Comparison of the effect of reporting cytoplasmic patterns as anti-nuclear antibody positive and anti-nuclear antibody negative on reflex test ordering

Vergleich der Auswirkungen des Ergebnisberichts über zytoplasmatische Muster bei positiven und negativen antinukleären Antikörpern auf die Anordnung eines Reflextests

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ABSTRACT

Objective Anti-nuclear antibody (ANA) patterns are classified as nuclear, cytoplasmic or mitotic. The International Consensus on ANA patterns (ICAP) suggests three parameters for ANA reporting – assay type, results and advice for reflex testing – but has not yet reached a consensus on the reporting of cytoplasmic/mitotic patterns as ANA-negative or positive. We investigated the effect of ICAP's two proposals for reporting cytoplasmic patterns as ANA-positive and negative with a view to the recommendation for reflex testing in a country that has no national reimbursement policies for automatic reflex testing.

Methods This non-interventional descriptive study included 1241 patients with positive cytoplasmic ANA patterns. 442 patients were reported as ANA-negative and 799 as ANA-positive. Patients were followed up for a two-year period to determine testing recommendations based upon nuclear and cytoplasmic patterns. For statistical analysis, the t-test was used, with a significance threshold of p-value < 0.05.

Results Appropriate reflex orders were seen more commonly with cytoplasmic patterns reported as ANA-positive (27.30 %) than with those reported as ANA-negative (5.51 %, p-value < 0.05). However, ANA-positive reports led to higher ordering of nuclear pattern reflex tests (12.97 %) compared with ANA-negative reports (1.10 %, p-value < 0.05). A large group of patients (59.73 % ANA-positive, 93.39 % ANA-negative) did not receive reflex testing.

Conclusion Reporting cytoplasmic patterns as ANA-positive was considered more significant, but reading the result report without considering the pattern and recommendation notes could lead to inappropriate reflex testing. Besides reaching a consensus for reporting cytoplasmic patterns as ANA-negative or positive, it is important to consider solutions to reimbursement policies for automatic reflex testing to decrease the impediments in reporting cytoplasmic ANA patterns.

ZUSAMMENFASSUNG

Hintergrund Antinukleäre Antikörper (ANA) werden als nukleäre, zytoplasmatische oder mitotische Muster klassifiziert. Der internationale Konsens zur ANA-Bestimmung (ICAP) schlägt drei Parameter für die ANA-Berichterstattung vor – die Art des Tests, dessen Ergebnisse und Empfehlungen für Reflextests. Für die Meldung zytoplasmatischer/mitotischer Muster als ANA-negativ oder -positiv wurde jedoch noch kein Konsens

erzielt. Wir untersuchten die Auswirkungen der beiden Vorschläge des ICAP für die Meldung von zytoplasmatischen Mustern als ANA-positiv und -negativ auf die Empfehlung für einen Reflextest in einem Land, das über keine nationalen Erstattungsrichtlinien für automatische Reflextests verfügt.

Methoden Diese nicht-interventionelle deskriptive Studie umfasste 1241 Patienten mit positiven zytoplasmatischen ANA-Mustern. 442 Patienten wurden als ANA-negativ eingestuft und 799 als ANA-positiv. Zur Ermittlung der Testempfehlungen auf Grundlage der nukleären und zytoplasmatischen Muster wurden die Patienten über einen Zeitraum von zwei Jahren nachbeobachtet. Für die statistische Analyse wurde der t-Test mit einer Signifikanzschwelle von p-Wert < 0,05 angewendet.

Ergebnisse Ordnungsgemäße Anordnungen von Reflextests wurden häufiger bei zytoplasmatischen Mustern gesehen, die als ANA-positiv angezeigt wurden (27,30 %) als bei negativen (5,51 %, p-Wert < 0,05). Bei ANA-positiven Befunden wurde jedoch häufiger ein Kernmuster-Reflextest angeordnet (12,97 %) als bei ANA-negativen Befunden (1,10 %, p-Wert < 0,05). Bei einer großen Gruppe von Patienten (59,73 % ANA-positiv, 93,39 % ANA-negativ) wurden keine Reflextests angeordnet.

Schlussfolgerung Die Meldung von zytoplasmatischen Mustern als ANA-positiv wurde als aussagekräftiger erachtet. Das Lesen des Ergebnisberichts ohne Berücksichtigung des Musters und der Empfehlungshinweise könnte jedoch zu unangebrachten Reflextests führen. Neben der Erzielung eines Konsenses zur Meldung zytoplasmatischer Muster als ANA-negativ oder -positiv ist es wichtig, Lösungen für die Erstattungsrichtlinien für automatische Reflextests zu prüfen, um die Hindernisse bei der Meldung zytoplasmatischer ANA-Muster zu verringern.

Introduction

Determining anti-nuclear antibodies (ANA) using indirect immunofluorescence assays (IIFA) on human epithelial cells (HEp- 2) is a "gold" standard test for first level screening of systemic autoimmune rheumatic diseases (SARD) [1–3]. The IIFA technique on HEp-2 cells has the advantage of added clinical value, since it works as a "natural array" that allows the detection of more than 30 different nuclear, cytoplasmic and mitotic cell patterns, by presenting numerous native antigens [1, 2].

ICAP provides a recommended model of the nuclear patterns, cytoplasmic and mitotic apparatus patterns ANA Test Report with clinically relevant content to harmonize for a meaningful report in accordance with ISO 15,189, which is a challenge in autoimmunity laboratories [1, 2].

The recommendations for reporting nuclear, cytoplasmic, and mitotic apparatus ANA patterns by IIFA should be concise and traceable and that the report should consist of a minimum of five well-defined parts: patient and referring physician identifiers, ANA patterns (ICAP AC code and pattern descriptor), titer, reference range, and comments or remarks [2–4]. While the nomenclature for ANA patterns has reached a consensus within the International Consensus Of ANA Patterns (ICAP) workshops, neither the American College of Rheumatology (ACR) nor the European Autoimmunity Standardization Initiative/International Union of İmmunological Societies (EASI/IUIS) recommendations state a clear position on which cytoplasmic/mitotic apparatus patterns should be considered ANA negative or positive [1–3, 5]. If an ANA positive reporting proposal is adopted, the pattern name could be written under the cytoplasmic ANA subtitle [2]. Adding the subtitle allows for many clinically important cytoplasmic patterns to be now reported as positive, calling the necessary attention of the ordering physician to meaningful results.

ANA testing using the IIFA technique necessitates the characterization of positive ANA results through immunoassays for the detection of specific nuclear and cytoplasmic autoantibodies in the framework of the recommended two-tier approach [6, 7]. There are several biological and non-biological limitations inherent to the IIFA method. Visual evaluation is time-consuming, inter-observer variability may occur, ad-hoc training is needed, and expert morphologists are required. In addition, the photobleaching effect, the lack of automated procedures, low predictive value of the ANA test, the variability of cellular substrates, the need for visual determination of the pattern, and lack of specificity all contribute to the difficulties of this method [1, 8–15]. The combination of HEp-2 IIFA and solid phase assays (SPA) with the most relevant nuclear and cytoplasmic antigens has balanced the limitations and increased the specificity [16]. The two-tier approach fits a reflex test which is a ''cascade'' diagnostic approach where a positive initial (first level) test automatically triggers further (second level) tests based on predefined rules applied to information systems [7, 17, 18]. However, the two-tier approach has proved to be laborious despite apparent advantages such as only requiring a single visit to the doctor and laboratory, allowing for a rapid clinical diagnosis, as well as potential economic profit resulting from a decrease in unneceassary second-level tests [1, 7, 17–19]. Many laboratories cannot integrate reflex tests into their testing algorithm, mostly due to a lack of reimbursement policies. In these circumstances, the second line of testing is advised based on the report, as is done in our country of Turkey [17].

For nuclear and cytoplasmic ANA patterns, the reflex test cascade would be different. For nuclear ANA patterns, the reflex test cascade is mostly ENA testing (Extractable Nuclear Antigens) (by ELISA or immunoblotting methods). For cytoplasmic patterns, the reflex cascade is variable. For cytoplasmic reticular patterns, the reflex test cascade is an anti-mitochondrial antibody (AMA) (by the IIFA method); for cytoplasmic linear patterns, it is anti-smooth muscle antibodies (actin antibodies; ASMA) (by the IIFA method) and for cytoplasmic dense fine speckled patterns, the reflex test cascade is mostly ENA testing (Extractable Nuclear Antigens) (by ELISA or immunoblotting methods) for anti-ribosomal P protein antibody [17].

Reflex testing is advised for all relevant ANA patterns, including cytoplasmic ones which could be reported as ANA positive or ANA negative according to the ICAP recommendations. The effect of different reporting layouts of cytoplasmic ANA patterns on reflex

testing is discussed in this study. We compared the number of reflex test orders (AMA/ASMA) recommended between the positive reported cytoplasmic reticular/linear ANA patterns and the negative ones.

Materials and Methods

Clinical Sample Selection

Fifty one (51) different primary, secondary, and tertiary healthcare centers in all seven geographic regions of Turkey sent blood samples to our Clinical central laboratory for ANA IIFA testing. In our single central laboratory, all the analysis were performed. The selection of the patients, testing proc

edures and follow-up period took 5 years between 2014 and 2019.

In this study, 1241 patients who were monitored with suspicion of autoimmune liver disease and who had positive cytoplasmic (reticular and linear fibrillar ANA patterns) ANA patterns were included. 442 patients were reported as ANA negative and 799 as ANA positive.

The type of assay used (IIFA), reported pattern, antibody levels and the advice for reflex testing (noted as "AMA/ASMA reflex test was recommended in case of suspicion of autoimmune hepatitis/biliary disease.") were identical for all patients, as per ICAP recommendations. The laboratory wrote the pattern name under the cytoplasmic ANA subtitle **(▶ Fig. 1)**.

At the reports of totally 215 (= 79 from ANA negative reported group + 136 from ANA positive reported group) patients, the advice for reflex testing note have not written, since the reflex test (AMA/ASMA) ordered already with ANA test. For the next two years, we followed up the patients that we recommended reflex tests at their reports for AMA/ASMA and ENA reflex orders.

We prepared a questionare with two questions for the clinicians who haven't order any reflex;

1-Did you read the recommendation note at the report?

2- If answer is yes, why did you not order any reflex test?

2 i- clinically not needed

2 ii-patient stop follow-up

ANA and Reflex Testing

ANA tests were performed using the HEp-2 Standard kit for Helios automated IFA systems (Aesku, Wendelsheim, Germany). Helios automated IFA systems were used to capture images of the ANA slides and were added to the report through a Laboratory Information System (LIS) and then stored **(▶ Fig. 1).** ANA pictures could add value, improving the report layout and moving towards a better education of the ordering physician. Additionally, two IIFA experts (one laboratory technician and a doctor) examined the slides blindly using a Led Microscope (Motic, Hong Kong). In the case of non-conformity between the two readings, an ANA test was run using the Mosaic HEP-20–10/Liver (Monkey) (Euroimmun, Lüebeck, Germany).

AMA/ASMA tests were performed with rLKS–Rat wrapped (Rat/Monkey) kits for Helmed IFA systems (Aesku). Slides were read by two IIFA experts (one laboratory technician and a doctor) blindly on a Led Microscope (Motic, Hong Kong). In the case of non-conformity between two readings, AMA/ASMA test were run with the Euro plus LKS Mosaic (Euroimmun).

ENA tests were performed with ANA-17 comp kits using Helmed Blot systems (Aesku). In the case of non-conformity between the ANA and ENA results, ENA tests were run with the Euroline ANA Profile 1 (IgG) kit (Euroimmun).

Statistical Analysis

For Statistical analysis, the IBM SPSS Statistics software Version 26 (SPSS Inc., Chicago, IL) was used. Pearson chi square test for categorical variables was used for the comparison of reflex testing between the two groups. The results were evaluated within a confidence interval of 95 %, and a p value of less than 0.05 was considered statistically significant.

Results

Demograhic analysis of 1241 are summarized at ▶ **Table 1**. 442 patients were reported as ANA negative and 799 as ANA positive. % 83 of ANA negative reported group is female and %83,97 of ANA positive. The mean of ages in years at ANA negative reported group is 47,3 and ANA positive is 46,8.

Analysis of AMA/ASMA Patient Testing

Numbers of AMA/ASMA tests performed were analyzed bi-annually (▶ **Table 2**). AMA/ASMA test orders were higher for cytoplasmic reticular/linear patterns reported as ANA positive than negative (p-value < 0.05). In the positive group with recommendation note for reflex (AMA/ASMA) testing (n = 663), AMA/ASMA (n = 181, 27.30 %) tests were ordered. In the negative group (n = 363), 52 AMA/ASMA (n = 20, 5.51 %) tests were ordered

Analysis of ENA Patient Testing

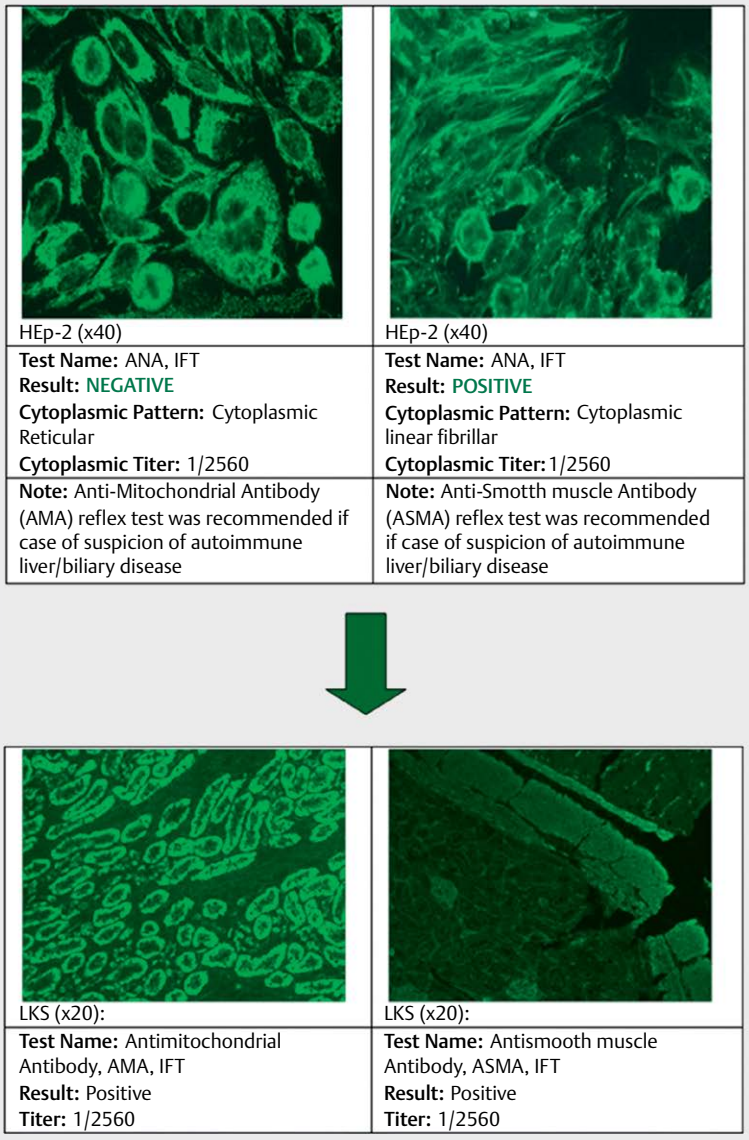
Although at the reports there was a recommendation note as "AMA/ASMA reflex test is recommended in case of the suspicion of autoimmune hepatitis/biliary disease," the order numbers of ENA tests even not mentioned at the reports was higher. ENA test orders for cytoplasmic patterns reported as ANA positive group, were higher than those for the ANA negative group (p-value < 0.05) (▶ **Table 2**). In the ANA positive group (n = 663), the clinicians ordered 86 ENA (12.97 %) tests, and 4 ENA (1.10 %) tests in the ANA negative group (n = 363).

Despite of the significant differences of reflex test ordering numbers, the result distribution of AMA/ASMA and ENA reflex testing were smiliar in both group (▶ **Table 3**) (p > 0.05). Ratio of positive AMA/ASMA reflex test results was 74.03 % in ANA positive group and 75.00 % in ANA negative group. Ratio of positive ENA results was 1.16 % in ANA positive group and 0.00 % in ANA negative group.

Patients With No Reflex Tests Ordered

In a large group of patients, any reflex test neither AMA/ASMA nor ENA tests were performed (▶ **Table 2**). To analyze this omission, we contacted with their clinicians; however, not all these clinicians were available for comment. Totally 20 of them filled the questionare (▶ **Table 4**).

The result of the questionare filled with the clinicians who didn't order reflex tests;



▶ **Fig. 1** ANA reporting algoritm.

▶ **Table 1** Demographic information of patients Abbrevation: ANA : anti-nuclear antibody.

	ANA Positive n: 799	ANA Negative n:442
Gender	Female n:671 (83,97 %)	Female n:367 (83,03 %)
Age, years	46,8 (15–89)	47,3 (17–90)

1–70 % from the positive and 85 % from the negative groups, admitted that they did not read the recommendation notes.

2–30 % from the positive and 10 % from the negative groups declared that they did not need any further tests according to the patient's clinical results.

3–0 % from the positive and 5 % from the negative groups said that their patients failed to report for the follow-up visits.

More than half declared that they did not read the recommendation notes. This number was even higher in the ANA negative group (p-value < 0.05); however, positive reporting alone is not enough to understand the recommendation notes.

Discussion

The ICAP has proposed that cytoplasmic ANA patterns can be reported as either positive or negative. However, they recommend reflex testing (ICAP recommendation 13) to improve the utility of the serological evaluation regardless of whether ANA is reported as positive or negative [1, 4]. Results of the ANA should be communicated to clinicians through reflex test recommentation notes , bridging the technical knowledge of the laboratory with the clinical relevance, and so

▶ **Table 2** Distribution of reflex test ordering at cytoplasmic ANA patterns reported as positive and negative group. Abbrevation: ANA : anti-nuclear antibody, AMA: anti-mitochondrial antibody, ASMA: anti-smooth muscle antibody, ENA: Extractable nuclear antigens.

Patients with positive cytoplasmic ANA patterns	ANA Positive	With recommendation note for reflex (AMA/ASMA) testing	AMA/ASMA Reflex test ordering n:181 (27.30 %) (p-value < 0.05)
n: 1241	n: 799	n: 663	ENA Reflex test ordering
			n:86 (12.97 %) (p-value < 0.05)
			No reflex test order
			n:396 (59.7 %3)
		Without recommendation note for reflex (AMA/ASMA) testing	
		n: 136	
	ANA Negative	With recommendation note for reflex (AMA/ASMA) testing	AMA/ASMA Reflex test ordering
	n:442	n: 363	n:20 (5.51 %) (p-value < 0.05)
			ENA Reflex test ordering
			n:4 (1.10 %) (p-value < 0.05)
			No reflex test order
			N:339 (93.39 %)
		Without recommendation note for reflex (AMA/ASMA) testing	
		n: 79	

▶ **Table 3** Distribution of AMA/ASMA and ENA reflex testing results. Abbrevation: ANA : anti-nuclear antibody, AMA: anti-mitochondrial antibody, ASMA: anti-smooth muscle antibody, ENA: Extractable nuclear antigens.

	ANA Positive	ANA Negative
AMA/ASMA Positive	134 (74.03 %)	12 (75.00 %)
AMA/ASMA Negative	47 (25.97 %)	4 (25.00 %)
ENA Positive	1 (1.16 %)	0 (0.00 %)
ENA Negative	85 (98.84 %)	4 (100.00 %)

▶ **Table 4** Distribution of the reasons why clinician do not order reflex testing. Abbrevation: ANA : anti-nuclear antibody.

	ANA Positive n:396	ANA Negative n: 339
Not clinically needed	30.05 %	12.97 %
Not read the note	67.68 %	82.89 %
Not followed	2.27 %	4.14 %

adding value to clinical decision-making,. Also we allocated time for additional communication between the author and the clinician only for exceptional cases as a consequence of our extensive sample collection network (51 different centers) and an intensive work load (Our ANA test number per month is over 10,000). We wanted to analyse how clinical perception, attraction, and attention impacted clinical decision. If a report attracted the attention of the clinician, he read the whole of the report and advised the ordering of a reflex test whenever clinically needed. Paying attention to the advice notes is crucial in our country of Turkey, since there is no reimbursement policy for the reflex cascade algorithm. So we compared the recommended reflex test ordering frequency between two types of cytoplasmic pattern reports; ANA positive and negative [1].

In our study, we found that an ANA positive cytoplasmic reticular/linear pattern led to a higher AMA/ASMA reflex testing rate when compared to the ANA negative group (p-value < 0.05). Positive reports attracted more attention from the clinician and, consequently, more ICAP recommended reflex tests were ordered. Parallel to our findings, The ICAP believes that if the laboratory reported such a pattern as negative, the additional information in the report on the pattern and titer might go unnoticed because clinicians tend to pay less attention to negative results [2].

We also found that reporting cytoplasmic reticular/linear patterns as ANA positive led to a higher ENA reflex testing rate than ANA negative reports (p-value < 0.05). ENA reflex tests were recommended for nuclear ANA patterns, which are more common than cytoplasmic patterns, as The ICAP recommends reporting nuclear ANA patterns as positive [17].

We found that reporting cytoplasmic ANA patterns as positive was not enough for clinicians to read the reflex test recommendations by The ICAP. Clinicians may pay attention to the result and not the additional information in the report [2]. If a clinician received a positive ANA report and didn't pay attention to the pattern/notes, he may assume it was a nuclear pattern, since nuclear patterns are more frequent and always reported as positive, and would therefore order the ENA reflex test. Paying attention to the results but not the pattern/notes could be a reason for requesting the ENA reflex test. Else, clinicians could read the results, pattern/titer information, and recommendation notes, and still could order the ENA test according to the clinical data of the patient. In our study, the positivity rate of ENA tests was too low (1.16 % and 0.00 %) to support the second scenario.

ICAP declared that some rheumatologists thought that ANA patterns were not so important, they just payed attention to the ANA results and the titer; second, patterns were considered as irrelevant, because with an ANA positive, most of the doctors would order anti-ENA tests; third, information obtained from staining patterns was subjective, and varied according to the reader and dilution. This thesis explained our study results. In our sttudy we found that the doctors who did not pay attention to the patterns also did not pay attention to the notes.

Advice resulting from this study on reflex testing could be redundant until Turkey develops a national reimbursement policy. Before

solving the reimbursement problems, advice about reflex testing could be redundant. After development of national regulations, we could achieve efficient reflex test numbers by decreasing unnecessary orders and increasing the appropriate requests. Unnecessary ENA reflex tests conducted at the same visit as ANA testing could be reduced by this regulation. The cost of non-effective ENA reflex tests could be decreased (12.97 % in the case of a positive ANA test and 1.10 % in the case of a negative ANA test). More importantly, the chance of probable diagnosis could be increased, particularly in cases where clinicians do not read testing recommendation notes (59.73 % of positive ANA reports and 93.39 % of negative ANA reports). In a study, Among 108 laboratory respondents, 55 (51 %) adopt the practice of reflex testing (tests for specific autoantibodies, performed after a positive ANA, and without a new order from the prescribing physician) or follow-up testing (the same, after a new prescription). Some laboratories practicing the reflex testing strategy integrate their ANA results into a general autoimmune report or directly uploaded from the LIS into the patient's EMR, with all specific autoantibody tests conducted, technique used, and respective result. So a covetable ratio of ideal reflex test numbers could be achieved. But this is not feasible in many countries: the physician must order specific tests in a second step, according to the ANA pattern reported and recomented reflex tests.

The ANA-reflex test is different from other reflex tests in terms of its algorithm construction. Although it has very low predictive values, it serves a role in the diagnosis of several systemic autoimmune rheumatic diseases as a first-line test [17]. Additionally, the first test can be positive in up to 20–30 % of healthy subjects, especially some regular patterns as dense fine speckled-70, which are not associated with systemic autoimmune disorders [17, 20, 21]. Despite these drawbacks, the application of ANA-reflex testing has many objective advantages, such as simplifying the patient workup, with only a single visit to the doctor's surgery and laboratory required, enabling a more rapid clinical diagnosis. the ANA-reflex algorithm allows for more appropriate use of second-level testing [3].

As an alternative to the national reimbursement policy for the reflex cascade algorithm, the Italian model could be applied, which decreases bureaucratic or administrative problems. Laboratories can calculate the cost of reflex tests by considering the number and type of further tests. The fee for the ANA-reflex test covers all of the possible second line tests, so additional payment problems can be avoided [18]. An efficient workflow for reflex testing at the auto-immunology laboratory must be compatible with the growing request for autoimmune diagnostic tests, regional restrictions, and limited reimbursement [22]. We can adapt the ANA-reflex request modality to our national health policy and culture to provide rapid, complete diagnostic information with a critical impact on the clinical decision [17].

Positive reporting of cytoplasmic reticular/linear ANA patterns resulted in an incremental increase in AMA/ASMA test ordering (p-value < 0.05), and an incremental increase in ENA test ordering ($p < 0.05$) into consideration.

Reporting of cytoplasmic pattern with the ANA test has been suggested by some groups. The Italian Forum Interdisciplinare per la Ricerca nelle Mallattie Autoimmuni (FIRMA) and the second Brazilian consensus ICAP (although this changed in the third consensus) recommended a cytoplasmic pattern to be considered ANA positive. However, the European Consensus Finding Study Group on Laboratory Investigation in Rheumatology (ECGSG), a member of the European League Against Rheumatism (EULAR), advocates cytoplasmic pattern to be considered ANA negative due to the problems during reimbursement, classification criteria, and EQC programs; simply put, there is a conflict with the reporting of the test [2]. The major concern with respect to reporting cytoplasmic patterns as ANA positive is that in some jurisdictions, existing guidelines and diagnostic/classification criteria for systemic lupus erythematosus (SLE), Sjogren's syndrome, mixed connective tissue disease (MCTD), systemic sclerosis (SSc), and autoimmune hepatitis (AIH) [13, 14] are based on restricting ANA to nuclear patterns. Problematically, no guidance has been provided about the interpretation of cytoplasmic and mitotic patterns. Diagnosis can be affected by positive reported cytoplasmic and mitotic ANA patterns [2, 4, 23–30]. Diagnostic criteria for AIH clearly define that only nuclear patterns are to be interpreted as ANA [28–30]. A positive AMA result gives a negative 4 points [28–30]. This scoring results in a paradox if AMA is reported as ANA positive [2, 4, 28]. A third problem relates to the external quality control (EQC) programs that accept cytoplasmic patterns reported as ANA negative. With some alternative immunoassays, surprisingly, ACR and EASI/IUIS recommendations allow a positive result with relevant cytoplasmic ANA patterns [2].

At a multinational study, it was found that there were more expert-level laboratory professionals (61 %) than competent-level laboratory professionals (46 %) that considered cytoplasmic patterns as ANA positive ($p < 0,05$). The fraction of laboratory professionals that considered cytoplasmic ANA patterns as ANA positive was higher in non-European countries (63 %) than in European countries (48 %) ($p < 0,05$). The reasons was maintained for historical name problem (the name 'antinuclear' for the HEp-2 cell IIF test does not take into consideration that autoantibodies to cell compartments other than the nucleus) as well as for laboratory coding and invoicing [31, 32].

As written in an ICAP publication [50], jurisdictional and reimbursement reasons may be at play in many countries, so that there is no universal consensus of the ICAP experts for reporting cytoplasmic patterns.

Conclusion

Achieving an interim consensus between the clinical societies for diagnostic/classification criteria in distinct diseases, as well as political solutions to reimbursement policies, could decrease blockades to reporting cytoplasmic and mitotic ANA patterns. In order to achieve this consensus between all stakeholders, it is important to note that positive cytoplasmic ANA patterns have a constructive influence on clinical perceptions but do not preclude the need to read the recommendation notes. Not reading recommendation notes from positive cytoplasmic ANA patterns could lead to various diagnostic and management problems.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] 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

[2] Damoiseaux J, Mühlen C, Torre I et al. International consensus on ANA patterns (ICAP): the bumpy road towards a consensus on reporting ANA results. Autoimmune Highlights 2016; 7: 1–8

[3] Solomon DH, Kavanaugh AJ, Schur PH. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum 2002; 47: 434–444

[4] Chan E.K.L, Damoiseaux J. de Melo Cruvinel, et al. Report on the second International Consensus on ANA Pattern (ICAP) workshop in Dresden 2015. Lupus. 2016; 25: 797–804

[5] Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69: 1420–1422

[6] Scholz J, Grosmann K, Knütter I et al. Second generation analysis of antinuclearantibody (ANA) by combination of screening and confirmatory testing. Clin Chem Lab Med 2015; 53: 1991–2002

[7] Bonaguri C, Melegari A, Ballabio A et al. Italian multicentre study for application of a diagnostic algorithm in autoantibody testing for autoimmune rheumatic disease: conclusive results. Autoimmun Rev 2011; 11: 1–5

[8] Rigon A, Infantino M, Merone M et al The inter-observer reading variability in anti-nuclear antibodies indirect (ANA) immunofluorescence test: A multicenter evaluation and a review of the literature. Clin Chem Lab Med 2015; 53: 1991–2002

[9] Bizzaro N, Tozzoli R, Tonutti E et al. Variability between methods to determine ANA, anti-dsDNA and anti-ENA autoantibodies: a collaborative study with the biomedical industry. J Immunol Methods 1998; 219: 99–107

[10] Song L, Hennink E, Young IT et al. Photobleaching kinetics of fluorescein in quantitative fluorescence microscopy. Biophys J 1995; 68: 2588–2600

[11] Kavanaugh A, Tomar R, Reveille J et al. American College of Pathologists. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. Arch Pathol Lab Med 2000; 124: 71–81

[12] Copple SS, Giles SR, Jaskowski TD et al. Screening for IgG antinuclear autoantibodies by HEp-2 indirect fluorescent antibody assays and the need for standardization. Am J Clin Pathol 2012; 137: 825–830

[13] Tonutti E, Bassetti D, Piazza A et al. Diagnostic accuracy of ELISA methods as an alternative screening test to indirect immunofluorescence for the detection of antinuclear antibodies. Evaluation of Five Commercial Kits. Autoimmunity. 2004; 37: 171–176

[14] Tan EM, Smolen JS, McDougal JS et al. A critical evaluation of enzyme immunoassays for detection of antinuclear autoantibodies of defined specificities. I. Precision, sensitivity, and specificity. Arthritis Rheum 1999; 42: 455–464

[15] Satoh M, Chan EK, Ho LA et al. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 2012; 64: 2319–2327

[16] Meroni PL, Chan EK, Damoiseaux J et al. Unending story of the indirect immunofluorescence assay on HEp-2 cells: old problems and new solutions? Ann Rheum Dis. published online April 17, 2018

[17] Tonutti E, Bizzaro N, Morozzi G et al. The ANA-reflex test as a model for improving clinical appropriateness in autoimmune diagnostics. Autoimmun Highlights. 2016; 7: 9

[18] Bizzaro N, Morozzi G. A proposed model for effective collaboration between rheumatologists and clinical pathologists for the diagnosis of autoimmune rheumatic diseases. Rheumatol Int 2009; 29: 849–851

[19] Damoiseaux J, Agmon-Levin N, Van BM 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

[20] Mariz HA, Sato EI, Barbosa SH et al. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011; 63: 191–200

[21] Fabris M, Zago S, Tosolini R et al. Anti-DFS70 antibodies: a useful biomarker in a pediatric case with suspected autoimmune disease. Pediatrics. 2014; 134: 706–708

[22] Infantino M, Meacci F, Grossi V et al. The burden of the variability introduced by the HEp-2 assay kit and the CAD system in ANA indirect immunofluorescent test. Immunol Res 2017; 65: 345–354

[23] Petri M, Orbai AM, Alarcón GS et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 2012; 64: 2677–2686

[24] Shiboski SC, Shiboski CH, Criswell L et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. Arthritis Care Res 2012; 64: 475–487

[25] Amigues JM, Cantagrel A, Abbal M et al. Comparativestudy of 4 diagnosis criteria sets for mixed connectivetissue disease in patients with anti-RNP antibodies. AutoimmunityGroup of the Hospitals of Toulouse. J Rheumatol 1996; 23: 2055–2062

[26] Van den Hoogen F, Khanna D, Fransen J et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/ European League against Rheumatism collaborative initiative. Arthritis Rheum 2013; 65: 2737–2747

[27] Meroni PL, Biggioggero M, Pierangeli SS et al. Standardization of autoantibody testing: aparadigm for serology in rheumatic diseases. Nat Rev Rheumatol 2014; 10: 35–43

[28] Alvarez F, Berg PA, Bianchi FB et al. International Autoimmune HepatitisGroup Report: review of criteria for diagnosis of autoimmunehepatitis. J Hepatol 1999; 31: 929–938

[29] Hennes EM, Zeniya M, Czaja AJ et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008; 48: 169–176

[30] Liberal R, Grant CR, Longhi MS et al. Diagnostic criteria of autoimmune hepatitis. Autoimmun Rev 2014; 13: 435–440

[31] Van Hoovels L, Broeders S, Chan EL et al. Current laboratory and clinical practices in reporting and interpreting anti-nuclear antibody indirect immunofuorescence (ANA IIF) patterns: results of an international survey. Autoimmun Highlights 2020; 11: 17, doi: 10.1186/s13317-020-00139-9

[32] Von Mühlen CA, La Torre IG, Infantino M. How to report the antinuclear antibodies (anti-cell antibodies) test on HEp-2 cells: guidelines from the ICAP initiative. Immunologic Research 2021; 69: 594–608