease, calcinosis, facial teleangiectasies, linear hyperkeratosis and dry syndrome.[31,34,47,53,54]

Anti Mi-2: auto-antibody against a nuclear complex that controls cell proliferation - a chromatin. Anti-Mi-2 presents as homogenous nuclear FANA and occurs in 15% of dermatomyositis bearers. The presence of anti-Mi-2 indicates the giagnosis of dermatomyosistis in 95% of instances. It is less frequent in bearers of isolated poliomyositis.[31,53,54]

Other antibodies are possible[16,26] to be represented by FANA positivity, albeit with a yet undefined interpretation, thus needing further studies. For instance, anti-Mas, anti-NOR, anti-Ki-67, anti-Wa, anti-fibrilarine, anti-topoisomerase II, anti-SR, are some that have been under investigation for over two years.

ANTINUCLEAR FACTOR INTERPRETATION SCHEME

When there is suspicion of a collagen disease, the initial screening exam is always FANA. The best diagnosis can be obtained by the association of FANA results and clinical findings. Therefore, when it is obtained:

Negative FANA + clinical suspicion -> request anti-Ro/SSA and/or antiJo-1 and/or antiphospholipid. Consider the possibility of negative-FANA LE

or presence of plasmatic auto-antibodies (antiphospholipid), in addition to cytoplasmatic auto-antibodies with discontinuous fluorescence (antiJo-1).

Positive FANA, low title + inespecific clinical findings -> request anti-Ro/SSA and/or antiJo-1 or antiphospholipid. Find out the specificity that best fits clinical hypothesis.

Positive FANA, low title + highly suspected clinical findings of -> request:

LE -> antiDNAn, anti-Sm, anti-Ro/SSA

Drug-induced LE -> antiDNA histone

Sclerodermia -> anti-Scl-70, anticentromere, anti-PM-Scl

Dermatomyosite -> antiJo-1, antiPM-Scl

Mixed Syndrome -> anti-U1RNP, anti-RNA-synthetase

Sjögren's Syndrome -> anti-Ro/SSA, anti La/SSB

Neonatal LE -> anti-Ro/SSA, antiLa/SSB, antiU1RNP

Positive FANA, high title FANA + highly suspec-

ted clinical findings: evaluation of location and deposit pattern can be sufficient to establish a correlation between FANA specificity and diagnostic conclusion; or request specific auto-antibodies for confirmation, in the same fashion as the described above.

CONCLUSION

Without a doubt, FANA is the most important exam for the diagnosis of collagen diseases; however, it must always be correlated with clinical findings. Its isolated presence has no pathological meaning, even though it does have a predictive value indicating a higher probability of an individual developing a collagen disease throughout the years. Its negativity cannot be conclusive of the absence of collagen disease, either. The FANA assay, as well as its interpretation, is dynamic, with time-variable positivity and characteristics. More refined laboratorial techniques have been increasing accuracy of both the method and interpretation, being an indispensable laboratorial element as a support for better clinical diagnosis of collagen diseases. ❏

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