Advances in Rheumatology

POSITION ARTICLE AND GUIDELINES

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VI Brazilian consensus guidelines for detection of anti-cell autoantibodies on HEp-2 cells

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Abstract

Background: The VI Brazilian Consensus on Autoantibodies against HEp-2 cells for determination of autoantibodies against cellular constituents on HEp-2 cells was held on September, 2019, in Fortaleza (CE, Brazil). The guidelines in this edition were formulated by the group of Brazilian experts discussing the classification of complex patterns, the classification of the nuclear discrete dots (few and multiple), the identification of the discrete fine speckled pattern (AC-4a) and improvements on the ANA report.

Mainbody: Sixteen Brazilian researchers and experts from universities and clinical laboratories representing the various geographical regions of Brazil participated in the meeting. Four main topics were discussed: (1) How to classify patterns with fluorescence in more than one cell compartment considering three relevant categoris: composite patterns, mixed patterns and multiple patterns; (2) The splitting of the discrete nuclear dots pattern into the multiple discrete nuclear dots (AC-6) and few discrete nuclear dots (AC-7) patterns, respectively; (3) Inclusion of a novel nuclear pattern characterized by discrete fine speckled pattern highly associated with antibodies to SS-A/Ro60, classified as AC-4a. In addition, adjustments on the Brazilian Consensus nomenclature were implemented aiming to harmonize the designation of some patterns with the International Consensus on ANA Patterns (ICAP). Furthermore, the designations of the PCNA-like pattern (AC-13), CENP-F-like pattern (AC-14) and Topo I-like pattern (AC-29) were adjusted in accordance to ICAP. Finally, there was a recommendation for adjustment in the test report in order to address the status of nuclear envelope staining. For all topics, the aim was to establish specific guidelines for laboratories and

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clinicians. All recommendations were based on consensus among participants. All recommendations from the V Consensus were maintained and there was relevant progress in the BCA/HEp-2 guidelines and further harmonization with ICAP.

Conclusion: The VI BCA/HEp-2 edition was successful in establishing important recommendations regarding the classification of complex patterns, in supporting the identification of a novel pattern within the AC-4 group and in the harmonization process with the ICAP terminology.

The V Brazilian Consensus for determination of Autoantibodies against HEp-2 cells (BCA) started the discussion about the harmonization between the recommendations of BCA [1] and the International Consensus on ANA Patterns (ICAP) [2]. The V BCA/HEp-2 established the basis for an overall harmonization with the ICAP for interpretation of IFA HEp-2, filling important gaps between both initiatives.

Actually, the BCA/HEp-2 recognizes all patterns classified by ICAP except for AC-28 [1], and includes additional patterns not recognized by ICAP. BCA/HEp-2 harmonizes this point by creating preliminary alphanumeric codes (BAC-#: Brazilian anti-cell autoantibodies) to classify the patterns not yet recognized by ICAP such as the *quasi*-homogeneous speckled nuclear pattern (BAC-3) and the reticular coarse nuclear pattern (BAC-4) [1].

The V BCA sustained the original arrangement of groups of patterns (nuclear, nucleolar, cytoplasmic, mitotic and complex patterns) in the classification tree [1]. Unlike the ICAP classification tree, which includes the group of nucleolar patterns within the nuclear [2], BCA prefers to keep the nucleolar patterns in a separate group, aiming to drive the attention of the analyst to classify the different patterns with focus to positivity of the four cell compartments (nucleus, nucleolus, cytoplasm and mitotic apparatus) and dividing cells [1].

The BCA adapted the local test designation to anti-cell autoantibodies (ANA HEp-2), in Portuguese "*FAN—Fator Antinúcleo*" and adopted the abbreviation HEp-2 IFA, for Indirect Immunofluorescence Assay on HEp-2 cells. This decision aimed the harmonization with the ICAP recommended designation HEp-2 IFA used in the most recent publications [1, 3, 4]. There was also an adaptation on the recommendations for the test report, aiming greater practicality for the clinician's interpretation [1]. The recommendation was that the test report expresses on the top the name of each observed pattern followed by the ICAP/BAC code and the respective titer. The V BCA/HEp-2 maintained the recommendation to discriminate the fluorescence pattern in each cell compartment and at the chromosome metaphase plate [1]. Various recommendations on the technical procedure and the use of quality control strategies were maintained in the V BCA/HEp-2 [1]. It was emphasized that different HEp-2 slide brands and even different lots of the one brand may present variation in the display of some patterns, and this should be controlled by assaying a panel of samples known to yield the most relevant patterns [1, 5]. The screening dilution was recommended as 1/80 and the need for titration of the conjugate with each new brand and each new lot of the same brand was emphasized. This action is very important to assure consistency of titer in kits of different lots and especially if a different slide brand is used [1, 5]. Finally, based on the IV BCA recommendation, the V BCA also emphasized the need to choose carefully the method for autoantibodies identification in antigen-specific immunoassays, considering the high sensitivity of enzyme-linked immunosorbent assays (ELISA) chemiluminescent immunoassays and other solid-phase immunoassays, which may generate inappropriate positive results in some samples. [1, 5].

The sixth edition of the BCA/HEp-2 maintained the focus on the harmonization processes with ICAP and additionally addressed the discussion about relevant topics related to the pattern classification on the day-to-day routine, which have important repercussion for the clinicians who interpret the test. In this context, the VI BCA/HEp-2 discussed the classification of complex patterns previously designated as mixed patterns on III BCA/HEp-2 [6]. Additional items were the suggestion of splitting the classification of the nuclear discrete dots pattern, the classification for AC-4a pattern with peculiar fluorescence configuration suggestive of anti-SS-A/Ro60 autoantibodies, and finally an indication for obligatory reporting the status of the nuclear envelope staining. This paper presents the recommendations of the VI BCA/HEp-2, enabling Brazilian clinical laboratories to update the latest recommendations, as well as, to provide an update for clinicians who order and interpret the test.

Methods

On September 4, 2019, during the XXXVI Brazilian Congress of Rheumatology held in Fortaleza (CE, Brazil), 16 experts on the HEp-2 IFA test from university centers and clinical laboratories from different areas in Brazil participated in a full-day workshop with the purpose of discussing and approving the new BCA/HEp-2

recommendations. The selected discussion points were previously presented to the members and have been selected according to comments and suggestions directed to the BCA/HEp-2 team: (1) definition of the group of complex patterns and its subdivisions; (2) sub classification of the nuclear discrete dots pattern; (3) classification of a subgroup of BAC-4 pattern highly associated with anti-SS-A/Ro60 antibodies; (4) harmonization of the nomenclature for "antigen-like" patterns and (5) improvement in the HEp-2 IFA report structure.

During the VI BCA/HEp-2 session, the group of specialists approached the problems that had been presented to members and widely discussed in order to reach a consensus among several participants. Discussions were based on previous review of the literature concerning the subjects of interest, as well as presentation of relevant data by specially designated members. All topics underwent broad discussion followed by a voting process or spontaneous consensus. All points in the agenda achieved successful consensus as follows.

Recommendations

Definition of group of complex patterns and its subdivisions

The reactivity of autoantibodies is not limited to the recognition of autoantigens in a single cell compartment (nucleus, nucleolus, cytoplasm or mitotic apparatus). There are different possibilities, for example, more than one autoantibody reacting with different cell compartments, more than one autoantibody reacting with antigens in the same cell compartment, or a single autoantibody recognizing an antigen in different cell compartments. Each of these situations display distinct morphological scenarios that may be classified as multiple, mixed and composite patterns, respectively.

The II BCA/HEp-2 included in the classification algorithm the group of mixed patterns, classified as patterns with staining of more than one cell compartment (fluorescence of nucleus, nucleolus, cytoplasm or mitotic apparatus) [7]. In view of the complexity and relevance of this group of patterns and the need to update this classification, the VI BCA/HEp-2 discussed this group of complex patterns, discriminating the various possible scenarios. It is noteworthy that an expressive part of patterns in this group has important clinical relevance in the medical investigation and, therefore, it is necessary to coherently identify this group of patterns in the clinical laboratories.

For the general understanding of the classification algorithm for the group of complex patterns introduced in the VI BCA/HEp-2, it is important to highlight that in most situations, the HEp-2 IFA patterns are elementary, that is, they reflect the staining of a single autoantibody in a single cell compartment and have conserved basic morphological characteristics that allow their identification according to the ICAP guidelines. However, in the day-to-day routine there are situations that escape this simple rule, with morphological characteristics that do not allow the classification as elementary patterns. These patterns are classified as complex and the three possibilities are detailed below. The first situation is when we observe the concomitant presence of two or more elementary concomitant patterns in the same biological sample, and it is possible to distinguish each one of them. In this case the complex pattern is classified as a multiple pattern, for example, a sample containing anti-SS-A/Ro (yielding AC-4 pattern) and anti-centromere (yielding AC-3 pattern) antibodies. Another example of multiple patterns is the simultaneous presence of anti-Sp100 (yielding AC-6 pattern) with anti-mitochondria (yielding AC-21 pattern) antibodies. The morphological analysis of such cases allows the characterization of both elementary patterns individually, and the codes of each one is presented in the report (AC-4/AC-3 and AC-6/AC-21, respectively). Thus, multiple patterns are those characterized by the presence of more than one AC pattern, and it is clearly possible to identify each individual pattern. The schematic representation of the concept of multiple patterns can be seen in Fig. 1.

Another situation occurs when we have a mixture of autoantibodies in the sample reacting with different autoantigens in the same cell compartment, and the resultant morphological aspect does not reflect the individual classic patterns traditionally associated with each of the autoantibodies. Such a situation can be observed, for example, in a sample with the presence of anti-nDNA and anti-U1-RNP antibodies. In such a case, the resultant morphological aspect frequently does not allow the recognition of AC-1 (traditionally associated with anti-nDNA) and AC-5 (traditionally associated with anti-U1-RNP). The morphology resulting from the coexistance of these two autoantibodies does not allow the identification of respective elementary patterns. In these cases the observed pattern is classified as Mixed Pattern and it is not possible to indicate the corresponding AC codes. For these cases, the BCA/HEp-2 Consensus recommends the indication on the report of the areas with reactivity, and the description of fluorescence as a mixed pattern at the respective cell compartment, e.g., nuclear mixed pattern.

The third possibility of complex patterns corresponds to composite patterns. These are situations where a single autoantibody recognizes elements in different cell compartments in such a characteristic way that such composite configuration is highly associated with that autoantibody. For example, anti-NuMa antibodies react with the mitotic spindle in metaphase cells and with

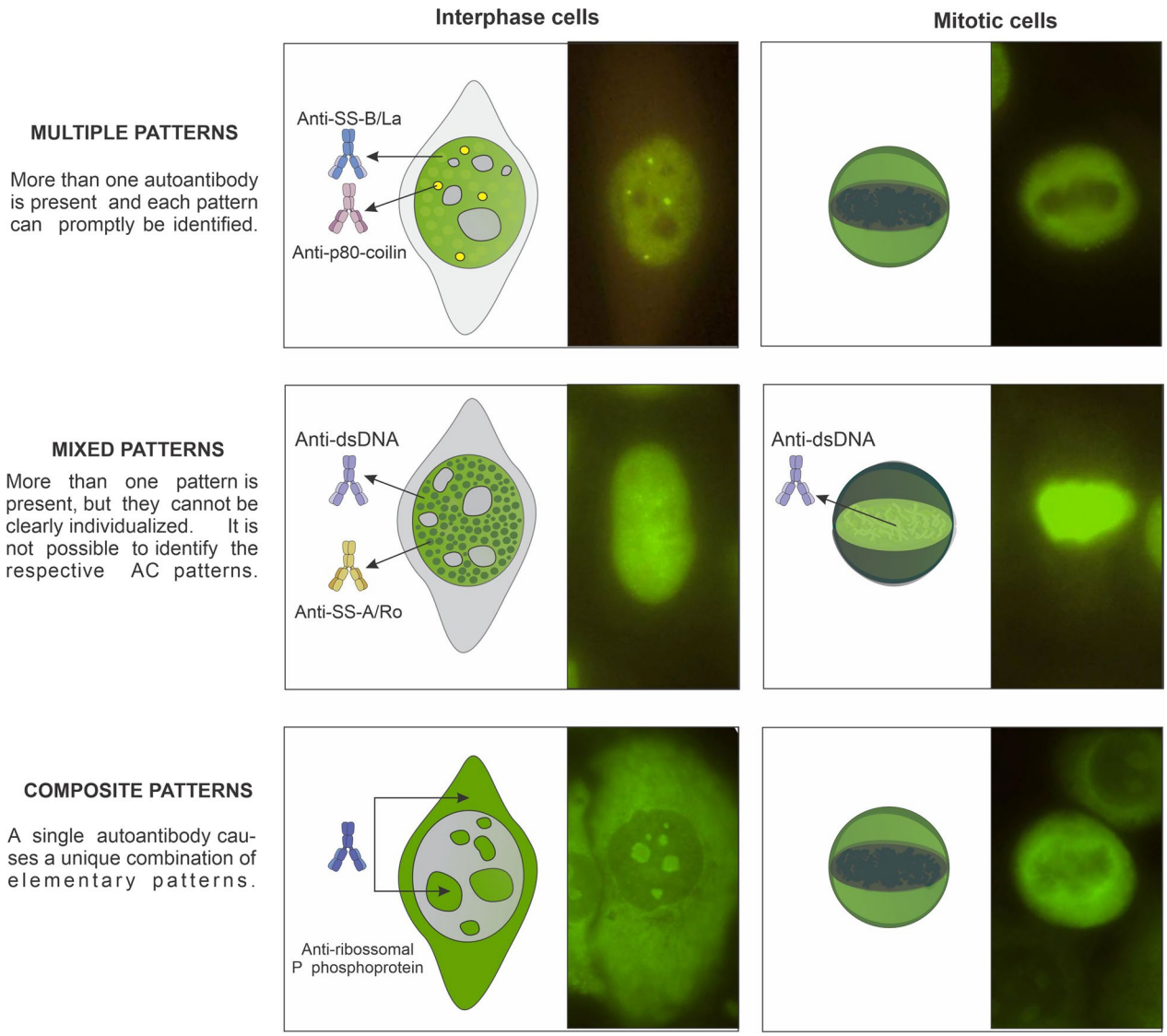


Fig. 1 Schematic conception of three examples of complex patterns (multiple, mixed and composite) showing relevant details about staining on interphase and mitotic cells

the cell nucleus in interphase cells, yielding a composite pattern that has been classified as AC-26 in ICAP. Another example is the composite pattern caused by antibodies to ribosomal-P protein that comprehend a dense fine speckled in the cytoplasm and a faint homogeneous staining in the nucleoli. The VI BCA/HEp-2 has grouped the composite patterns in a separate branch of the classification tree, with remarks for their related immunological identity, characteristics of the fluorescence pattern and clinical relevance. Figure 1 provides a schematic representation of the three categories in the group of complex patterns.

Subclassification of the nuclear discrete dots pattern

The VI BCA/HEp-2 recommends the classification of the discrete nuclear dots patterns into multiple discrete nuclear dots (AC-6) and few discrete nuclear dots (AC-7). The previous recommendation for classification in a single group came from the III BCA/HEp-2 [8] and was based of occasional difficulty in discriminating both patterns. However, considering the differences in the clinical relevance between the two patterns and that most Brazilian laboratories report expertise in distinguishing them, the VI BCA/HEp-2 now recommends, in harmonization with ICAP, the discrimination of the

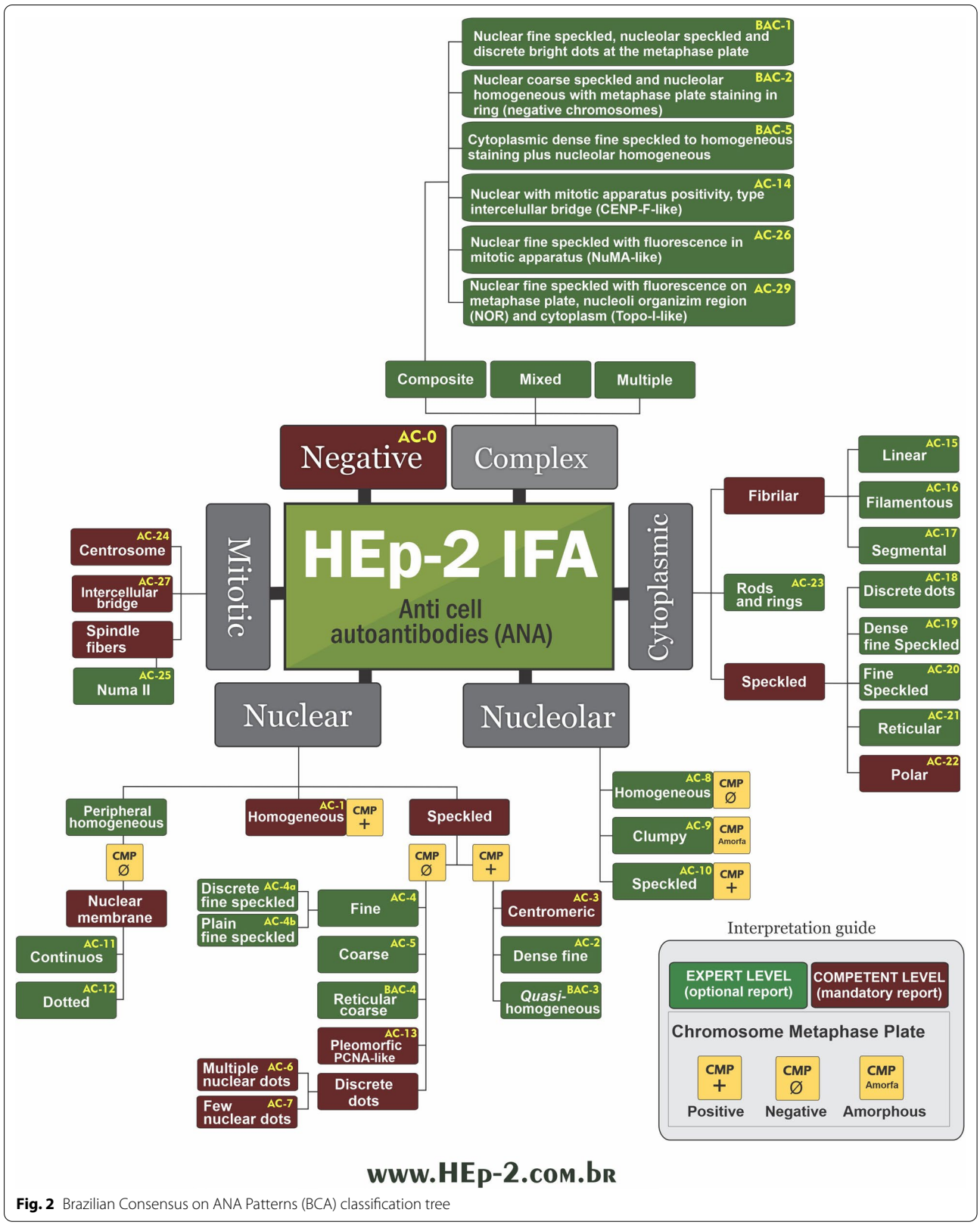


Fig. 2 Brazilian Consensus on ANA Patterns (BCA) classification tree

two patterns classification on BCA decision tree (Fig. 2) [9].

The multiple discrete nuclear dots (AC-6) consists in a spectrum of IFA patterns characterized by a variable size and number of nuclear dots (6–20) distributed over the cell nucleus, sparing the nucleoli, and the chromosomes in mitotic cells [10, 11]. This pattern is usually observed in the presence of anti-Sp100 and/or anti-PML antibodies. The AC-6 pattern may occur in patients with primary biliary cholangitis (PBC), autoimmune hepatitis, some forms or myositis and undifferentiated connective tissue diseases. The AC-6 pattern may occur in association with other patterns [12], especially with the cytoplasmic reticular/AMA pattern (AC-21) in PBC.

There are at least three different antigenic targets related with the multiple nuclear dots pattern (AC-6). Sp100 is the main autoantigen and consists of a 53 kDa nuclear protein ([29]) related with the cell transcription activation [13]. Another autoantigen, the promyelocytic leukemia protein (PML), is a transformation and cell growth suppressing protein, expressed in promyelocytic leukemia cells, that co-localizes with Sp-100 in nuclear domains (dots), coexisting in many cases in the same serum [14, 15]. Another autoantigen associated with AC-6 pattern is the 140 kDa nuclear matrix protein 2 (NXP-2), previously identified as MJ autoantigen [16] with diverse nuclear functions including RNA metabolism and maintenance of nuclear architecture [17]. NXP-2 recruits and activates p53 protein regulating the cellular senescence [18], and the autoantibody against it has been described as an important serological marker of juvenile dermatomyositis [16].

In a ICAP publication on the clinical relevance of HEp-2 IFA patterns [9], the multiple nuclear discrete dots pattern (AC-6) was associated with a broad spectrum of diseases, including PBC, autoimmune myopathy/dermatomyositis (AIM/DM), as well as other inflammatory conditions. The titer can vary over the years and there seems to be no correlation between the antibodies titer and the disease activity [12].

If PBC is clinically suspected, it is recommended to perform follow-up tests for anti-Sp100 (and PML/Sp140) antibodies; in particular anti-Sp100 antibodies have good clinical association with PBC and have added value, especially when associated with anti-mitochondria antibodies (AMA) [9, 19, 20].

Considering the suspicion of dermatomyositis, it is recommended to perform a follow-up test for anti-MJ/NXP-2 antibodies, which are highly specific for autoimmune myopathies (AIM), and they, are found in up to one third of patients with juvenile DM. They were also reported to be associated with malignancies in adult AIM patients [9, 21–23].

The few discrete nuclear dots pattern (AC-7) consists in an IFA pattern characterized by 1 to 6 nuclear dots distributed over the interphase cell nucleus, often in close proximity to nucleoli. Characteristically, cells in the late S/G2 phase of the cell cycle present very few and large dots, whereas cells at G1 present a higher number of smaller dots (4–6) and the metaphase chromatin in mitotic cells show no dots in most cases [24]. These nuclear dots are Cajal bodies (formerly known as coiled bodies) and the target antigen has been identified as an 80 kD protein localized predominantly in the Cajal body and the primary antigen is p80-coilin [25].

Another autoantigen in the Cajal body related to the AC-7 pattern is the survival of motor neuron (SMN), related with critical role in assembly of the snRNPs which are important for pre-mRNA splicing. Deletion or mutation of SMN is known to cause spinal muscular atrophy [26, 27].

Concerning the clinical relevance, the AC-7 pattern has low positive predictive value for autoimmune diseases [9, 28, 29]. In fact, AC-7 and anti-p80-coilin antibodies appear not to be associated with any specific clinical condition. Commercial immunoassays for anti-p80-coilin antibodies are not available at the moment [9]. The comparison of the main characteristics of the AC-6 and AC-7 patterns can be seen in Fig. 3.

Classification of nuclear discrete fine speckled pattern

The VI BCA/HEp-2 recommends for the expert level laboratories (optional report) the classification of the nuclear discrete fine specked pattern, in Portuguese "*nuclear pontilhado fino de pontos distintos*". This pattern belongs to the group of speckled nuclear patterns, such as the fine (AC-4), coarse (AC-5), and reticular-coarse speckled pattern, which are differentiated according to the size, number and distribution of the speckles in interphase cells. The distinctive features of all speckled patterns are the granular staining of the nucleoplasm of interphase cells and no staining of the metaphase chromatin plate [2].

The coarse speckled pattern (AC-5) is characterized by dense intermediate sized speckles in the nucleus associated with larger bright speckles throughout the nucleoplasm of interphase cells. The nucleoli and mitotic chromatin are not stained. The fine speckled pattern shows a fine granular variably dense speckled staining of the nucleus in a uniform distribution. Nucleoli may stain (e.g., SS-B/La or Ku antibodies) or are negative. The chromatin plate is usually negative [2]. The reticular coarse speckled pattern shows large speckles arranged in a netwise configuration across the nucleus and has been designated by some authors as the nuclear matrix pattern classified as BAC-4 by the Brazilian Consensus [1].

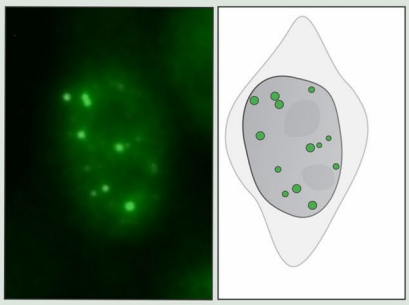
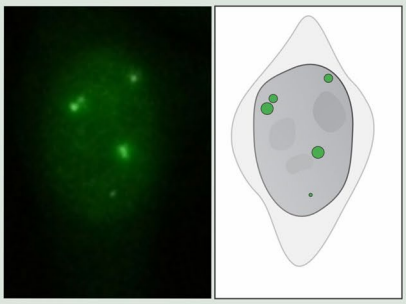
	AC-6	AC-7
Associated Antigens	Sp-100 PML proteins MJ/NXP-2	p80-coilin SMN
IFA Pattern description	Countable discrete nuclear speckles (6 to 20 nuclear dots per cell) distributed over the cell nucleus, sparing the nucleoli, and the chromosomes in mitotic cells. The other cell compartments are not fluorescent and metaphase chromatin in mitotic cells usually negative. These are known as the PML bodies.	Countable discrete speckles (1 to 6 nuclear dots/cell in most cells). These are known as Cajal bodies or coiled bodies. The other cell compartments are not fluorescent and metaphase chromatin in mitotic cells usually negative.
Aspect of Interphase cells and schematic view		
Clinical relevance	Broad spectrum of autoimmune diseases, including primary biliary cholangitis, autoimmune myopathy / dermatomyositis, as well as other inflammatory conditions.	Low positive predictive value for any disease.
Follow-up test	If primary biliary cholangitis is clinically suspected, it is recommended follow-up tests for anti-Sp100 (and PML/Sp140) antibodies. If dermatomyositis is clinically suspected, it is recommended follow-up test for anti-MJ/NXP-2 antibodies.	Specific immunoassays for these autoantibodies are currently not commercially available.

Fig. 3 Features of AC-6 and AC-7 patterns based on ICAP (www.anapatterns.org)

In contrast, the nuclear discrete fine specked pattern is characterized by myriad very tiny speckles uniformely distributed all over the interphase nucleus. Due to its distinctive appearance and strong association with anti-SS-A/Ro60 antibodies this pattern was incorporated into the BCA/HEp-2 classification tree as AC-4a. AC-4a is characterized by myriad of tiny discrete speckles (Fig. 4) distributed across the nucleoplasm and not staining the chromatin mass in mitotic cells. The nucleoli, cytoplasm and mitotic apparatus are also not stained. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass not stained [30, 31].

The AC-4a pattern is highly associated with anti-SS-A/Ro60 antibodies, since 98.8% of 86 sequentially selected AC-4a serum samples presented the SS-A/Ro60 reactivity. The AC-4a classification by laboratories is of special

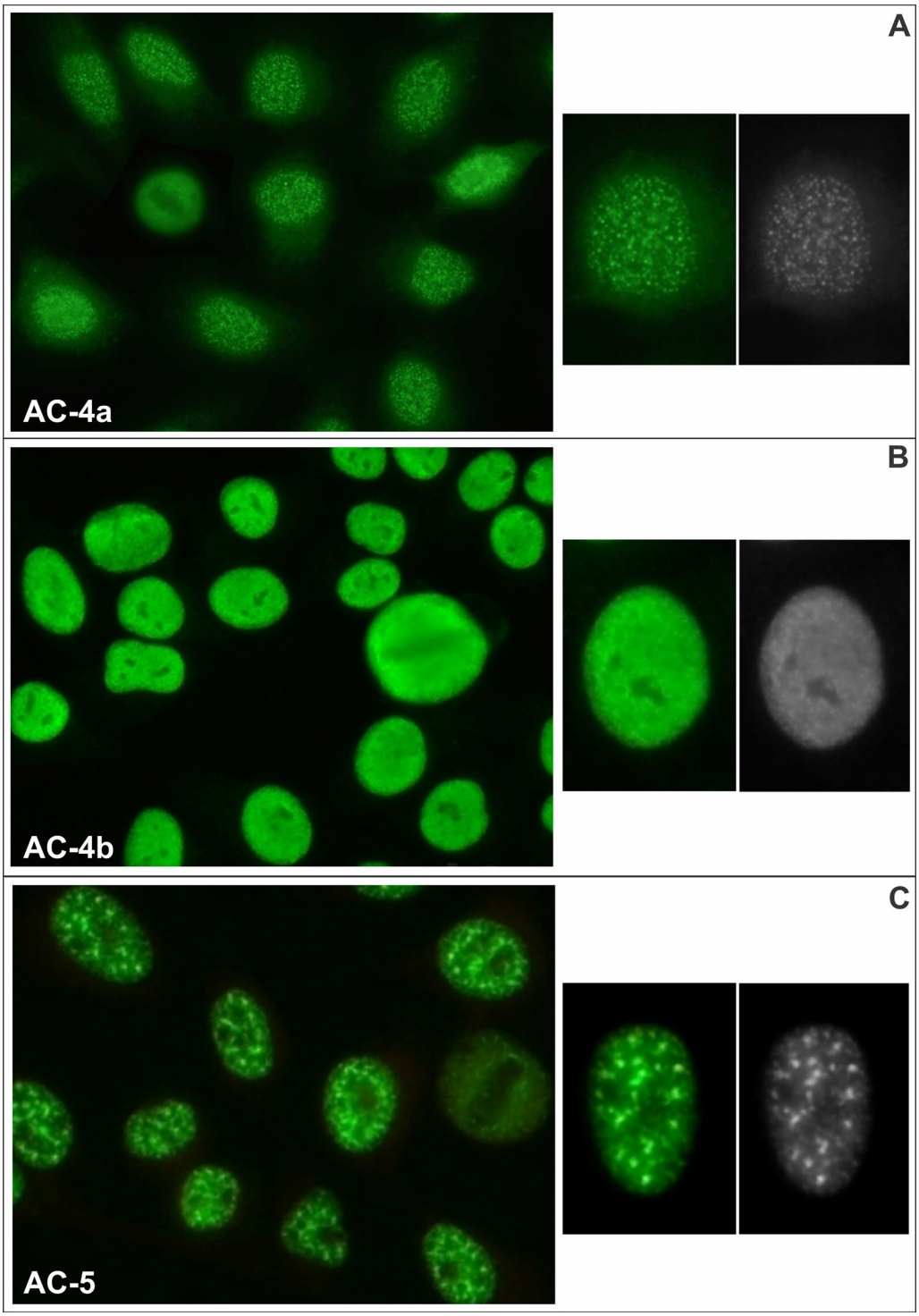


Fig. 4 Comparison of nuclear speckled patterns. **A**. Nuclear discrete fine specked pattern (AC-4a). **B**. Nuclear plain fine speckled pattern (AC-4b). **C**. Large/coarse speckled nuclear pattern (AC-5). Panels on the right side depict in greater details the fine characteristics of each pattern on interphase cells. Image **A**: credits to Alessandra Dellavance. Images **B** and **C**: credits to ICAP (Werner Klotz, Alessandra Dellavance and Luis Andrade)

clinical interest because it offers the opportunity of suspecting of the presence of anti-SS-A/Ro60 antibodies in the sample, indicating the appropriate antigen-specific immunoassays for the autoantibody confirmation. Fine speckled nuclear patterns that do not show the peculiar features of the AC-4a pattern can be classified as plain fine speckled pattern (AC4-b) that is much less frequently associated with anti-SS-A/Ro60 and may be associated with a variety of autoantibodies, including Mi-2, TIF-1ɣ, and Ku. In addition, frequently no defined specificity can be demonstrated in samples with the AC-4b, which is one of the most frequent patterns in healthy individuals with a positive HEp-2 IIF result. AC-4a and AC-4b are under the umbrella of AC-4 pattern and the latter classification can be used for samples where one cannot confidently dicriminate AC-4a and AC-4b [11].

The AC-5 pattern is associated with different antigenic targets (hnRNP, U1RNP, Sm, RNA polymerase III) related to distinct systemic autoimmune rheumatic diseases (SARD), in particular systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), SSc-AIM overlap syndrome, and undifferentiated connective tissue disease (UCTD) [9, 32]. The same can be said about all AC-4 group including many antigenic targets (SS-A/Ro, SS-B/La, Mi-2, TIF1γ, TIF1β, Ku) present in distinct SARD, in particular Sjögren's syndrome (SjS), SLE, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, congenital heart block, dermatomyositis, SSc, and SSc-AIM overlap syndrome [9]. Considering the differences in the antigenic targets associated with AC-4 and AC4a patterns, the recommendation of reporting the AC-4a on report is helpful since this pattern will drive the specific investigation of anti-SS-A/Ro60 antibodies in the patient serum and save time in the clinical investigation.

Harmonization of the nomenclature for "Antigen-Like" patterns

ICAP recommends that no pattern is designated strictly according to the cognate autoantigen of the associated autoantibodies. This aims to avoid an inexact notion that patterns can be 100% specific for any given autoantibody. The BCA/HEp-2 has the same understanding and the Brazilian algorithm has been established strictly based on the morphological characterization of patterns.

However, some patterns were named after their most relevant autoantibody association, e.g., PCNA pattern, CENP-F pattern and Topo I pattern. In order to harmonize this item with ICAP, the designation of these patterns was adjusted to PCNA-like pattern (AC-13), CENP-F-like pattern (AC-14) and Topo I-like pattern (AC-29), respectively.

Update on ANA report

The VI BCA/HEp-2 maintained the report structure approved in the IV Brazilian consensus [1], with the additional recommendation that laboratories should include the report of the ICAP codes, whenever available, based on information published at the www.hep2.com.br and www.anapatterns.org webpages [33]. The recommendation is that the report states the pattern name followed by ICAP code as well as the titer as the first information. Thereafter, the fluorescence patterns in each cell compartment should be stated. In addition to the five cell compartments recommended in the previous editions of BCA/HEp-2, the nuclear envelope compartment should be aggregated to the test report. This recommendation aims to drive the attention of the HEp-2 IFA analyst to focus the nuclear envelope in addition to the other cell compartments: nucleus, nucleolus, cytoplasm, mitotic apparatus and the metaphase plate.

The following example shows a typical report for the homogeneous nuclear pattern (AC-1). The VI BCA/HEp-2 also suggests that the report includes the BAC/HEp-2 and ICAP electronic addresses for consulting of clinical relevance and other useful information (Fig. 5).

Final considerations

In view of the worldwide acceptance of ICAP, nowadays adopted in most countries, the specialists of the BCA/HEp-2 have set to seek the progressive harmonization between both initiatives, while preserving some of the advances in relevant actions in the national context. With this aim, the VI BCA/HEp-2 edition was successful in establishing important recommendations regarding the definition of the group of complex patterns and classification of its components (multiple, mixed and composite), in supporting the identification of the novel nuclear discrete fine specked pattern AC-4a, a variant pattern within the speckled nuclear group, in the splitting of the nuclear discrete dots pattern into multiple nuclear dots pattern (AC-6) and few nuclear dots pattern (AC-7), and improvement in the report structure of HEp-2 IFA. Additionally, the designation of the PCNA-like pattern (AC-13), CENP-F-like pattern (AC-14) and Topo I-like pattern (AC-29) was adjusted in accordance to ICAP. By harmonizing both initiatives, we contribute to the strengthening of the harmonization of nomenclature and optimal interpretation of the HEp-2 IFA test with the correspondent benefits to patients under investigation for systemic autoimmune diseases.

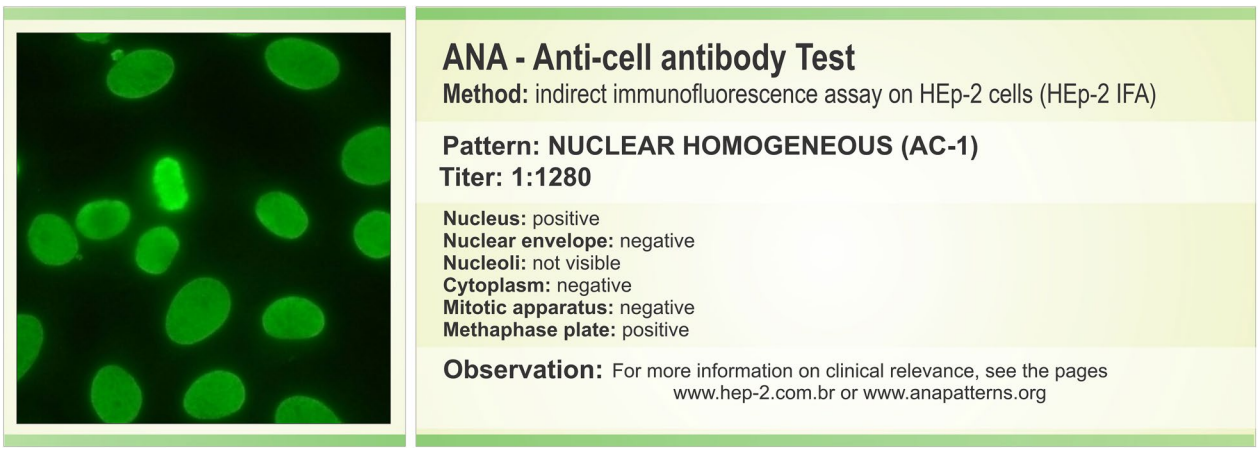


Fig. 5 Example of report based on VI BCA recommendation

Abbreviations

AIM: Autoimmune myopathy; AMA: Anti-mitochondria antibodies; ELISA: Enzyme-linked immunosorbent assays; BAC: Brazilian anti-cell autoantibodies; BCA: Brazilian Consensus on ANA; DM: Dermatomyositis; HEp-2: Human epithelial type 2; ICAP: International Consensus on ANA Patterns; IFA: Indirect Immunofluorescent Assay; SjS: Sjögren's syndrome; MCTD: Mixed connective tissue disease; NXP-2: Nuclear matrix protein 2; PBC: Primary biliary cholangitis; PML: Promyelocytic leukemia protein; SARD: Systemic autoimmune rheumatic diseases; SLE: Systemic lupus erythematosus; SMN: Survival of motor neuron; SSc: Systemic sclerosis; UCTD: Undifferentiated connective tissue disease.

Acknowledgements

The authors would like to thank the Sociedade Brasileira de Reumatologia (SBR), the SBR's Laboratory Commission and the Sociedade Brasileira de Patologia Clínica e Medicina Laboratorial (SBPC/ML).

Author contributions

Design of the meeting: WMC, LECA, CMG, RAN, PLCF. Participation in overall discussions: WMC, LECA, AD, ACX, CDAB, CLPM, EB, FAB, HAM, LMEAV, SGP, VV, WFSS, CMG, RAN, PLCF. Elabration of the first Draft in Portuguese: WMC, LECA. Translation into English and first review: WMC, LECA, AD, CLPM, SGP, CMG, RAN, PLCF. Final review of the manuscript: LECA, EB, WFSS, PLCF. Elaboration of figures: WMC. All authors read and approved the final manuscript.

Funding

There is no funding to be declared, except for the support of the Brazilian Society of Rheumatology in providing the infra-structure and a slot in the timeframe of the XXXVI Brazilian Congress of Rheumatology for the development of the activities of the V Brazilian Consensus on ANA.

Availability of data and materials

This manuscript refers to the proceedings of a meeting with a panel of experts and, therefore, there is available research data or materials.

Declarations

Ethics approval and consent to participate

This manuscript refers to the proceedings of a meeting with a panel of experts and, therefore, there is no pertinent research ethical involvement. Consent for publication All authors comply with the content of the manuscript.

Consent for publication

All authors comply with the contento of the manuscript.

Competing interests

The authors of the manuscript declare that they have no competing interests.

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Received: 6 July 2022 Accepted: 24 August 2022
Published online: 07 September 2022

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