REVISTA BRASILEIRA DE REUMATOLOGIA

www.reumatologia.com.br

SOCIEDADE BRASILEIRA DE REUMATOLOGIA

Editorial

Shedding fluorescent light into the antinuclear antibody world

Derramando luz fluorescente no mundo dos anticorpos antinúcleo

Autoantibodies are valuable biomarkers for the clinical research of systemic autoimmune diseases (SARD).[1] The screening for antinuclear antibodies (ANA) is frequently performed using the classic technique of indirect immunofluorescence assay on HEp-2 cells (HEp-2 ANA assay). The HEp-2 ANA assay is recommended by the American College of Rheumatology as the gold standard test for autoantibody screening.[2] The popularization and expansion in the use of this test by several specialists, for the past 15 years or so, have caused anxiety and perplexity in several cases in which a positive test does not match the clinical presentation. Some specialists in the field have suggested that the correct interpretation of the HEp-2 ANA pattern is helpful to differentiate a positive test in autoimmune and non-autoimmune individuals. In particular, the Brazilian group has taken the lead on building a nation-wide consensus of the ANA pattern nomenclature classification. This initiative has been successful and related programs have been developed in other parts of the world. On this issue, the journal brings the proceedings of the fourth Brazilian Consensus on ANA Pattern Nomenclature.

The charisma of the ANA-HEp-2 test

Probably due to historical reasons, the ANA test has gained a very strong reputation among clinicians and nonprofessionals. This may be partially understood if we revisit the 1960s and 1970s scenario when medical science began to understand the potential diagnostic role of autoantibodies. Autoantibodies were initially identified in SARD. Specifically, rheumatoid factor was identified as a marker for rheumatoid arthritis,[3] and the LE cell phenomenon was linked to systemic lupus erythematosus (SLE).[4] Soon it was realized that the basis for the LE cell phenomenon was the reaction of autoantibodies against deoxyribonucleoprotein (equivalent to what we call nowadays anti-nucleosome or anti-chromatin antibodies). The early indirect immunofluorescence assays of antinuclear antibodies (ANA) were performed on animal tissue sections and they showed limited sensitivity and a restricted array of immunofluorescence patterns. Nevertheless, the indirect immunofluorescence ANA assay soon replaced the LE Cell test and was included as one of the classification criteria for SLE.[5]

One important aspect to be considered is that the ANA test was ordered mostly by rheumatologists, nephrologists, and dermatologists; usually to test patients with high diagnostic probability for SLE. The high accuracy in the ANA results related to the pre-test probability, contributed to a favorable evaluation of the test. It is no surprise, therefore, that the ANA test achieved a prominent reputation as a useful specific diagnostic assay for SLE and related diseases.

In the 1980s there was a shift to HEp-2 cells as substrate for the assay. This added a whole new dimension to the ANA test due to the fact that it added a higher sensitivity and a vast array of new immunofluorescence patterns. It was progressively realized that, in addition to the nucleus itself, there were dozens of patterns associated with other cell compartments; including the nucleolus, cytoplasm, nuclear membrane, and mitotic apparatus. The monolayer cultures of HEp-2 cells offered a unique display of the several cellular domains integrated to the immunofluorescence system. The charming images obtained in the HEp-2 ANA assay definitely contributed the charisma of the test.

One important consequence of this shift was that autoantibodies associated with a larger spectrum of autoimmune diseases, within and beyond rheumatology, were readily detected in the HEp-2-ANA test. As a consequence, a broader group of physicians started ordering the test.

The idiopathic positive ANA syndrome

Contrast between reality and reputation-based expectation is not infrequent, and this is also true for parameters used in

0482-5004/$ - see front matter.
http://dx.doi.org/10.1016/j.rbre.2014.02.016

medicine for diagnosis and for the management of patients. In fact, contrast is precisely what we are dealing with today at the HEp-2 ANA testing. Nothing can be more unrealistic than the idea that a positive HEp-2 ANA test is a specific evidente for the diagnosis of SLE, or any other systemic autoimmune disease. However, this misconception is firmly embedded in the minds of many physicians and patients for the very reasons exposed in the previous section. In the case of the HEp-2 ANA test, the contrast comes from several factors that, include the shifts in assay sensitivity, the clinical scenario in which the test is ordered, and the phenomenon of bias due to a generalization of the scientific observations.

The HEp-2 ANA test proved to be much more sensitive than the LE cell assay and the rodent tissue-based ANA test. The gain in sensitivity was accompanied by a loss in specificity since a positive HEp-2 ANA test may be obtained in patients with several inflammatory conditions, such as infectious and neoplastic diseases, as well as in apparently healthy individuals. Several studies have consistently shown that a positive HEp-2 ANA test occurs in 13 to 20% of the normal population in relation to gender and age.[6,7] But at the same time, the HEp-2 ANA test has become more and more popular among several other specialists. Currently this test is ordered oftenly by gynecologists, neurologists, internists, psychiatrists, ENT specialists, dermatologists, gastroenterologists and pulmonologists among others.[8] The widespread use of the test results in a low pre-test trust.[9] The consequence of the relatively high frequency of positive HEp-2 ANA test in non-SARD patients plus its widespread use is a high number of "false-positive" results. This phenomenon has been described as the idiopathic positive ANA syndrome.

The idiopathic positive ANA syndrome is a double-edged sword. Many patients with muscle-skeletal complaints and other symptoms that may be associated to SARD have the opportunity to be seen by a rheumatologist because of a positive ANA result ordered by the general practitioner or other consultant. However, much stress and confusion has been caused by positive ANA results in individuals with no evidence of SARD, or in patients with an ill-defined clinical evaluation. Physicians often face difficulty in interpreting a positive ANA test in such circumstances.

The stethoscope and the three dimensions of the HEp-2 ANA test

Cardiac murmurs are well known signs of heart disease and are carefully investigated in the clinical examination. However, it is widely acknowledged that not all murmurs are clinically significant. In fact, it is well recognized that many healthy individuals present some form of heart murmur. To help in the interpretation of heart auscultation, there is a wide collection of parameters that need to be taken into consideration. For example, a low-intensity mild meso systolic murmur heard at the fourth intercostal space adjacent to the left sternal border in a 7-year old boy is probably meaningless as opposed to a rough diastolic murmur with pre-systolic surge heard at the heart apex in a 30 year-old woman. Trained cardiologists and internists will pay attention to these nuances and make decisions about further investigation accordingly.

Similarly to the HEp-2 ANA test, the stethoscope and the heart auscultation procedure represent a quite charismatic aspect in Medicine. However, charisma is not the only feature in common between the stethoscope and the HEp-2 ANA test. As in the heart auscultation, a multitude of nuances are available for interpretation in the HEp-2 ANA test. These aspects can be divided into three dimensions. The careful analysis of these offers a useful guide for the correct interpretation of this serological evaluation. The first and most obvious one is the qualitative result: negative or positive. The second one, also quite intuitive, is semi quantitative and refers to the concentration of autoantibodies in the analyzed sample. This dimension is usually expressed as the titer and reactivity of antibodies. A higher titer means the higher the concentration of autoantibodies in the sample. The intuitive rationale that a higher titer is more clinically relevant than a lower titer HEp-2 ANA results is generally correct, however there are several exceptions that make this a very relative rule. In fact, there are healthy individuals with a positive HEp-2 ANA test at dilutions as high as 1/5.120, as well as SLE patients with a positive test results with low titer, such as 1/80 or 1/160.

The third dimension of the HEp-2 ANA test refers to the immunofluorescence pattern and offers a very rich collection of information. It reflects the topographic distribution of the antigens recognized by the autoantibodies present in the sample. The several autoantigens have specific distribution patterns within the cell and this relationship is enriched if one analyzes the dynamic behavior along the successive stages of the cell cycle. The careful analysis of the immunofluorescence pattern frequently allows to suggest certain autoantibody specificities with variable degrees of confidence. However, not all immunofluorescence patterns allow a strong affirmation about possible associated autoantibody specificities. The strength of the relationship between a given immunofluorescence pattern and the associated autoantibodies varies.

Great emphasis has been given to the distinctive HEp-2 ANA patterns in which there is staining of the mitotic plates. Since chromatin, the very substance of chromosomes, is a major target of SLE associated autoantibodies (anti-native DNA, anti-nucleosome, anti-histones), patterns circling the whole nucleus and the metaphase plate might be interpreted as indicative of anti-chromatin or anti-native DNA antibodies. However, morphological nuances disclose a heterogeneous scenario. A smooth hyaline staining of the mitotic plates and a homogeneous staining of the interphase nucleus are in fact compatible with anti-native DNA and anti-nucleosome antibodies. In contrast, a dense fine speckled staining of the interphase nucleus and a dense coarse staining of the mitotic plates are compatible with anti-DFS70/LEDGF antibodies (10,11). The first pattern indicates a strong possibility of SLE and the second pattern almost discards this diagnosis.[6,12]

The relationship between HEp-2 ANA patterns and specific autoantibodies achieves a high degree of reliability in complex patterns. For example, autoantigen distribution occurs in more than one cell compartment in such a dynamic and peculiar fashion throughout the cell cycle that makes it virtually unique to that autoantigen. Since very few macromolecules share the same "cellular choreography", very specific associations can be observed. Examples of such situation include the

centromere pattern,[13] the proliferating cell nuclear antigen (PCNA) pattern (14), the NuMA-1 and NuMA-2 patterns,[15,16] the CENP-F pattern,[17] and the Scl-70 pattern.[18]

The importance of the correct evaluation and classification of the several HEp-2 ANA patterns was soon acknowledged by several specialists in our country. The first Brazilian Consensus on HEp-2 ANA Pattern took place in the year 2000,[19] by initiative of Paulo Francescantonio, from Universidade Católica de Goiás, under the stimulus of Paulo Leser, from Escola Paulista de Medicina. Thereafter, three editions of the Brazilian Consensus have taken place, each one advancing and complementing the achievements of the previous editions.[20-22] In each edition, several specialists from academic and private laboratories discuss thoroughly several aspects, including the definition, nomenclature, immunological associations, and clinical relevance of the several HEp-2 ANA patterns. Every edition has issued a final document approved by all participants, all of which have been widely publicized in diverse media. The recommendations of the Consensus Group have been adopted by the majority of clinical and university laboratories in the country. Interested clinicians have had access to this information via scientific articles, websites, and lectures. The broadcast of the Consensus recommendations has largely contributed to improve the ability in the interpretation and characterization of the several HEp-2 ANA patterns by analysts across the country. This issue of Revista Brasileira de Reumatologia brings the proceedings of the fourth Brazilian Consensus on HEp-2 ANA Pattern, occurred at the XXIX Brazilian Congress of Rheumatology in 2012 in Vitoria. In addition to important discussions regarding critical aspects in the methodology for determination of autoantibodies, novel HEp-2 ANA patterns were characterized and incorporated into the classification algorithm.

Related initiatives have been undertaken in different parts of the world. The French initiative, published in 2005, reports the progressive improvement and harmonization in the methodology for the execution of the assay, as well as for titer and pattern report by over 600 laboratories across the country.[23] Only nuclear patterns were considered: homogenous, speckled, and centromeres. The German initiative emphasized the technical recommendations for assay performance and considered seven nuclear patterns (nucleolus was considered a type of nuclear pattern) and four cytoplasmic patterns. The recommendations were published in 2009, including a detailed and ingenious proposition to associate HEp-2 ANA patterns, autoantibody specificities and potential diseases.[24] The North European initiative is the one that has the most similarities with the Brazilian Consensus. Specialists from the Denmark, United Kingdom, and Sweden are involved in this initiative. Its recommendations were published in 2010 and offer an extremely detailed array of nuclear, nucleolar, cytoplasmic, and mitotic apparatus patterns. High quality pictures are available for each pattern, and associations between autoantibody specificities and clinical associations are established.[25] Most recently, the Argentinian initiative has been published and addresses a carefully organized algorithm that has large resemblance to the Brazilian Consensus.[26]

These independent initiatives in several countries reflect the importance of the subject. In addition it reflects the concern of specialists for the need to match the information provided by the analysis in charge of the HEp-2 ANA test. In fact, these several isolated initiatives indicate that the time has come to build a World Consensus on ANA Pattern. This observation has been taken into account by the Autoantibody Standardization Committee, along with the International Union of Immunology Societies(IUIS) and the World Health Organization (WHO). Accordingly, the Autoantibody Standardizing Committee has commissioned the 12th International Workshop on Autoantibodies and Autoimmunity (IWAA) to implement the International Consensus on an ANA Patterns (ICAP) workshop. With the participation of renowned specialists from all over the world, the ICAP will take place during the first day of the 12th IWAA, which will occur in São Paulo from August 28th to 30th 2014. This will be a landmark event that shall pave the way for significant improvement in the serological diagnosis of systemic autoimmune diseases.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Tan EM. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. Adv Immunol 1982; 33:167-240.
2. Solomon DH, Kavanaugh AJ, Schur PH. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum 2002; 47(4):434-44.
3. Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep corpuscles. Acta Pathol Microbiol Scand 1940; 17(2):172-88.
4. Hargraves M, Richmond H, Morton R. Presentation of two bone marrow components, the tart cell and the LE cell. Mayo Clin Proc 1948; 23(2):25-8.
5. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF et al: The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; 25(11):1271–7.
6. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011; 63(1):191–200.
7. Satoh M, Chan EK, Ho LA, Rose KM, Parks CG, Cohn RD et al: Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012; 64(7):2319-27.
8. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. Am J Med 2013;126(4):342-8.
9. Meroni PL, Chan EK, Tincani A, García de la Torre I, Andrade LE: Antinuclear antibody test: when to order? Am J Med. 2013 126(10):e17. http://dx.doi.org/ 10.1016/j.amjmed.2013.04.022
10. Ochs RL, Stein TW Jr, Peebles CL, Gittes RF, Tan EM. Autoantibodies in interstitial cystitis. J Urol 1994;151(3):587-92.
11. Dellavance A, Viana VS, Leon EP, Bonfa ES, Andrade LE, Leser PG. The clinical spectrum of antinuclear antibodies associated with the nuclear dense fine speckled immunofluorescence pattern. J Rheumatol 2005; 32(11):2144-9.

12. Mahler M, Parker T, Peebles CL, Andrade LE, Swart A, Carbone Y, et al. Anti-DFS70/LEDGF antibodies are more prevalent in healthy individuals compared to patients with systemic autoimmune rheumatic diseases. J Rheumatol 2012;39(11):2104-10.
13. Moroi Y, Peebles C, Fritzler MJ, Steigerwald J, Tan EM. Autoantibodies to centromere (kinetochore) in scleroderma sera. Proc Natl Acad Sci USA 1980; 77(3):1627-31.
14. Takasaki Y, Fishwild D, Tan EM. Characterization of proliferating cell nuclear antigen recognized by autoantibodies in lupus sera. J Exp Med 1984;159(4):981-92.
15. Andrade LE, Chan EK, Peebles CL, Tan EM. Two major autoantigen-antibody systems of the mitotic spindle apparatus. Arthritis Rheum 1996; 39 (10):1643-53.
16. Whitehead CM, Winkfein RJ, Fritzler MJ, Rattner JB. The spindle kinesin-like protein HsEg5 is an autoantigen in systemic lupus erythematosus. Arthritis Rheum 1996;39(10):1635-42.
17. Casiano CA, Humbel RL, Peebles C, Covini G, Tan EM. Autoimmunity to the cell cycle-dependent centromere protein p330d/CENP-F in disorders associated with cell proliferation. J Autoimmun 1995; 8(4):575-86.
18. Dellavance A, Gallindo C, Soares MG, da Silva NP, Mortara RA, Andrade LE. Redefining the Scl-70 indirect immunofluorescence pattern: autoantibodies to DNA topoisomerase I yield a specific compound immunofluorescence pattern. Rheumatology (Oxford, England) 2009;48(6):632–7.
19. Dellavance A. Gabriel Jr A, Cintra AFU, Ximenes AC, Nuccitelli B, von Mühlen CA et al. The first Brazilian Consensus for Standardization of ANA in HEp-2 cells. J Bras Patol Med Lab 2002; 38(3):207-16.
20. Dellavance A, Gabriel Jr G, Cintra AFU, Ximenes AC, Nuccitelli B, Taliberti BH et al. II Brazilian Consensus on Antinuclear Antibodies in HEp-2 Cells. Definitions for standardization of autoantibody testing against the nucleus (ANA HEp-2), nucleolus, cytoplasm, and mitotic apparatus, as well as its clinical associations. Rev Bras Reumatol 2003;43(3):129-40.
21. Dellavance A, Gabriel Jr A, Nuccitelli B, Taliberti BH, von Mühlen CA, Bichara CDA et al. Third Brazilian Consensus for autoantibodies screening in HEp-2 cells (ANA). Recommendations for standardization of the autoantibody testing in HEp-2 cells, quality control, and clinical associations. Rev Bras Reumatol , 2009; 49(2):89–109.
22. Francescantonio PLC, Cruvinel WM, Andrade LEC, Dellavance A, Taliberti BH et al. IV Brazilian Guidelines for autoantibodies on HEp-2 cells. Rev Bras Reumatol 54: 44-50.
23. Pham BN, Albarede S, Guyard A, Burg E, Maisonneuve P. Impact of external quality assessment on antinuclear antibody detection performance. Lupus 2005;14(2):113-9.
24. Sack U, Conrad K, Csernok E, Frank I, Hiepe F, Krieger T, et al. Autoantibody detection using indirect immunofluorescence on HEp-2 cells. Ann N Y Acad Sci 2009;1173:166-73.
25. Carballo GO, Igénito FB, Ginaca AA, Carabajal P, Costa MA, Balbaryski J. First Argentine Consensus for standardization of Antinuclear Antibodies by Indirect Immunofluorescence–HEp-2. Acta Bioquím Clín Latinoam 2012;46(1):3-13.
26. Wiik AS, Hoier-Madsen M, Forslid J, Charles P, Meyrowitsch J. Antinuclear antibodies: a contemporary nomenclature using HEp-2 cells. J Autoimmun 2010;35(3):276-90.

Luís Eduardo Coelho Andrade[a,b,c]
[a] *Rheumatology Division, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil*
[b] *International Union of Immunology Societies (IUIS)*
[c] *World Health Organization (WHO)*
E-mail: rbreumatol@terra.com.br (L.E.C. Andrade).