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SPECIAL ARTICLE

Report on the second International Consensus on ANA Pattern (ICAP) workshop in Dresden 2015

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The second meeting for the International Consensus on Antinuclear antibody (ANA) Pattern (ICAP) was held on 22 September 2015, one day prior to the opening of the 12th Dresden Symposium on Autoantibodies in Dresden, Germany. The ultimate goal of ICAP is to promote harmonization and understanding of autoantibody nomenclature, and thereby optimizing ANA usage in patient care. The newly developed ICAP website www.ANApatterns.org was introduced to the more than 50 participants. This was followed by several presentations and discussions focusing on key issues including the two-tier classification of ANA patterns into competent-level versus expert-level, the consideration of how to report composite versus mixed ANA patterns, and the necessity for developing a consensus on how ANA results should be reported. The need to establish on-line training modules to help users gain competency in identifying ANA patterns was discussed as a future addition to the website. To advance the ICAP goal of promoting wider international participation, it was agreed that there should be a consolidated plan to translate consensus documents into other languages by recruiting help from members of the respective communities. *Lupus* (2016) **25,** 797–804.

Key words: Antinuclear antibodies; autoantibodies; autoimmunity; consensus; standardization

Introduction

The assay for antinuclear antibodies (ANA) is commonly used in the screening of autoantibodies in systemic autoimmune diseases,[1,2] and the indirect immunofluorescence assay utilizing HEp-2 cell substrates remains the recommended methodology.[3,4] HEp-2 cells grown as a semi-confluent monolayer exhibit prominent intracellular structures and is the traditional ANA substrate of choice for most diagnostic laboratories. With appropriately equipped microscopes, these features contribute to optimal detection and ready recognition of many subcellular structures. The International Consensus on ANA staining Patterns (ICAP) initiative originated as a session of the 12th International Workshop on Autoantibodies and Autoimmunity (IWAA) held in São Paulo, Brazil, in 2014. More than 60 participants took part in the discussion during that meeting. The consensus nomenclature and representative 28 patterns are established and available on-line at the ICAP website: www.ANApatterns.org.[5] Patterns are categorized into three major groups (nuclear, cytoplasmic, and mitotic patterns) and each pattern has been defined and described in detail.[5] The second ICAP meeting was held a day prior to the 12th Dresden Symposium on Autoantibodies in Dresden, Germany on 23–26 September 2015.[6] The present report summarizes the majority of

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Figure 1 An overview of the ICAP home webpage at www.ANApatterns.org. The web link A on the toolbar connects to the ICAP nomenclature and classification tree (see Figure 2). Link B allows selection for nuclear, cytoplasmic, or mitotic patterns to provide full descriptions. Link C provides selection from the list of all 28 alphanumeric coded patterns to access specific description and images. Link D is the key word search function. Link E will access the website into selected languages still under construction. Link F will access available free posters upon user registration. Link G provides access to ICAP publications.

issues discussed and the consensus initiatives for future action. The ultimate goal of ICAP is to promote harmonization of autoantibody test nomenclature and interpretation, and to optimize ANA usage in patient care.

The www.ANApatterns.org website and free instructional posters

At the onset of the 2nd ICAP meeting, the ANApatterns.org website was introduced by Wilson de Melo Cruvinel (Brazil), who has been primarily responsible for the development and operation of this website (Figure 1). Note that the ANApatterns.org site is also formatted to be accessible on mobile devices including cell phones and tablets. A general overview of the website was provided and instructions for its use were included as an introductory chapter in the Proceedings of the Dresden Symposium.[6] Paulo Francescantonio and Marvin Fritzler had previously commented that the actual implementation of the ICAP recommendations by diagnostic service laboratories would require ICAP recommendations be taught to trainees and technologists at our respective institutions, and that there needs to be persistence in presenting the cohesive, consistent recommendation messages at both national and international scientific symposia. In keeping with these goals, additional items were introduced to the website, including several free

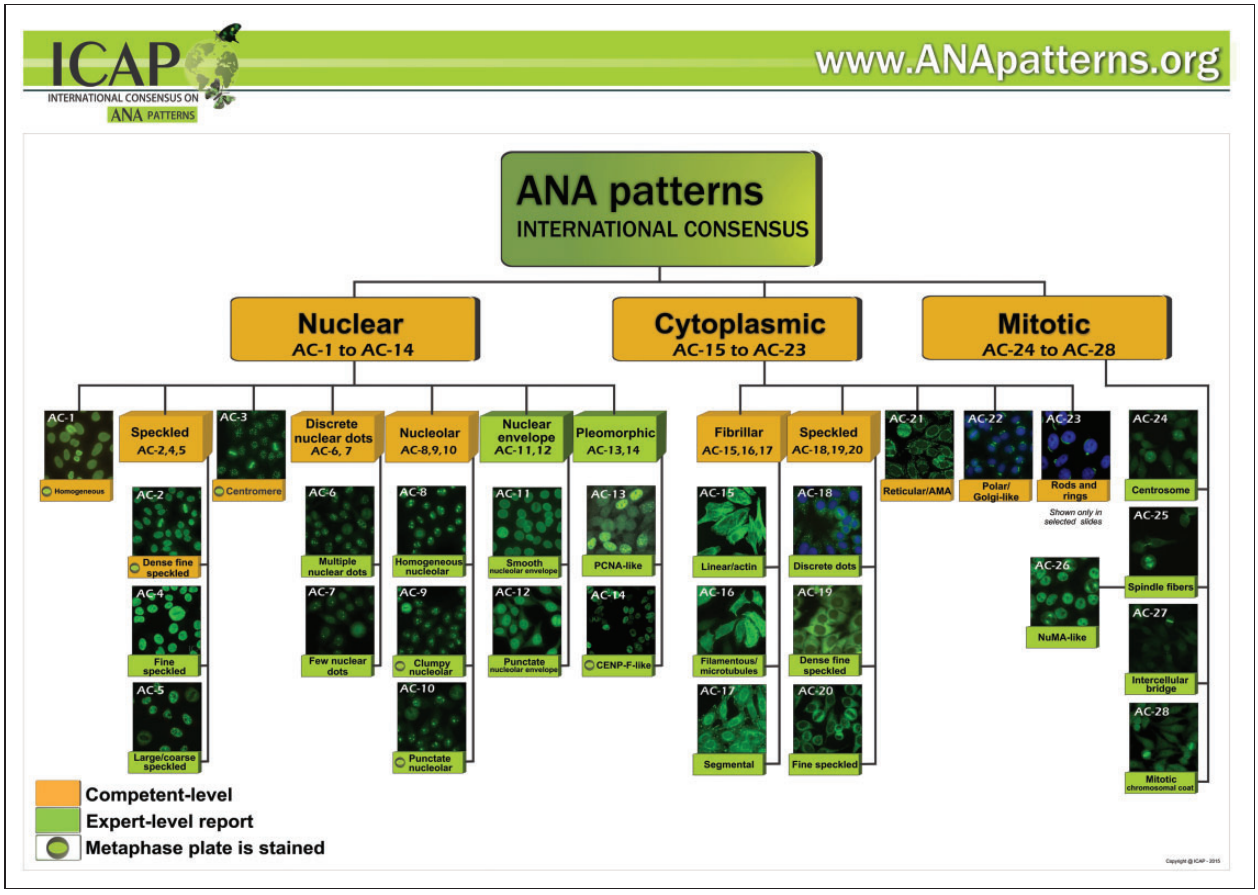

Figure 2 The nomenclature and classification tree for 28 HEp-2 cell patterns. The 28 ICAP patterns are designated with alphanumeric AC code for each from AC-1 to AC-28. Boxes with amber background are recommended as competent-level reporting, whereas those with olive green background are considered for expert-level reporting. AC, anti-cell.

posters that are available upon user registration at the website: http://anapatterns.org/download_files.php. For example, one of the figures showing the consensus nomenclature and the 28 patterns are available for download as a full-size poster (Figure 2). Additional slide sets to present the ICAP patterns will be available in the future via direct download from the website. These slide sets will be useful for teaching purposes, for example.

The website had 14,288 page views from 19 May to 11 September 2015. This represented a total of more than 3000 visits from 94 countries within these first 4 months (25 visits/day). The top three countries accessing the website during this period were USA (21.80%), Brazil (12.13%), and Germany (5.22%) (Figure 3(a)). The website usage was mostly on personal computers (91.6%); website usage on mobile cell phones and tablets was 7.61% and 0.76%, respectively (Figure 3(b)). This preliminary access data is encouraging, and clearly suggests that the ICAP website represents a useful and acknowledged source of information.

Generally, comments for the ANApatterns.org website have been highly positive regarding the overall quality of the website. There were some useful feedback comments. First, the number of representative images per anti-cell (AC) pattern provided at the initial setup for the website is limited to two. There were several requests to increase the number of images for each pattern. Hence, there are plans to obtain additional images that will be evaluated and approved by a working committee as before. The record for each image will be documented to indicate the source of images plus other relevant information. Second, a suggestion was made to provide an interpretive clinical description for each AC pattern. This description could be used as a standard comment to be included in the ANA reports and there was a consensus to move forward with this initiative. Third, it was suggested to add a comment section on the website to allow for comments from users. At a later stage, potentially these comments may be incorporated into a frequently asked questions

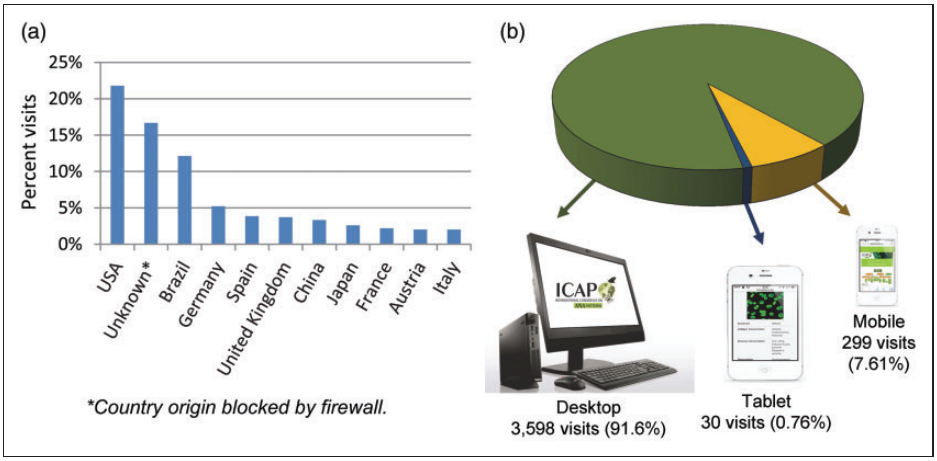

Figure 3 Summary of initial access to www.ANApatterns.org. A. Plot of countries with most visits to the website. B. Access of the website categorized based on the type of device.

(FAQ) section of the website. This latter initiative will be introduced in the coming year and will involve members of the committee as well as other interested groups.

Distinction of competent-level versus expert-level patterns

In the first ICAP report[5] patterns were divided into competent level versus expert level, with the intention that ANA readers should be trained to minimally recognize all the patterns that are listed under the competent level. Edward KL Chan (USA) and Manfred Herold (Austria) were assigned to re-address the issues regarding the decision on which patterns are to be considered competent level. Recommendations for clinical immunology laboratories to become capable of reporting patterns in the competent level include using available standard sera, such as those of the IUIS/CDC ANA standards, available from the Autoantibody Standardization Committee via www.AutoAb.org,[7] reference to images from the ICAP website at ANApatterns.org,[5] and use of additional subcellular markers (i.e. monoclonal antibodies) for co-staining validations.

The classification of patterns as competent level did not follow strict criteria, but took into consideration the clinical relevance and the morphological consistency of the patterns. Some of the competent-level patterns elicited further discussion at the second ICAP meeting. The nuclear dense fine speckled pattern (AC-2) is considered particularly important given its strong association with anti-DFS70/LEDGF antibodies and the fact that many investigators either have not heard of it or do not know the relevance of its negative correlation to systemic autoimmune rheumatic diseases.[8,9] There was some concern over the apparent lack of clinical relevance of Golgi-like pattern (AC-22)[10–12] or the rods and rings pattern (AC-23),[13–16] and yet they represent distinctive and consistent patterns that belong to the competent-level group. It has been proposed that the competent level and expert level are equivalent to the more simple description of "basic" and "advanced" levels and should not depend strictly on the clinical relevance of the pattern. It should be stated that the division between both levels may be considered rather arbitrarily and most certainly can be changed in the future as new information is available. It is acknowledged that users may not be considered as "competent" versus "expert" based solely on the ability to identify ANA patterns. Manfred Herold brought up his concerns that a critical piece of becoming "competent" in reading ANA is the ability to discriminate between positive and negative, which is a topic worthy of further discussion on the ICAP website and in subsequent ICAP meetings.

The concept of composite patterns as a separate category did not reach consensus

Luis EC Andrade (Brazil) and Karsten Conrad (Germany) were assigned to discuss whether certain patterns should be considered "composite" patterns. The discussion focused on how composite patterns are defined and the potential advantages in adopting the "composite pattern" category. Composite patterns would be defined as those in

which a single autoantibody specificity elicits the staining of more than one cell compartment. For example, NuMA (AC-26) may be considered a ''composite'' pattern because both nucleoplasmic and mitotic spindle poles are stained, with the implication that the staining in the two different compartments is characteristic of a single autoantibody specificity;[17] in this example, anti-NuMA monoclonal antibodies have shown the same staining pattern. One clear advantage of adopting the category of composite patterns can also be illustrated by the NuMA pattern, as NuMA is classified as a Mitotic Pattern in the ICAP classification tree despite the fact that all interphase cells show a strong nucleoplasmic staining. Another relevant advantage is the fact that the simultaneous occurrence of a consistent set of features in the composite patterns may increase the stringency of association with the cognate autoantibody specificities. In fact, the recognition of the multiple features of the NuMA pattern is virtually pathognomonic of anti-centrophilin/NuMA antibodies. Other patterns that could be classified as composite patterns include the CENP-F-like (AC-14), Scl-70-like (not yet classified in the ICAP classification tree), and the peculiar cytoplasmic/nucleolar staining pattern associated with anti-ribosomal P antibodies. The potential vulnerability for the category ''composite pattern'' is the confusion with ''mixed'' patterns as generated by sera that contain distinct autoantibodies to different antigens in different compartments. In addition, some specialists noted the lack of necessity to assign a distinct ''composite pattern'' category, as the subcellular localization of a given protein/antigen to different compartments at various stages of the cell cycle or under different physiological conditions is well documented. In any case, no consensus was obtained and it was decided that the category of composite patterns will not be consented at this time.

Caution on association of ANA patterns with diseases

Minoru Satoh (Japan) and Jan Damoiseaux (The Netherlands) were assigned to discuss the advantages and limitations of ANA patterns in relation to disease associations. For example, a nucleolar pattern (AC-8–AC-10) is considered to be associated with systemic sclerosis (SSc) primarily because in this disease autoimmunity may be directed to several nucleolar antigens, like Th/To,[18] U3-snoRNP/fibrillarin, and PM-Scl.[19] However, the association between SSc and the nucleolar patterns is very weak because the nucleolar pattern is often observed, even in high titer, without any clinical signs of SSc. It was further acknowledged that a given ANA pattern should suggest what the targeted autoantigens are in order to enable directed reflex testing or appropriate advice to do so for the clinician. It is the identification of autoantibodies to these self-antigens that are best associated to certain diseases, while the ANA patterns alone may be insufficiently linked to these diseases. The centromere pattern (AC-3) may be an exception, as this pattern is strongly associated with reactivity towards the CENP-B protein and, for that reason, many laboratories do not perform any antigen-specific testing for this pattern.[20,21] However, even in this case the association between the centromere pattern and SSc is not absolute. Nevertheless, the disease associations are primarily based on the target antigens recognized by autoantibodies that reveal a particular ANA pattern.[5]

Guideline for new patterns on the horizon to be considered

Prior to the second ICAP meeting, members assigned the specific task to focus on subcellular compartments were asked to re-visit their topics and provide an update for additional patterns to be considered for inclusion in the future. Karsten Conrad summarized ANA patterns that are associated with the recently defined myositis-specific autoantibodies.[22] Some of these autoantibodies do not reliably reveal novel indirect immunofluorescence (IIF) patterns and are ANA negative.[4,23,24] Karsten Conrad also made a case to include a more specific SS-A/Ro-like ANA pattern characterized by a distinctive fine speckled nuclear pattern resembling a myriad of multiple nuclear dots.[25] No consensus was achieved regarding addition of these patterns to the ICAP at this time.

Although there was no consensus on adding new patterns to the existing 28 patterns from the first ICAP, it was agreed that guidelines are needed for nomination and inclusion of novel patterns in the future. There was little discussion due to time limitation, but patterns associated with autoantibodies with clinical relevance obviously should be considered a higher priority. Other ''new'' patterns will need to await further documentation, as in publications or otherwise, with well-defined markers for validation.[26–32] The availability of multiple

consensus IIF images for new patterns is also necessary.

An unresolved issue in ANA reporting

Jan Damoiseaux, Carlos A von Mühlen (Brazil), and Ignacio Garcia-De La Torre (Mexico) were tasked to discuss how ANA reporting should come to an international consensus. With respect to the reporting of ANA, there was agreement that the test result is to be reported as negative or positive, and if positive, the IIF pattern (according to the ICAP nomenclature) and fluorescence intensity or titer are to be included. In addition, there was consensus that the report should include information on the test system applied, and, where appropriate, relevant contemporary literature provided to the clinician; for example, the laboratory might suggest that the test be repeated within a year or sooner if clinical parameters change (i.e. if ANA is at borderline positive). It was suggested that ICAP may be a good platform for preparing clinical comments to be added to the distinct patterns defined.

There was no consensus as to whether cytoplasmic and mitotic patterns are to be reported as ANA negative or positive, although it was widely agreed that some cytoplasmic patterns are clinically relevant and that this information should not be overlooked. The major concern with respect to reporting cytoplasmic patterns as ANA positive is that, in some jurisdictions, existing guidelines and diagnostic/classification criteria are based on restricting ANA to nuclear patterns. The most striking example is the diagnostic criteria for autoimmune hepatitis (1999), which is based on a scoring system.[33] A positive ANA, depending on the titer, gives positive points, while the presence of anti-mitochondrial antibodies requires subtraction of points. This evidently results in a paradox if anti-mitochondrial antibodies are reported as ANA positive. A separate report has been compiled for publication on the full discussion and proposals.[34]

On-line assessment for users to gain competence in HEp-2 cell patterns

Edward Chan and Wilson de Melo Cruvinel lead this discussion. It is acknowledged that training is needed to ensure that all laboratories are able to report patterns at the competent level (basic-level training) as well as training to help advance all laboratories to recognize expert-level patterns (advanced-level training). It is clear that multiple training programs already exist for ANA pattern recognition and reporting. However, to date, none has been adapted to the newly established ICAP nomenclature or recommendations. The general roadmap on ANA pattern training should be an open system allowing different groups to participate. Wilson de Melo Cruvinel has compiled a draft proposal for an ICAP educational program with a goal of developing an on-line assessment tool for users to gain competence in ANA determination at both basic and advanced-level training. For example, every 3 months, participants will access the ICAP website restricted by password login for the on-line immunofluorescence images. Users will examine the images provided and complete analysis by filling out a form according to ICAP guidelines. The users will be provided with a quality assessment report, which will show (1) the correct answers and results rated among the peer group; (2) the detailed characterization of the pattern; (3) autoantigen association and clinical relevance. In the US and many other countries, the program may be developed to provide Continuing Education (CE) credits and perhaps an ICAP (or IUIS/IWAA) certificate of competence. Yearly refresher courses with CE credit may also be considered.

Moving forward with ICAP internationalization

In order for ICAP to be recognized and be taken full advantage of at an international level, continuous improvement, extension, updating and maintenance of the ANApatterns.org site is essential. In addition, translation into different languages will be necessary to further promote the effort to achieve general consensus. The discussion organized by Edward Chan, Ignacio Garcia-De La Torre, and Wilson de Melo Cruvinel focused on establishing a draft guideline for each language translation project. To date, there are already projects on translation into German, Spanish, and Portuguese. The guidelines consist of: (1) for each language project, the translation should be handled by a team rather than a single individual to promote acceptance and inclusion in daily practice; (2) whenever appropriate, individuals from different countries sharing the same language should be invited to participate; (3) at different stages of the process, potential users should be involved in a "beta test" of the draft translation. This may

mean that it takes a longer time to achieve consensus in translation, but it helps to spread the message regarding the ICAP initiative. A draft letter to ''recruit'' members in the participation of translation should introduce the general description of the ICAP, the Frontiers Immunology publication[5] and the ANApatterns.org site; (4) further promotion is recommended. For example, the translation team may consider planning a manuscript/report in regional/local journals to announce the translated work. The primary target audience should be clinical immunology laboratories including organizations similar to the American Medical Laboratory Immunology.

During the Dresden Symposium on Autoantibodies, the issue of translation of the ANApatterns.org was discussed at the European Autoimmune Standardization Initiative (EASI) session where it was generally agreed that the national EASI teams[35] will help with the translation of the relevant European languages. This action also enables to invite the national EASI teams for feedback on the content of the website.

Planning of future meetings

The ICAP executive members will meet again at the time of the 10th International Congress of Autoimmunity in Leipzig, Germany, where the main agenda item will be the preparation for the 3rd ICAP meeting.

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References

1 Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* 1989; 44: 93–151.
2 Satoh M, Chan EKL, Sobel ES, *et al*. Clinical implication of autoantibodies in patients with systemic rheumatic diseases. *Exp Rev Clin Immunol* 2007; 3: 721–738.
3 Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69: 1420–1422.
4 Agmon-Levin N, Damoiseaux J, Kallenberg C, *et al*. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *Ann Rheum Dis* 2014; 73: 17–23.
5 Chan EKL, Damoiseaux J, Carballo OG, *et al*. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015. *Front Immunol* 2015; 6: 412.
6 Chan EKL, de Melo Cruvinel W, Andrade LEC. The international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns (ICAP) initiative - Current state and perspectives. In: Conrad K, Chan EKL, Andrade LEC, Steiner G, Pruijn GJM, Shoenfeld Y (eds), *12th Dresden Symposium on Autoantibodies*. Dresden, Germany: Pabst Science Publishers, 2015. pp. 282–288.
7 Chan EKL, Fritzler MJ, Wiik A, *et al*. Autoantibody Standardization Committee in 2006. *Autoimmun Rev* 2007; 6: 577–580.
8 Mahler M, Meroni PL, Andrade LE, *et al*. Towards a better understanding of the clinical association of anti-DFS70 autoantibodies. *Autoimmun Rev* 2016; 15: 198–201.
9 Ochs RL, Mahler M, Basu A, *et al*. The significance of autoantibodies to DFS70/LEDGFp75 in health and disease: integrating basic science with clinical understanding. *Clin Exp Med* 2015. Epub ahead of print, DOI: 10.1007/s10238-015-0367-0.
10 Stinton LM, Eystathioy T, Selak S, Chan EKL, Fritzler MJ. Autoantibodies to protein transport and messenger RNA processing pathways: endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes, and GW bodies. *Clin Immunol* 2004; 110: 30–44.
11 Nozawa K, Fritzler MJ, Chan EKL. Unique and shared features of Golgi complex autoantigens. *Autoimmun Rev* 2005; 4: 35–41.
12 Nozawa K, Casiano CA, Hamel JC, Molinaro C, Fritzler MJ, Chan EKL. Fragmentation of Golgi complex and Golgi autoantigens during apoptosis and necrosis. *Arthritis Res* 2002; 4: R3.
13 Keppeke GD, Nunes E, Ferraz ML, *et al*. Longitudinal study of a human drug-induced model of autoantibody to cytoplasmic rods/rings following HCV therapy with ribavirin and interferon-alpha. *PLoS One* 2012; 7: e45392.
14 Covini G, Carcamo WC, Bredi E, von Muhlen CA, Colombo M, Chan EKL. Cytoplasmic rods and rings autoantibodies developed during pegylated interferon and ribavirin therapy in patients with chronic hepatitis C. *Antivir Ther* 2012; 17: 805–811.
15 Carcamo WC, Ceribelli A, Calise SJ, *et al*. Differential reactivity to IMPDH2 by anti-rods/rings autoantibodies and unresponsiveness to pegylated interferon-alpha/ribavirin therapy in US and Italian HCV patients. *J Clin Immunol* 2013; 33: 420–426.
16 Carcamo WC, Calise SJ, von Muhlen CA, Satoh M, Chan EKL. Molecular cell biology and immunobiology of mammalian rod/ring structures. *Int Rev Cell Mol Biol* 2014; 308: 35–74.
17 Andrade LEC, Chan EKL, Peebles CL, Tan EM. Two major autoantigen-antibody systems of the mitotic spindle apparatus. *Arthritis Rheum* 1996; 39: 1643–1653.
18 Mahler M, Fritzler MJ, Satoh M. Autoantibodies to the mitochondrial RNA processing (MRP) complex also known as Th/To autoantigen. *Autoimmun Rev* 2015; 14: 254–257.
19 Kayser C, Fritzler MJ. Autoantibodies in systemic sclerosis: unanswered questions. *Front Immunol* 2015; 6: 167.
20 Damoiseaux J, Agmon-Levin N, Van Blerk M, *et al*. From ANA-screening to antigen-specificity: an EASI-survey on the daily practice in European countries. *Clin Exp Rheumatol* 2014; 32: 539–546.

21 Damoiseaux JG, Tervaert JW. From ANA to ENA: how to proceed? *Autoimmun Rev* 2006; 5: 10–17.
22 Satoh M, Tanaka S, Ceribelli A, Calise SJ, Chan EKL. A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. *Clin Rev Allergy Immunol* 2015. Epub ahead of print, DOI: 10.1007/s12016-015-8510-y.
23 Mahler M, Dervieux T. Comments on recent advances and recommendations for the assessment of autoantibodies to cellular antigens referred as antinuclear antibodies. *Ann Rheum Dis* 2014; 73: e36.
24 Mahler M, Meroni PL, Bossuyt X, Fritzler MJ. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J Immunol Res* 2014; 2014: 315179.
25 Dellavance A, Alvarenga RR, Rodrigues SH, *et al*. Autoantibodies to 60kDa SS-A/Ro yield a specific nuclear myriad discrete fine speckled immunofluorescence pattern. *J Immunol Methods* 2013; 390: 35–40.
26 Covini G, Chan EKL, Nishioka M, Morshed SA, Reed SI, Tan EM. Immune response to cyclin B1 in hepatocellular carcinoma. *Hepatology* 1997; 25: 75–80.
27 Zhang JY, Megliorino R, Peng XX, Tan EM, Chen Y, Chan EKL. Antibody detection using tumor-associated antigen mini-array in immunodiagnosing human hepatocellular carcinoma. *J Hepatol* 2007; 46: 107–114.
28 Muro Y, Chan EKL, Landberg G, Tan EM. A cell-cycle nuclear autoantigen containing WD-40 motifs expressed mainly in S and G2 phase cells. *Biochem Biophys Res Commun* 1995; 207: 1029–1037.
29 Furuta K, Chan EKL, Kiyosawa K, Reimer G, Luderschmidt C, Tan EM. Heterochromatin protein HP1Hsbeta (p25beta) and its localization with centromeres in mitosis. *Chromosoma* 1997; 106: 11–19.
30 Fritzler MJ, Kerfoot SM, Feasby TE, *et al*. Autoantibodies from patients with idiopathic ataxia bind to M-phase phosphoprotein-1 (MPP1). *J Investig Med* 2000; 48: 28–39.
31 von Muhlen CA, Chan EKL, Peebles CL, Imai H, Kiyosawa K, Tan EM. Non-muscle myosin as target antigen for human autoantibodies in patients with hepatitis C virus-associated chronic liver diseases. *Clin Exp Immunol* 1995; 100: 67–74.
32 Soo Hoo L, Zhang JY, Chan EKL. Cloning and characterization of a novel 90 kDa 'companion' auto-antigen of p62 overexpressed in cancer. *Oncogene* 2002; 21: 5006–5015.
33 Alvarez F, Berg PA, Bianchi FB, *et al*. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; 31: 929–938.
34 Damoiseaux J, von Muhlen CA, Garcia-De La Torre I, *et al*. International consensus on ANA patterns (ICAP): the bumpy road towards a consensus on reporting ANA results. *Autoimmun Highlights* 2016; 7: 1.
35 Shoenfeld Y, Cervera R, Haass M, *et al*. EASI - The European Autoimmunity Standardisation Initiative: a new initiative that can contribute to agreed diagnostic models of diagnosing autoimmune disorders throughout Europe. *Ann N Y Acad Sci* 2007; 1109: 138–144.