International Consensus on Antinuclear Antibody Patterns in Portugal

International Consensus on Antinuclear Antibody em Portugal

ARTIGO ORIGINAL

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ABSTRACT

Introduction: Screening for autoantibodies in HEp-2 cells by indirect immunofluorescence is currently accepted as the gold-standard test for the diagnosis of systemic autoimmune diseases. The main objective of the International Consensus on ANA Patterns is to achieve a consensus on the nomenclature and description of antinuclear antibody morphological patterns. This work aims to build on the International Consensus on ANA Patterns project to establish a nomenclature consensus in Portugal, thus contributing to harmonization in autoimmune diagnosis and promoting diagnostic quality in autoimmune systemic rheumatic diseases.

Material and Methods: Participating laboratories identified all the nuclear and cytoplasmic pattern designations in the International Consensus on ANA Patterns (including the anti-cell pattern code), and matched them with the corresponding Portuguese nomenclature in use. The results were aggregated and used as a foundation for nomenclature harmonization work. Consensus meetings followed an iterative process, until a final consensual proposal was drafted.

Results: Prior agreement between laboratories was over 75% for 23 of the total 29 anti-cell patterns. The degree to which each laboratory is aligned with the International Consensus on ANA Patterns international reference ranges from 22.1% to 100%. It was possible to write a consensual version of the International Consensus on ANA Patterns nomenclature for Portugal.

Discussion: There was a good consensus basis for the nomenclature in the International Consensus on ANA Patterns, despite relevant differences with some translations. The study highlights the need for collaboration among laboratories towards an unambiguous description of laboratory results.

Conclusion: This study shows that there is good potential for collaboration between laboratories in order to produce the consensus needed to improve diagnosis and patient follow-up.

Keywords: Antibodies, Antinuclear/analysis; Fluorescent Antibody Technique, Indirect/methods; Portugal

RESUMO

Introdução: A pesquisa de autoanticorpos em células HEp-2 através de imunofluorescência indireta é o teste padrão atualmente aceite como a ferramenta central para o diagnóstico das doenças autoimunes sistémicas. O *International Consensus on Antinuclear Antibody* (ANA) *Patterns* tem como objetivo principal alcançar um consenso na nomenclatura e na descrição dos diferentes padrões morfológicos de anticorpos antinucleares. Este trabalho tem como objetivo ampliar o projeto do *International Consensus on* ANA *Patterns* de forma a estabelecer um consenso em Portugal para a sua nomenclatura, procurando contribuir para a harmonização no diagnóstico autoimune e promover a qualidade diagnóstica nas doenças reumáticas sistémicas autoimunes.

Material e Métodos: Os laboratórios participantes identificaram cada designação de padrão citoplasmático e nuclear do *International Consensus on* ANA *Patterns* (incluindo o código padrão anti-célula), e fizeram corresponder a cada uma a respetiva nomenclatura portuguesa em uso. Os resultados foram agregados e serviram de base ao trabalho de harmonização da nomenclatura. Seguiram-se reuniões de consenso, num processo iterativo até à redação de uma proposta final consensualizada.

Resultados: A concordância prévia entre laboratórios era superior a 75% para 23 do total de 29 padrões anti-célula. O grau em que cada laboratório está alinhado com a referência internacional do *International Consensus on* ANA *Patterns* varia entre 22,1% e 100%. Foi possível elaborar uma versão consensualizada da nomenclatura do *International Consensus on* ANA *Patterns* para Portugal.

Discussão: Existia uma boa base de consenso para a nomenclatura do *International Consensus on* ANA *Patterns*, mas com diferenças importantes em algumas das traduções da terminologia. O estudo realça a necessidade de colaboração entre laboratórios para uma descrição inequívoca dos resultados laboratoriais.

Conclusão: Este trabalho mostra o potencial positivo da colaboração entre laboratórios para gerar consensos que contribuam para a melhoria do diagnóstico e acompanhamento dos doentes.

Palavras-chave: Anticorpos Antinucleares/análise; Portugal; Técnica Indireta de Fluorescência para Anticorpo/métodos

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INTRODUCTION

Autoantibody testing using indirect immunofluorescence (IIF) on HEp-2 cells has been used since 1950, when Coons and Kaplan first described it[1] and became the standard test when it migrated from animal tissues to human HEp-2 epithelial cells and is now accepted as the major tool for the diagnosis of systemic autoimmune diseases.[2]

Recommendations for the correct evaluation and interpretation of cellular autoantibodies, historically known as antinuclear antibodies (ANA), were issued in 2013 by a group of international experts (laboratory specialists, scientists and physicians), considering indirect immunofluorescence as the reference method for ANA screening. Since then, antibody testing targeted to nuclear and non-nuclear elements has been referred to as ANA,[2] or HEp-2 IIF.[3]

Alan Wiik has described in the article *Guidelines for antinuclear antibody testing* (2016) that the ANA terminology had been used for all cellular antibodies that can be visualised by IIF on HEp-2 substrate[4] and that although the cell contains thousands of different proteins, few have autoantigenic properties. Subsequently, ANAs can be divided into five groups, including i) those that recognise organelles of the nucleoplasm, ii) nucleolus, iii) cell membrane antigens, iv) mitotic apparatus and v) cytoplasm.[4] The different types of ANA generate different and individually characteristic patterns in HEp-2 cells, depending on the cellular location and the properties of the target antigen in the sample.

The ICAP (International Consensus on Antinuclear Antibody (ANA) Patterns) initiative was carried out in São Paulo, Brazil, in 2014, hosted by the 12th International Workshop on Autoantibodies and Autoimmunity (IWAA). This initiative was held by members of the Autoantibody Standardization Committee (ASC), which is part of the Quality Assessment and Standardization Committee of the International Union of Immunological Societies (IUIS), linked to the World Health Organization (WHO). The ICAP initiative mainly aimed at reaching a consensus on the nomenclature and description of the different ANA morphological patterns, with highly variable details when observed by IIF on HEp-2 cells.[5] Another major objective was achieving harmonisation in the way ANA test results are reported,[6] achieved by describing the patterns and sub-patterns, which are assigned anti-cell (AC) pattern codes ranging from AC-1 to AC-29, into three categories: i) nuclear pattern, ii) cytoplasmic pattern and iii) mitotic pattern. The initiative's work was shared on the

Table 1 – Nuclear patterns – antigen and disease associations

Nuclear pattern (ICAP)	Code	Antigen association	Disease association
Homogéneo	**AC-1**	dsDNA, nucleosomes, histones	SLE, drug-induced lupus, juvenile idiopathic arthritis
Mosqueado	**AC-2, 4, 5**	hnRNP, U1RNP, Sm, SS-A/Ro (Ro60), SS-B/La, RNA polymerase III, Mi-2, Ku	MCTD, SLE, SjS, DM, SSc/PM overlap syndrome
Mosqueado fino denso	**AC-2**	DFS70/LEDGF	Rare in SLE, SjS, SSc
Mosqueado fino	AC-4	SS-A/Ro (Ro60), SS-B/La, Mi-2; TIF1Y, TIF1β, Ku, RNA helicase A, Replication protein A (RPA)	SjS, SLE, DM, SSc/PM overlap syndrome
Mosqueado grosseiro	AC-5	hnRNP, U1RNP, Sm, RNA polymerase III	MCTD, SLE, SSc
Centrómero	**AC-3**	CENP-A/B (C)	Limited cutaneous SSc, PBC
Pontos nucleares discretos	**AC-6, 7**		
Múltiplos pontos nucleares	AC-6	Sp100, PML proteins, MJ/NXP-2	PBC, SARD, PM/DM
Raros pontos nucleares	AC-7	p80-coilin, SMN	SjS, SLE, SSc, PM, asymptomatic individuals
Nucleolar	**AC-8, 9, 10**		
Nucleolar homogéneo	AC-8	PM/Scl-75, PM/Scl-100, Th/To, B23/ nucleophosmin, nucleolin, No55/SC65	SSc, SSc/PM overlap syndrome
Nucleolar aglomerado	AC-9	U3-snoRNP/fribrillarin	SSc
Nucleolar ponteado	AC-10	RNA polimerase I, hUBF/NOR-90	SSc, SjS
Envelope nuclear	**AC-11, 12**		
Membrana nuclear linear	AC-11	Lamins A, B, C, or lamin-associated proteins	SLE, SjS, seronegative arthritis
Membrana nuclear ponteada (complexo poro-nuclear)	AC-12	Nuclear pore complex proteins (i.e., gp210)	PBC
Pleomórfico	**AC-13, 14**		
Pleomórfico (tipo PCNA)	AC-13	PCNA	SLE, other conditions
Pleomórfico (tipo CENP-F)	AC-14	CENP-F	Cancer, other conditions

SLE: systemic lupus erythemathosus; MCTD: mixed connective tissue disease; SjS: Sjögren's syndrome; DM: dermatomyositis; SSc/PM: systemic sclerosis / polymyositis overlap syndrome; SSc: systemic sclerosis; Limited cutaneous SSc: limited cutaneous systemic sclerosis; PBC: primary biliary cholangitis; SARD: systemic autoimmune rheumatic disease; PM: polymyositis; PM/DM: polymyositis/dermatomyositis

internet, including translations in English, French, Italian, Spanish, Dutch, Mandarin and Portuguese (Brazilian and European), available at www.anapatterns.org.

Current international consensus

Nuclear patterns

Nuclear patterns were based on any staining of the nuclei of HEp-2 cells in HEp-2 interphase. Nomenclature for nuclear patterns is based primarily on the reactivity observed in the nucleoplasm (e.g. homogeneous or speckled) and nuclear subcomponents (e.g. centromere or nucleolar). Nuclear patterns include homogeneous, speckled, centromere, discrete nuclear dots, nucleolar, nuclear envelope and pleomorphic patterns. Dense Fine Speckled 70 (DFS70) is included in the speckled group. The centromere pattern is included in the discrete nuclear dots, even though within its own group due to its characteristic pattern and frequent presence in a particular clinical setting. The nuclear pattern groups are further divided into subgroups including fine or coarse, multiple or rare nuclear dots, homogeneous nucleolar, clustered or dotted; linear or dotted nuclear envelope and pleomorphic patterns similar to Proliferating Cell Nuclear Antigen (PCNA) or Centromere Protein F (CENP-F). This classification of the nuclear patterns provides information on antigen and disease association (Table 1). As an example, the homogeneous pattern is found in reactions with chromatin components such as double-stranded DNA, histones and/or nucleosomes. These autoantibodies are associated with SLE (systemic lupus erythematosus), drug-induced lupus and juvenile idiopathic arthritis. Other nuclear reactions can help in the definition of MCTD (mixed connective tissue disease), SjS (Sjögren's syndrome), SS (systemic sclerosis), PM (polymyositis), DM (dermatomyositis), PBC (primary biliary cholangitis) or other autoimmune diseases. Mixed patterns regard the presence of more than one autoantibody in the patient´s sample, such as autoantibodies to both centromere (nuclear) and mitochondria (cytoplasmic), frequently found in PBC.

Cytoplasmic patterns

Cytoplasmic patterns were based on any HEp-2 cytoplasm staining. Nomenclature is based primarily on the reactivity found in the cytoplasm (e.g. filamentous or segmental) and the cytoplasmic structure (e.g. rods and rings). Fibrillar, speckled, reticular/mitochondrion-like (AMA), polar/Golgi-like and rods and rings corresponded to the five major cytoplasmic patterns, while the fibrillar pattern is subdivided into linear, filamentous and segmental, as well as the speckled pattern into discrete dots, dense fine speckled and fine speckled. Cytoplasmic autoantibodies of different specificities are found in different disorders (Table 2) including MCTD, PM, DM, SLE, SS, PBC, Crohn's disease, ulcerative colitis and myasthenia gravis. Cytoplasmic patterns should be described in patient's test results and not described as ANA negative.

Table 2 – Cytoplasmic patterns – antigen and disease associations

Cytoplasmic pattern (ICAP)	Code	Antigen association	Disease association
Fibrilar	**AC-15, 16, 17**		
Linear/ actina	AC-15	Actin, non-muscle myosin MCTD	MCTD, active chronic hepatitis, liver cirrhosis, myasthenia gravis, Crohn's disease, PBC, long-term dialysis, rare in SARD, other than MCTD
Filamentoso/ microtúbulos	AC-16	Vimentin, cytokeratins	Infections or inflammatory conditions, long-term dialysis, alcohol-related liver disease, SARD, psoriasis, healthy controls
Segmentar	AC-17	Alpha-actin, vinculin, tropomyosin	Myasthenia gravis, Crohn's, ulcerative colitis
Mosqueado	**AC-18, 19, 20**		
Grânulos isolados	AC-18	SGW182, Su/Ago2, Ge-1	PBC, SARD, neurological and autoimmune diseases
Fino granular denso	AC-19	PL-7, PL-12, p-Ribosomal protein	Antisynthetase syndrome, PM/DM, SLE, Juvenile SLE, Neuropsychiatric SLE
Fino granular	AC-20	jo-1/histidyl-tRNA synthetase	Antisynthetase syndrome, PM/DM, Limited cutaneous SSc, idiopathic pleural effusion
Reticular/AMA	**AC-21**	PDC-E2/M2, BCOADC-E2, OGDC-E2, E1-alpha PDC subunit, E3BP/protein X	Common in PBC, SSc, rare in other SARD
Polar (tipo Golgi)	**AC-22**	Giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97, golgin-245	Rare in SjS, SLE, RA, MCTD, GPA, idiopathic cerebellar ataxia, paraneoplastic cerebellar degeneration, viral infections
Anéis e bastonetes	AC-23	IMPDH2, others	Patients with post-IFN HCV / ribavirin therapy, rare in SLE, Hashimoto and healthy individuals

MCTD: mixed connective tissue disease; PBC: primary biliary cholangitis; SARD: systemic rheumatic autoimmune disease; PM/DM: polymyositis / dermatomyositis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; Limited cutaneous SSc: limited cutaneous systemic sclerosis; SjS: Sjögren's syndrome; RA: rheumatoid arthritis; GPA: granulomatosis with polyangiitis; post-IFN HCV: post-interferon hepatitis C

Table 3 – Mitotic patterns – antigen and disease associations

Mitotic pattern (ICAP)	Code	Antigen association	Disease association
Centrossoma	AC-24	Pericentrin, ninein, Cep250, Cep110, enolase	Rare in SSc, Raynaud's phenomenon, infections (viral and mycoplasma)
Fuso mitótico	AC-25	HsEg5	Rare in SjS, SLE, other SARD
Fuso mitótico (NuMA-1)	AC-26	Centrophilin	SjS, SLE, others
Ponte Intercelular	AC-27	Aurora kinase B, CENP-E, MAS-2, KIF-14, MKLP-1	Rare in SSc, Raynaud's phenomenon, cancer
Envelope cromossómico	AC-28	Modified histone H3, MCA-1	Rare in discoid lupus erythematosus, chronic lymphocytic leukaemia, SjS and rheumatic polymyalgia

SSc: systemic sclerosis; SjS: Sjögren's syndrome; SLE: systemic lupus erythematosus; SARD: systemic autoimmune rheumatic disease

Mitotic patterns

Mitotic patterns were defined as patterns associated with cellular domains clearly related to mitosis. Mitotic patterns include the centrosome, the mitotic spindle with the nuclear mitotic apparatus subpattern (NuMA-1), the intercellular bridge and the chromosome envelope. Some mitotic patterns (e.g., centrosome) are not specifically associated with mitosis, rather showing very distinctive features in mitotic cells. Mitotic antibodies are rarely found in diseases such as SS, SLE, SjS and Raynaud's phenomenon (Table 3).

Confirmation of IIF test results

Positive ANA test results by IIF should be confirmed by further specific testing using different techniques such as multiplex immunoblot or enzyme-linked immunosorbent assay (ELISA) (Fig. 1).

Perspectives

The ICAP initiative laid the foundation for the creation of a harmonised nomenclature and improved description of ANA testing results. There are, of course, outstanding issues to be addressed in future editions, including the classification of composite and mixed patterns (e.g., topoisomerase I (Scl-70)), the inclusion of rare patterns or whether cytoplasmic and mitotic antibodies should be classified as ANA positive or reported separately. The optimisation of confirmation strategies will also need to be examined. Consensus is an ongoing process that is expected to mature into a global standard incorporating contributions from laboratories and clinicians worldwide. Another important contribution to the harmonisation of ANAs is the increasing use of pattern recognition software in laboratories. Consistency between different laboratories, as well as the speed and efficiency of the assessment procedure are increased with the automation of IIF assessment. Additionally, the software can also be adapted as the standards guiding interpretation evolve.

ANAs are associated with a wide variety of systemic autoimmune diseases, including SLE, MCTD, SjS, SS, PM, DM and PBC, representing an important diagnostic criterion; therefore, standardisation and harmonisation is a milestone for diagnostic quality in autoimmune systemic rheumatic diseases.

Apart from the participation in this working group and the translations, a group of Portuguese experts extended the ICAP project in order to establish a consensus in Portugal for its nomenclature, aimed at removing current discrepancies in the description of results and allowing an improvement both in quality of diagnosis and in the communication between professionals involved in diagnosis and treatment.

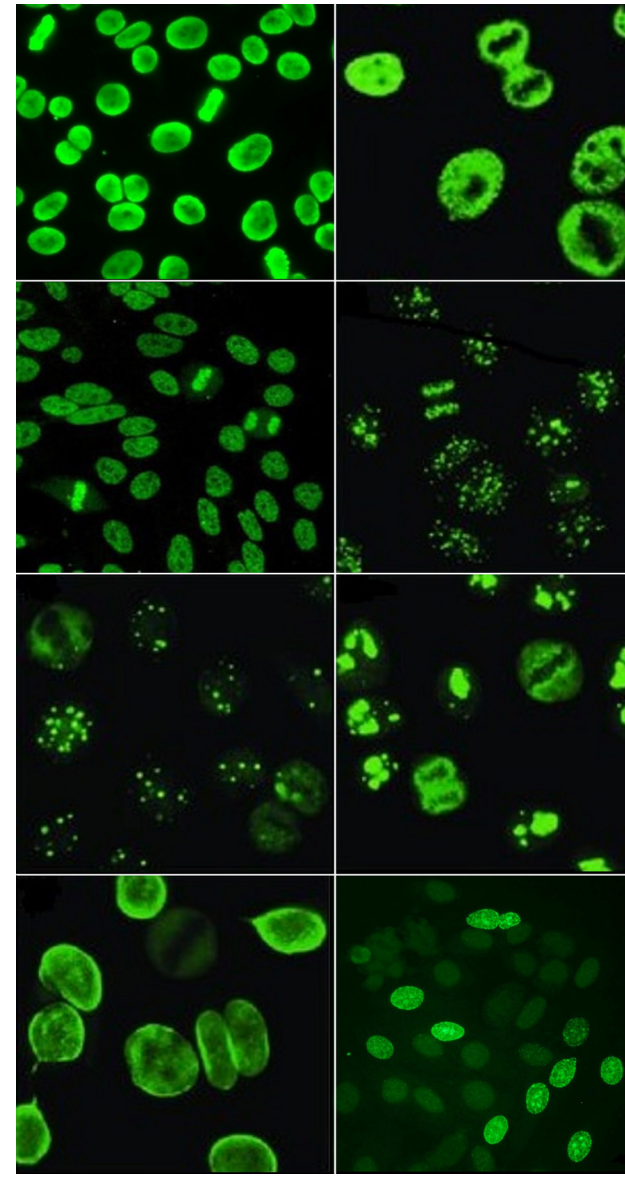

Figure 1 – Reflex test for autoantibodies based on nuclear patterns

MATERIAL AND METHODS

A consensus group has been held, including physicians and pharmacists working in the area of laboratory medicine in nine different locations in Portugal, with significant experience in IIF testing and ANA standards. Six public laboratories (hospitals or university hospitals) were involved, in addition to three private laboratories that were selected due to their capacity and experience with IIF testing on HEp-2 cells.

All participating laboratories were asked to identify each ICAP cytoplasmic and nuclear pattern designation (including the AC code), with the corresponding Portuguese nomenclature in use, for which there was no previous consensus or reference.

Before the first consensus meeting, the results of all laboratories were aggregated and sent to the working group in a report with descriptive statistics. The agreement rate for each standard was also described, allowing all participants to assess their level of consensus and to identify the most commonly used terminology prior to the second phase of this work.

Several consensus meetings based on the first report were carried out, in an iterative process supported by email documents, until a proposal was drafted. After agreement on Portuguese nomenclature, a final version was completed for an ICAP consensus in Portugal, with agreement from all nine laboratories for all cytoplasmic and nuclear pattern designations, shown below.

As no patients or the use of private or sensitive information were involved, there was no need to apply for ethics committee approval for this stud.

RESULTS

Overall, a high agreement has been found between the Portuguese laboratories, mostly above 75% (Fig. 2), with the exception of six AC patterns. There were no patterns with AN agreement within the first quartile range and there was one pattern within the second quartile. Twenty-three patterns (79.3%) were within the range of the last quartile. For the ACs regarding mitotic patterns, a degree of agreement ranging between 66.7% and 88.9% has been found. Only one nuclear pattern (AC-07) gathered 100% agreement between laboratories. This result was obtained in the same group (nuclear patterns) where the only

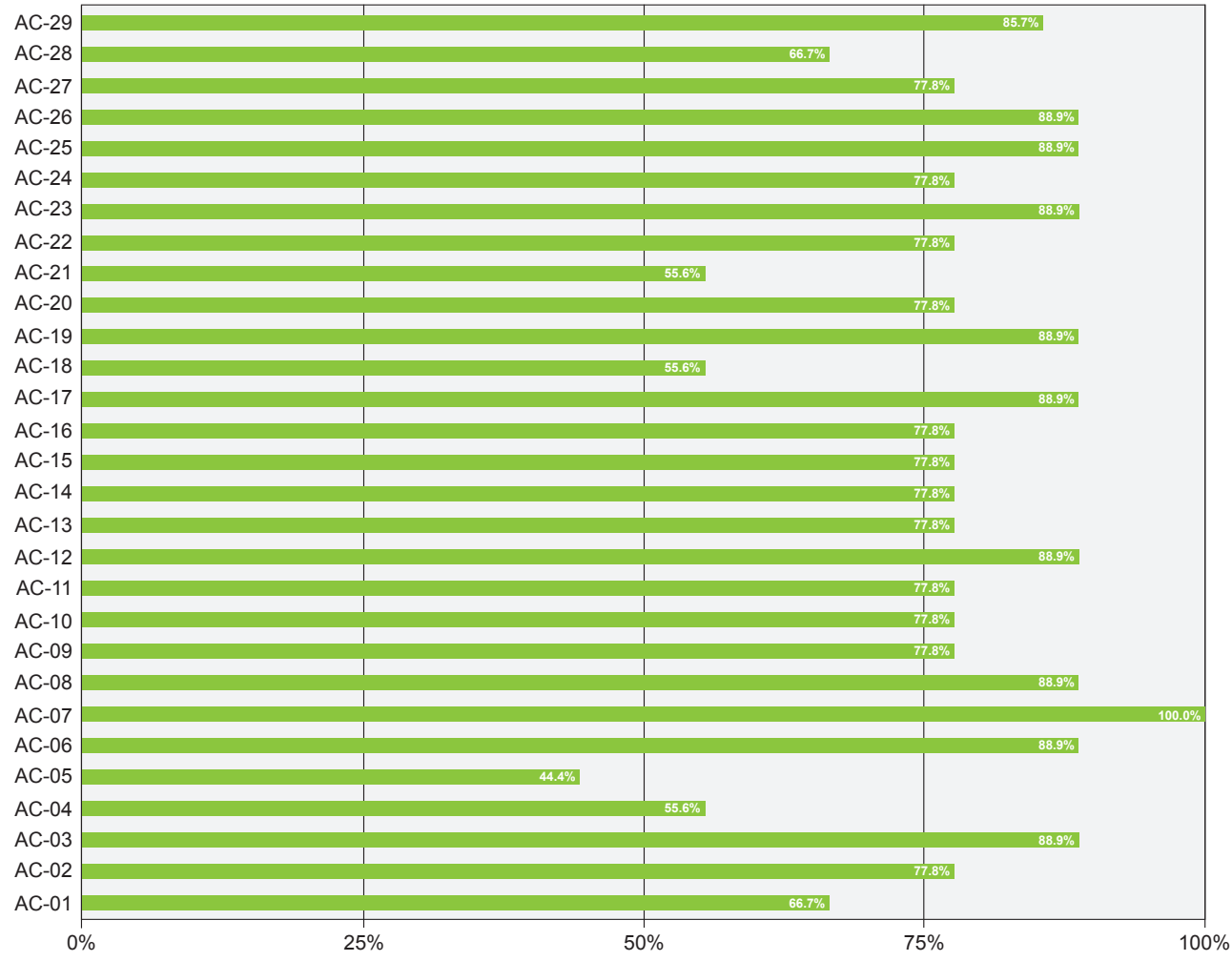

Figure 2 – Nomenclature concordance between laboratories for each AC pattern. Each bar shows the degree of agreement between laboratories for the nomenclature used in each AC pattern.

agreement level below 50% (AC-05) was found and where the agreement range was also higher among the three groups (44.4% - 100%). The cytoplasmic patterns ranged between 55.6% and 88.9%, mostly above 75%.

In addition to the agreement regarding nomenclature, the homology between the laboratories was also assessed before the consensus process (Fig. 3). Despite high degrees of overall agreement for AC standards in the country, significantly different alignments with the international ICAP reference were found. A degree of agreement below 75% has been found in only two laboratories, with all the remaining showing an agreement above 75%.

At the end of the process, a list was drawn up with the agreed nomenclature translated into Portuguese as a basis for consensus for all participating laboratories (Table 4).

DISCUSSION

An agreement above 75% has been found for 23 of the 29 AC standards, confirming that a good consensus for ICAP already existed in Portugal. This consensus is in line with other nationwide studies in the universe of quality assessment, regarding the test that was used and how to present the results.[7] However, differences in translations of the terminology used between the different laboratories were found, with an impact on how the results are reported, a phenomenon previously identified and widely discussed.[8-10]

Another relevant dimension of our results is associated with the differences in the nomenclature used for the set of all AC standards compared with the ICAP international reference before the consensus process. A degree of agreement ranging between 24 and 100% has been found, with an impact on the correct interpretation of results in the context of data sharing between institutions, previously justifying similar consensus processes of international scope.[5]

One limitation of this study was the absence of research of factors that could have an impact on the homology levels between laboratories, including geographical location, laboratory culture and organisational structure, in addition to any previous study with ICAP. These factors were not addressed due to the specific scope of this study and the methodological complexity involved in this assessment and should be analysed in future research. The number of laboratories involved is another limitation. Although only international examples of similar consensus processes exist,[5] with universes that have countries and not laboratories as the unit of study, increasing the number of participating laboratories will add relevance and impact in the medium and long-term.

This study has highlighted the importance of adopting consensus terminology when issuing ANA results and highlighted the importance of multi-centric work, reducing the risks associated with the lack of harmonisation.[11]

A more comprehensive and consensual agreement for all terminologies means a substantial improvement in the way laboratories contribute to the diagnostic process, as well as in terms of accuracy.

CONCLUSION

This work has shown the positive potential of collaboration between laboratories to generate consensus as a contribution to improved patient follow-up. The use and acceptance of the ICAP standards in Portugal will only be possible if the translation into Portuguese is accepted by a large number of laboratories and with a comprehensive involvement of physicians, who are the main users of laboratory results. As a next step, there should be a plan for disclosure and discussion of this information.

HUMAN AND ANIMAL PROTECTION

The authors declare that this project complied with the regulations that were established by the Ethics and Clinical Research Committee, according to the 2013 update of the Helsinki Declaration of the World Medical Association.

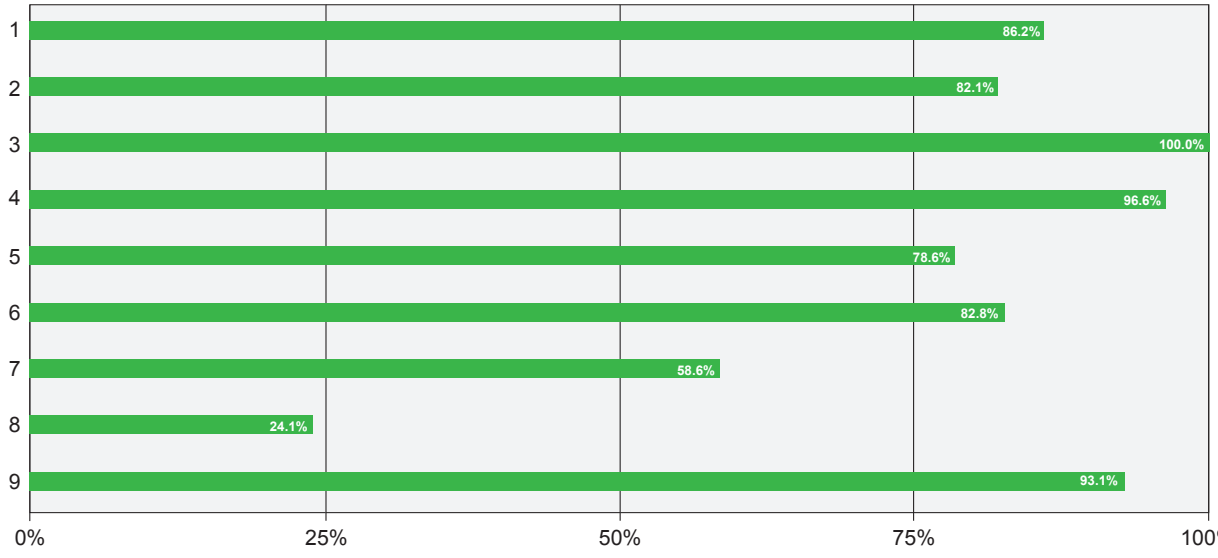

Figure 3 – Nomenclature homology by laboratory. Each bar shows the degree of agreement with the nomenclature for all AC patterns, by laboratory, prior to the consensus procedure.

Table 4 – Original English nomenclature and final consensus translation of AC patterns

	Pattern	*Padrão (consenso para Portugal)*
AC-01	*Nuclear homogeneous*	***Nuclear homogéneo***
AC-02	*Nuclear dense fine speckled*	***Nuclear mosqueado fino denso***
AC-03	*Centromere*	***Centrómero***
AC-04	*Nuclear fine speckled*	***Nuclear mosqueado fino***
AC-05	*Nuclear large/coarse speckled*	***Nuclear mosqueado grosseiro***
AC-06	*Multiple nuclear dots*	***Múltiplos pontos nucleares***
AC-07	*Few nuclear dots*	***Raros pontos nucleares***
AC-08	*Homogeneous nucleolar*	***Nucleolar homogéneo***
AC-09	*Clumpy nucleolar*	***Nucleolar aglomerado***
AC-10	*Punctate nucleolar*	***Nucleolar ponteado***
AC-11	*Smooth nuclear envelope*	***Membrana nuclear linear***
AC-12	*Punctate nuclear envelope*	***Membrana nuclear ponteada (complexo poro-nuclear)***
AC-13	*PCNA-like*	***Pleomórfico (tipo PCNA)***
AC-14	*CENP-F-like*	***Pleomórfico (tipo CENP-F)***
AC-15	*Cytoplasmic fibrillar linear*	***Citoplasmático filamentoso linear***
AC-16	*Cytoplasmic fibrillar filamentous*	***Citoplasmático filamentoso fibrilar***
AC-17	*Cytoplasmic fibrillar segmental*	***Citoplasmático filamentoso segmentar***
AC-18	*Cytoplasmic discrete dots/GW body-like*	***Citoplasmático granular (grânulos isolados)***
AC-19	*Cytoplasmic dense fine speckled*	***Citoplasmático granular fino denso***
AC-20	*Cytoplasmic fine speckled*	***Citoplasmático granular fino***
AC-21	*Cytoplasmic reticular/AMA*	***Citoplasmático reticular (tipo mitocondrial)***
AC-22	*Polar/Golgi-like*	***Citoplasmático polar (tipo Golgi)***
AC-23	*Rods and rings*	***Citoplasmático anéis e bastonetes***
AC-24	*Centrosome*	***Centrossoma***
AC-25	*Spindle fibers*	***Fuso mitótico***
AC-26	*NuMA-like*	***Fuso mitótico (NuMA-1)***
AC-27	*Intercellular bridge*	***Ponte intercelular***
AC-28	*Mitotic chromosomal coat*	***Envelope cromossómico***
AC-29	-	***Nuclear granular (tipo topoisomerase I/ ScL-70)***

DATA CONFIDENTIALITY

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in writing this manuscript.

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