Editorial

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The International Consensus on ANA Patterns (ICAP): from conception to implementation

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Introduction

Assays for anti-nuclear antibodies (ANA) have been introduced in the second half of the previous century, first on rodent tissue as substrate and next on the human laryngeal epidermoid carcinoma HEp-2 cell line [1]. The HEp-2 indirect immunofluorescence assay (IFA) has appeared of added value in the diagnosis of systemic autoimmune rheumatic diseases (SARD), in particular systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD) and systemic sclerosis (SSc), but to a lesser extend Sjögren's syndrome (SS) and idiopathic inflammatory myopathies (IIM) [2–4]. Next to SARD, HEp-2 IFA is also included in the diagnostic work-up of autoimmune liver diseases and juvenile idiopathic arthritis [5, 6]. Over the years alternative solid-phase immuno-assays (SPA) have entered the clinical laboratories, but HEp-2 IFA remained the gold standard as screening method [7, 8]. Nevertheless, more recently the combination of HEp-2 IFA with SPA seems to be of added value [3]. One advantage of HEp-2 IFA is the recognition of immunofluorescence patterns, not only restricted to the nucleus, but also including the cytoplasm and the mitotic apparatus. This pattern provides information on the autoantigens that might be recognized and in case of the centromere pattern even is part of classification criteria [9, 10]. Initiatives to harmonize the definitions and nomenclature of HEp-2 IFA patterns have culminated in the International Consensus on ANA Patterns (ICAP), an initiative that is increasingly being incorporated in routine clinical practice, as illustrated in the paper published by Infantino and Colleagues in this issue of the Journal describing the Italian experience of adopting the ICAP classification [11]. The current editorial elaborates on the history of ICAP, the major achievements in terms of implementation, and the anticipated perspectives.

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Historical perspective

The history of ICAP is with less than one decade rather short (Figure 1). The initiative of Luis Andrade (Sao Paulo, Brazil) and Ed Chan (Gainesville, Florida) was preceded by contributions of the late Allan Wiik (Copenhagen, Denmark), who recently was honored at the 16th Dresden Symposium on Autoantibodies (2023) by Johan Rönnelid (Uppsala, Sweden) for his legacy in autoimmune diagnostics [12, 13], as well as his humanity. In 2010 Allan Wiik published an extended contemporary nomenclature for HEp-2 IFA patterns which, in combination with the recommendations of the European Autoimmunity Standardisation Initiative (EASI), was the base for the ICAP classification [8, 14, 15]. For his contribution Allan Wiik was appointed “ICAP honorary member” at the 7th ICAP Workshop in Dresden (2023). Since consensus meetings on HEp-2 IFA patterns already took place in Brazil from 2000 onward [16], it was not surprising that the first ICAP workshop was organized in Sao Paulo as a satellite to the 12th International Workshop on Autoantibodies and Autoimmunity. As a result 28 HEp-IFA patterns were defined, divided in three categories: nuclear, cytoplasmic and mitotic [15]. Besides a name, each pattern was assigned an anti-cellular (AC)-code ranging from AC-1 to AC-28, analogous to the cluster of differentiation (CD) nomenclature for monoclonal antibodies that recognize leukocyte differentiation molecules. The first ICAP report was combined with the launch of the ICAP website (www.anapatterns.org) enabling widespread access to the initiative. In particular the recognition that also cytoplasmic patterns may be clinically relevant resurged the discussion if such autoantibodies are to be referred to as ANA [17]. As reviewed by Von Mühlén et al. the ICAP experts did not yet reach consensus on the terminology to be used [18]. At the subsequent six ICAP workshops the classification system was elaborated upon by defining a few new patterns (HEp-2 IFA negative [AC-0], HEp-2 IFA undefined [AC-XX], and anti-Topo I-like [AC-29]) and subpatterns for the nuclear fine speckled pattern (AC-4a and AC-4b), by rearranging the classification tree (removing the nuclear dense fine speckled [AC-2] and the nuclear anti-Topo I-like [AC-29] patterns from the category of nuclear speckled patterns and the cytoplasmic discrete dot pattern [AC-18] from the cytoplasmic speckled patterns) [19].
In addition, it was anticipated that the association between pattern and disease, as indicated in the first ICAP report [15], could better be replaced by clinical relevance of the individual patterns [20].

**Implementation in clinical practice**

The number of publications on ICAP enlisted in PubMed remains scarce with only 35 items found when searching for “ICAP HEp-2” and “ICAP ANA”. Eight of these publications originate from the ICAP committee itself, while 5 publications were the result of a close collaboration with the ICAP committee. Several of the remaining 22 publications, including the publication of Infantino et al. [11], address the topic of implementation in clinical practice. Obviously, the number of scientific publications is not necessarily representative of clinical implementation. Indeed, world-wide implementation is evident from the strong increase in users of the website, from 5,667 in 2015 to 181,782 in 2022 [11]. The excellent website, mastered by Wilson de Melo Cruvinel (Goiânia, Brazil), has strongly attributed to implementation of the ICAP initiative. The website is anticipated as very informative, up-to-date, and useful in clinical practice. Technicians and laboratory specialists that have to evaluate the slides have easy access to pattern definitions and representative images, while clinicians have easy access to clinical relevance of the patterns and recommendations for follow-up testing. It is to be expected that the recent launch of the ICAP-App for mobile phones will further enhance access to the content of the website and implementation of the ICAP classification of HEp-2 IFA patterns. Also the diagnostic industry and providers of external quality assessment (EQA), such as UK NEQAS, have adopted the ICAP classification, further propelling the conversion towards harmonization of HEp-2 IFA results. A recent international survey to evaluate reporting, familiarity, and considered clinical value of HEp-2 IFA patterns revealed that the major nuclear and cytoplasmic patterns are considered clinically important, but that there exist apparent differences in appreciation of the distinct patterns between laboratory specialists and clinicians [21]. Apparently, there remains a gap to be filled and this may be effectuated by the novel recommendations on the detection of ANA, a collaborative action from the European Federation of Laboratory Medicine (EFLM), EASI and ICAP [22]. These recommendations, as far as appropriate, follow the ICAP classification and it is stated that patterns should preferentially be reported according to the ICAP nomenclature.

Implementation is also evident from reports, such as the publication of Infantino et al. [11], on how ICAP is introduced in distinct countries. At the Medlab Asia meeting in Bangkok (2023) the implementation in Thailand was presented, while at the 7th ICAP Workshop in Dresden (2023) the Italian experience was presented. From these presentations, as well as from the translation of the website in many languages, it is apparent that minor changes are being introduced, either in the definition of the pattern or the position in the classification tree. Obviously, this may affect the goal of world-wide harmonization, but it at least aligns the test-results within a national health care system. However, shifting for instance the NuMA-like pattern (AC-26) from the mitotic category to the nuclear category, as being done in Thailand because of the evident nuclear staining in interphase cells, may impact on future epidemiological evaluations. Consistent assignment of the AC-codes may overcome this caveat. Of note, the NuMA-like pattern (AC-26) was by ICAP integrated in the mitotic category because parts of the mitotic apparatus, i.e., the spindle fibers, were recognized by the autoantibodies.

**Conclusions and future perspectives**

Broad implementation of the ICAP classification has been a major achievement within a time-frame of less than ten years. Infantino et al. suggested some further improvements, such as defining the competence level of technicians and
laboratory specialists and incorporation of multiple and mixed patterns in the ICAP classification [11]. The competence level might be assessed by future training modules that are planned. The issue on multiple patterns, due to two or more autoantibodies that react with distinct cellular compartments, or mixed patterns, due to two or more autoantibodies that react with the same cellular compartment, is highly relevant because patient sera often do not contain a single autoantibody revealing the currently defined HEP-2 IFA patterns. Especially in case of autoimmune liver diseases multiple patterns are quite common. Combinations of multiple nuclear dots (AC-6) and reticular/AMA (AC-21), centromere (AC-3) and reticular/AMA (AC-21), or nuclear homogeneous (AC-1) and cytoplasmic fibrillary linear (AC-15) are easily recognized and such combinations may even enhance the clinical relevance for primary biliary cholangitis and autoimmune hepatitis, respectively. Mixed patterns, on the other hand, might be more difficult to decipher as they include combinations of centromere (AC-3) and multiple nuclear dots (AC-6), or nuclear homogeneous (AC-1) and nuclear dense fine speckled (AC-2). For such combinations the clinical relevance will be more difficult to define. As a matter in fact, this shortcoming also becomes an issue if patterns are reported on competent level since clinical relevance is provided at the expert level [20]. Combining the clinical relevance of the nucleolar patterns (AC-8, AC-9, and AC-10) is not a problem, but this is different for nuclear dots (AC-6 and AC-7) and nuclear membrane (AC-11 and AC-12) for which one pattern (AC-6 and AC-12), but not the other (AC-7 and AC-11), has definite clinical relevance.

Another concern raised by Infantino et al. [11] involves the need for further expansion of the number of novel patterns. When looking at the analogy with the CD-system for monoclonal antibodies recognizing molecules expressed by leukocytes, the first Human Leukocyte Differentiation Workshop in Paris (1982) defined only 15 CDs, a number that expanded to 130 CDs after the fifth Workshop in Boston ten years later (1993). Interestingly, also the CD classification eventually subtyped in, for instance, CD11a, CD11b and CD11c. The question is if the consensus allows extension to many more patterns because they can be distinguished by the real experts, or if the consensus is to be more conservative and monitor the need in clinical practice. Personally, I do favor the latter in order to keep all current users on board.

References


