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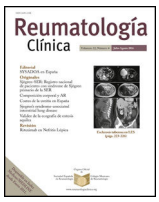

Editorial

Neither the anti-nuclear antibodies nor the anti-extractable nuclear antigens Are What They Used to Be. A Future Change of Nomenclature☆

Ni los anticuperpos antinucleares ni los anticuerpos dirigidos contra antígenos extraíbles del núcleoson lo que solían ser. Un futuro cambio de nomenclatura y recomendaciones para su determinación en práctica clínica habitual

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Antinuclear Antibodies

Antinuclear antibodies (ANA) were described for the first time by Coons and Kaplan,[1] and although it is not indicated by their name, they include autoantibodies (AAb) directed against cytoplasmic structures and the cell wall, which is to say that this is a term sustained by historic reasons for more than 60 years. In the classification of positive ANA staining patterns detected by immunofluorescence (IF), nuclear patterns are reported as much as cytoplasmic and mitotic patterns.[3,4]

Antibodies Directed Against Extractable Nuclear Antigens

Antibodies directed against extractable nuclear antigens (ENA) were described in 1959 by Holman and Robbins,[5] who with the utilization of saline solutions of low ionic strength extracted 4 protein antigens from the nucleus: SSA/Ro60, SSB7La, U1-ribonucleoprotein (RNP) and Smith (Sm).

Over time, this spectrum has grown and it is possible to detect AAb directed against other cytoplasmic or nuclear antigens and other cell compartments, such as receptors of

lamina B in the nuclear membrane, histones, other RNP and proteins that are expressed only when the cell is in mitosis (Sp100, Ku, Mi-2, cyclin, Scl-70, mitotic spindle, centrioles and kinetochores). This has benefited the introduction of multiple detection tests that vary depending on the company, according to the technological platform and the AAb to be evaluated.[2,3] At the present time, AAb detection panels usually include nuclear antigens (double-stranded DNA, ENA) and cytoplasm antigens (ribosomal-P Jo-1. In Spain, there are at least 5 companies that offer platforms for specific antigens (the so-called ENA profile), which utilize diverse native proteins, purified or human recombinant, such as Ro60 kDa, Ro52 kDa, La, U1-RNP, Sm, histones, centromere-B, Scl-70 and Jo-1.

Methodology for the Determination of Antinuclear Antibodies and Antibodies Directed Against Extractable Nuclear Antigens

In the majority of autoimmunity laboratories, both ANA and ENA are detected by IF, utilizing the laryngeal carcinoma Hep-2 cell line or one of its variants (for example, the Hep-2000 cell line), and report a maximum dilution in which the antigen-antibody reaction is observable, as is the IF staining pattern. It is considered that ANA are positive from a dilution of 1:80 in adults (although some guidelines recommend that a dilution of 1:160 is the inferior limit) and, in pediatrics, they are positive at dilutions starting at 1:10 or 1:20.[3,6–8] Subsequently, there is a second determination to evaluate the specificity of AAb using techniques such immunoblotting or enzyme-linked immunosorbent assay (ELISA). Because of the wide variability among the multiple assay platforms being marketed, it is indispensable to know the antigens that have been evaluated in each determination and information must be given about all of those utilized, whether the results were negative or positive.[2,3,6]

These determination provide us with extremely valuable information, given that AAb can develop years before the clinical manifestations of systemic lupus erythematosus, Sjögren's syndrome, scleroderma, mixed connective tissue disease and idiopathic or inflammatory myopathies (IIM). The findings are potentially relevant in the achievement of the diagnosis and for

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establishing the prognosis, the different forms of the clinical course and the eventual complications of these diseases.[7–10]

New Autoantibodies and a New Nomenclature?

The use of the nomenclature for ANA and ENA is currently considered imprecise; there are authors who refer to them as AAb directed against specific cell antigens,[6–8] and a change in the nomenclature is recommended.[6] The consensus on standardization for the evaluation of AAb was developed by two groups: the European Autoimmunity Standardization Initiative (EASI) and the International Union of Immunologic Societies/World Health Organization/Arthritis Foundation/Centers for Disease Control and Prevention (IUIS/WHO/AF/CDC). The result is the proposal of a change in the nomenclature and 25 recommendations for the harmonization of clinical and technical aspects of AAb determination, resolve discrepancies in the detection methods and stress the importance of communication between clinical specialists and the laboratory.[6]

However, what is the sense of adapting ourselves to employing a new nomenclature when we are at ease with the one we already know? In recent years, we have found that the number of new AAb associated with specific clinical conditions has continued to grow, a fact that means that we must be increasingly accurate when we refer to them. We should stress that some of these new AAb that are specific for IIM, such as anti-MDA5 (anti-melanoma differentiation-associated gene 5) antibodies and anti-HMGCR (anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase), are not associated with any IF pattern.[11,12]

Anti-Ro antibodies constitute another valid example to illustrate this case: the distinction between Ro52 and Ro60 was not defined until 1988, and they differ in their intracellular localization.[13] Anti-Ro60 antibodies have a fine speckled pattern in IF, whereas anti-Ro52 have no defined nuclear staining pattern[14]: would it not then be more useful, in this case, to say that they are specific antibodies?

On the other hand, the cytoplasmic pattern according to IF is defined like any cytoplasmic staining in HEp-2 cells, regardless of the positive or negative staining of the nuclei or mitotic cells, with 5 main pattern subgroups: 1) fibrillary; 2) speckled; 3) mitochondrial/reticular; 4) polar/Golgi apparatus; and 5) bars/rings. All should be included in the laboratory report, as well as the reactivity observed in the cytoplasm and the recognized cytoplasmic structure.[2–4] With this diversity, it may be worthwhile to change the manner in which we refer to AAb rather than to continue to utilize the term ANA.

The Special Case of Idiopathic Inflammatory Myopathies: Increasingly More Specific Autoantibodies

The formation of AAb has a cardinal role in the pathogenesis of IIM and they are markers that contribute to the diagnosis. With the relatively recent description of new AAb that are specific for IIM, it has been demonstrated that around 60% of these patients have specific AAb, such as anti-Jo-1, anti-MDA5, anti-HMGCR, anti-Mi-2, anti-TIF1 (transcription intermediary factors-1), anti-NXP2 (nuclear matrix protein 2), anti-SAE (small ubiquitin-related modifier [SUMO]-activating enzyme) and anti-SRP (signal recognition particle). At the present time, it is considered that their detection during early phases would be useful in predicting the clinical course of the disease.[11,12] It is important to point out that, according to IF, only some of these AAb were associated with a speckled or fine speckled cytoplasmic pattern; others were not detected by means of IF, but they were negative for ANA.

Due to the notable association between these AAb and the different clinical phenotypes, it is postulated that they are important not only for the classifications of IIM, but also as factors implicated in the mechanism that underlies their pathogenesis, such as induction, the perpetuation of muscle damage and the association between autoimmunity and oncogenesis.[11] Among these, anti-MDA5 has been related to certain phenotypes of dermatomyositis (DM), especially with clinically amyopathic DM and with a higher risk of rapidly progressive acute interstitial lung disease.[15,16] Anti-MDA5 is mutually exclusive with respect to other representative AAb detected in DM, in myopathy associated with cancer and in polymyositis.[11,12,15,16]

Perspectives

It is quite clear that, in the near future, we will continue to utilize the nomenclature to which we are accustomed, but it is worthwhile that we become familiar with the new proposals and adapt ourselves to them; the changes may possibly come from laboratory reports as we advance in the description of new AAb.

Changes in nomenclature is not a new situation in rheumatology; in 2012, we saw that the comprehension of the clinical and pathological characteristics of systemic vasculitis made it necessary to modify existing concepts in the International Chapel Hill Consensus Conference that revised the nomenclature of vasculitides for the purpose of offering a more appropriate nomenclature. We have gradually become adapted to this new nomenclature, and it is now the one we utilize in routine clinical practice.[17]

Therefore, if the specificity of an AAb is capable of indicating the road toward a clearer definition of the clinical characteristics, toward the prognosis and the outcome of certain conditions, we should be more specific with the terminology. This, moreover, could facilitate communication between clinicians and laboratory professionals, as well as the design of new and more dynamic diagnostic algorithms.

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