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Antinuclear factor: from diagnostic performance to predictive value for diagnosis of autoimmune diseases

Autoantibodies represent a serological markers of autoimmune diseases, and their detection and quantification have become a great laboratory instrument for both diagnostic utility and management of patients with rheumatic diseases. Considering their clinical relevance, some laboratorial immunologic findings are being included in the internationally established criteria for the diagnostic classification of many systemic autoimmune and organ-specific autoimune diseases.

Since the description of the LE cell phenomenon by Hargraves in 1948 in patients with systemic lupus erythematosus (SLE)[1] which is associated to the presence of autoantibodies specific to the histone-DNA complex, there has been a remarkable evolution of the laboratorial methodologies with an increase of their sensitivity and specificity. In fact, since the introduction of the indirect immunofluorescent (IIF) technique using animal tissue as substrate (mouse liver imprint) or more recently, of human cell lines, the progressive refinement in the techniques used for purification of autoantigens has allowed the establishment of even more sensitive methodologies to characterize autoantibody profile which include ELISA, immunoblotting, multiplex platform and proteomics.

Nevertheless, the experience in different laboratorial centers has shown that the IIF using HEp-2 cells is the gold standard test for the screening of autoantibodies in the connective tissue diseases showing increased sensitivity in relation to the murine tissue.

The introduction of human tumoral cells lines as the HEp-2 cells derived from larynx carcinoma in substitution to the murine liver tissue revealed an increasing in the reactivity spectrum of the autoantibodies in the connective tissue diseases. Besides the already recognized increase in the sensitivity of the test, the preparations using isolated cells showing various stages of the cell cycle present the following advantages over the use of animal liver: *1*. detect human autoantigens that are not present in rodent's tissue (e.g. Ro/SS-A protein); *2*. reveal new morphological immunofluorescent patterns such as the citoplasmatic (cytoskeleton, mitochondrial, ribosomal and Golgi apparatus) and those made evident only during mitosis (centromeric, spindle apparatus, centriolar); *3*. characterize pattern´s subtypes (e.g. homogeneous nucleolar, agglomerated and speckled); *4*. observe the topographical dynamics of some antigens during the cellular cycle; *5*. allows cellular manipulation by genetic engineering techniques for selective higher expression of target autoantigens.

This diversity of immunofluorescent patterns with more than 30 already described claimed the necessity of establishing standardization in the nomenclature for the emission of antinuclear antibody (ANA) reports. To that end, in 2001 the I National Consensus for autoantibodies Screening using HEp-2 Cells was published which initiated the uniformization of the nomenclature of the immunofluorescent patterns until then differing between the diverse centers that perform this test in Brazil. Thus, this first Consensus introduced topographic and morphologic criteria to be accordingly observed at the occasion of the test analysis and outlined decision algorithms for the emission of the report of nuclear patterns, nucleolar, citoplasmatic and the mitotic apparatus, as well as the establishment of a methodological standardization mainly regarding the serum screening dilutions and its final titering.[2] The II Brazilian Consensus for autoantibodies Screening using HEp-2 Cells ratified the decision algorithms for the analysis of the nuclear, nucleolar, citoplasmatic and mitotic apparatus immunofluorescent patterns, adding a new one related to the description of mixed patterns.[3] Other substantial contribution of the II Consensus was the denomination of the reports including the *citoplasmatic patterns*, now considered positive ANAs.

The increase in the sensitivity and in the antigenic repertoire allowed the extension of the IIF potentialities using HEp-2 cells in diseases related to other medical specialties such as Gastroenterology, Dermatology, Neurology and Hematology. The work by Laurino *et al*. published in this edition of the

Correspondence to: Vilma dos Santos Trindade Viana. Av. Dr. Arnaldo, 455, 3º andar, sala 3143, São Paulo-SP, *Zip Code*: 01246-903 – Brazil
Phone/Fax: (11) 30617498. E-mail: visatv@usp.br

Brazilian Journal of Rheumatology reports the experience in the application of these two consensuses in a Brazilian university hospital in which were analyzed 12.095 ANA tests in a four year period prior and after the implantation of the I and II Consensuses. This study evidenced a higher frequency of positive results after the implantation of the consensuses probably due to the introduction of new patterns in the descriptive reports of ANA, such as the citoplasmatic and those only observed during mitosis. Therewith, the data illustrated an increase of the ANA requests in other clinical specialties such as Dermatology, Gastroenterology and Hematology which could have been contemplated with the adoption of the more inclusive ANA reports.

The motivation for the organization of the 3rd Brazilian Consensus for autoantibodies Screening using HEp-2 Cells was to discuss strategies for a better standardization of the IIF technique, establish quality control, criteria, besides adequating the classification terminology of the patterns as well as explore and promote an update of its clinical associations in constant progression. The paper of Dellavance *et al*. published in this issue of the *Brazilian Journal of Rheumatology*, completely and didactically spreads this knowledge to the scientific-medical Brazilian community reflecting the vigorous research activity in the autoantibodies area.

Within this praised mobilization context to promote the progressive optimization of the ANA test, it should be considered the potencial relevance of the positivity detected among normal controls included in the tests. To that end, although the practical value of the presence of autoreactivity has been well recognized in some clinical situations, it has been underestimated in the apparently healthy individuals. Nevertheless, literature has increasingly supplied evidences of the presence of autoreactivity preceding in years the appearance of clinical manifestations associated to autoimmune diseases, providing a new perspective of the predictive value of the autoantibodies.[4-6]

Vilma dos Santos Trindade Viana
Cleonice Bueno
Jozélio Freire de Carvalho

Department of Rheumatology of Faculdade de Medicina da Universidade de São Paulo

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