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A cost-effective assessment for the combination of indirect immunofluorescence and solid-phase assay in ANA-screening

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Abstract

Objectives: Anti-nuclear antibodies (ANA) testing on indirect immunofluorescence (IIF) has been for a long time the gold standard assay in the diagnosis of rheumatic diseases; more recently different solid phase assays (SPA) have been recommended to increase specificity of positive results. The best combination of the different assays should both reduce the time to diagnosis and the costs of testing.

Methods: Serum samples from 995 unselected outpatients were analysed simultaneously using IIF and a fluorescent enzyme SPA as initial screening test. Any IIF or SPA positive sample was further analysed for individual antibody specificities and three algorithm models with different timelines were adopted. The cost-effectiveness assessment was performed by calculating the total number of positive patients and the cost of diagnosis for each algorithm.

Results: IIF and SPA were both positive in 112 (11.3 %) patients, and both negative in 597 (60 %) patients; 257 results (25.8 %) were conflicting between the two methods. The three algorithms resulted in a different number of positive patients and had a different cost per single diagnosis: the combined algorithm revealed the highest number of positive patients with a lower cost per diagnosis than the traditional one.

Conclusions: The combined approach of two different methods ensures the highest reliability of ANA screening test; however, specific appropriate SPA testing might be chosen according to IIF pattern as recommended in International guidelines. Each clinical laboratory should carefully evaluate its diagnostic algorithm for ANA testing on the volume and type of requests, eventually designing new cost-effective reimbursement models based on patients outcomes.

Keywords: anti-nuclear antibodies; connective tissue diseases; indirect immunofluorescence; solid-phase assay; laboratory management

Introduction

Anti-nuclear antibodies (ANA) testing on indirect immunofluorescence (IIF) on human epidermoid laryngeal carcinoma cells (HEp2 or HEp-2000 cells) has been considered the gold standard test in the diagnosis of rheumatic diseases for more than 40 years [1], but some recent papers have highlighted its limitations [1–4]. In the 2009 position paper of the American College of Rheumatology (ACR), IIF was recommended as the gold standard for ANA testing (Rheumatology, 2009); 10 years later, the ACR has stated that ANA is the entry criterion for the diagnosis of systemic lupus erythematosus (SLE) if a positive result is obtained on HEp-2 IIF or a solid phase assay (SPA) of equivalent performance [5]. However, it has already been highlighted that ANA IIF has revealed a lack of standardization, with a frequency of ANA negativity varying from 4.9 to 22.3 % between different commercial IIF assays [6]. Moreover, a recent meta-analysis confirmed a sensitivity of 89 % at 1:80 titer cut-off, but a lower specificity of 72 % [7]. In addition, ANA IIF on HEp2 may show different fluoroscopic patterns, and differences in substrates, preparation phases, and type of manual or automated microscope may increase the intra- and interlaboratory variability of the HEp-2 IIF pattern. For harmonization purposes, an international group of specialists has promoted an initiative to define the nomenclature of HEp-2 patterns, the International Consensus on ANA Patterns (ICAP) [8]. ICAP established a comprehensive classification of the most relevant HEp-2 IIF patterns that may be associated to specific autoantibodies, assigning an alpha-numeric code (AC-1, AC-2, etc.) and classified them into three main groups: the nuclear, the cytoplasmic and the mitotic [9]. Each group is further

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subdivided according to the pattern of IIF staining. An ICAP recently coded pattern is the one connected to anti-dense fine speckled 70 (DFS70) antibodies (AC-2), which are associated with a low likelihood of rheumatic disease when occurring as an isolated finding [10]. Given its presumed negative predictive value, the identification of AC-2 by IIF may be crucial, and various anti-DFS70 SPAs have been developed to differentiate AC-2 from its mimics [10].

Several SPAs were developed for quantitative detection of antibodies against extractable nuclear antigens (anti-ENA), including enzyme-linked immunoassays (ELISA), fluorescent enzyme immunoassays (FEIA), chemiluminescence assays (CLIA) [1] and, more recently particle-based multianalyte technology (PMAT) [11]. SPA have been developed to identify and quantify specific autoantibodies with a high positive predictive value for a connective tissue disease and are designed as multiplex testing. Various comparative studies showed a higher specificity for SPA vs. IIF, though a variable sensitivity and specificity between assay methods depending on single antibody specificity included and proposed cut-offs [12]; interestingly the authors highlighted different performances of SPA and IIF for each ANA-associated rheumatic disease (AARD) considered.

While the different methods were already used in autoimmune laboratories in sequential testing, the so-called "reflex" algorithms, mostly for confirmatory purposes, the combination of IIF and SPA for initial ANA screening has been evaluated in a large Italian multicentric study revealing an increase in each diagnostic performance considered [13], as it was previously demonstrated for well-characterised patients with established AARD [14]. Bizzaro et al. mentioned also a possible cost-effectiveness of the combination strategy [13].

The need for established algorithms in autoimmune laboratories emerged a few years ago in a landscape of constantly increasing test requests, not only from rheumatology or autoimmune diseases specialists but also from general practitioners: This issue, in combination with the availability of different techniques may result into an increased inappropriateness and waste of resources [15, 16].

The algorithm should allow to optimize the "patient-journey", contributing to reduce the time to diagnosis and assuring to the clinical laboratory the possibility to decide which tests perform to fulfill the clinician requests.

Our study aims to establish practical algorithms to manage the routine work-up of ANA-screening in a large clinical laboratory setting, combining two different assay methods, IIF and FEIA SPA, as first-line testing and additional confirmatory testing, when appropriated. Moreover, the ability of three following different diagnostic approaches to detect the highest number of patients with specific anti-ENA antibodies, that would be considered a surrogate measure of diagnostic effectiveness, will be explored with a cost-effective assessment:

(1) Traditional approach: ANA-IIF, then anti-ENA specificities testing (SPA Reflex panel) on each ANA-IIF+ result (SPA Screening not performed)
(2) Progressive approach: ANA-IIF, then SPA Screening on ANA-IIF+, then SPA Reflex on positive results at screening test
(3) Combined approach: ANA-IIF and SPA Screening followed by SPA Reflex on positive results, regardless of ANA-IIF results

Furthermore, the advantages of performing a SPA anti-DFS70 test in every ANA-IIF+ (regardless of the ANA-IIF pattern) and SPA Screening negative patient will be assessed.

Materials and methods

A prospective study has been conducted on diagnostic leftovers from 995 unselected adult outpatients consecutively referred to Laboratory Medicine of University-Hospital of Padova from general practitioners, rheumatologists or other medical specialists with a clinical suspicion of systemic autoimmune rheumatic disease and with a coincidental request for both ANA test and anti-ENA test, that was the only inclusion criteria. Serum samples were analysed simultaneously using IIF and a SPA. Written informed consent was obtained from all individual participants included in the study and samples were anonymized for data analysis.

IIF was performed with the Nova Lite HEp2 ANA kit (Inova Diagnostics, San Diego CA USA) or IFA HEp-2 (Euroimmun, Germany) at the starting dilution of 1:80; the cut-off for positivity was set at 1:160 titer to increase specificity and optimize the agreement, as previously described by other groups [13]; interpretation of ANA IIF was done on a manual LED fluorescence microscope (Eurostar III Plus, Euroimmun, Germany) with a consensus by two expert clinical pathologists. The FEIA SPA (EliA™ CTD Screen, Thermo Fisher) was performed according to the instructions of the manufacturer on Phadia 250 instrument (Thermo Fisher, Uppsala, Sweden). The cut-off ratio for positivity proposed by the manufacturer was ≥1 ratio, and it is suggested that the results between 0.7 and 1 should be considered borderline; for the cost-effective assessment these results were considered weak positive. The mixture of native or recombinant antigens is as follows: SSA/Ro 52, SSA/Ro 60, SSB/La, U1RNP (RNP70, A, C), Sm, centromere B, Scl70, Jo-1, Rib-P, fibrillarin, RNA Pol III, PM-Scl, PCNA and Mi-2 recombinant human proteins; dsDNA native purified antigen.

After the initial screening test, any sample with a CTD Screen test result ≥0.7 and any sample CTD Screen <0.7 but ANA-IIF positive was further analysed for individual antibody specificities testing in a Reflex panel composed by the antigens previously described for the CTD Screen and hereafter named "CTD Reflex". Cut-off values for the single antibody specificity (considering also weak positive) are: for dsDNA 10 kU/L; for SSA/Ro 52, SSA/Ro 60, SSB/La, Sm, centromere B, Scl70, Jo-1, Rib-P, fibrillarin, RNA Pol III, PM-Scl, PCNA, Mi-2 7 kU/L; for U1RNP (RNP70, A, C) 5 kU/L.

Patients with CTD Screen <0.7 and ANA-IIF+ were also tested for anti-DFS70 antibodies, also with FEIA (EliA™DFS70, Thermo Fisher) with the cut-off >10 kU/L.

The cost-effectiveness assessment was performed by calculating the total number of CTD Reflex panel positives and the percentage of positive CTD Reflex panel related to the number of patients tested with CTD Reflex and the cost of each of the three approaches and the relative "Cost of Diagnosis" (CoD), calculated as the total cost of reimbursement following the update 2023 database of Veneto Region (Italy) of the approach for the whole population tested divided for the number of CTD Reflex panel positive patients.

Moreover, the number of patients with EliA™ CTD Screen borderline (ratio ≥0.7 and <1 with any CTD Reflex positive) was reported along with the specific antibody positive result.

Finally, the prevalence of ANA-IIF+ and CTD Screen−, that are positive to EliA™DFS70 was reported and the costs to include EliA™DFS70 in the laboratory algorithm was calculated as the cost of reimbursement for DFS70 multiplied for the number of ANA IIF+/CTD screen− and divided for the actual number of DFS70 positive patients.

Results

Total ANA-IIF+ results were 342 (34.4 %) and total CTD Screen ≥1 were 153 (15.4 %) (Table 1). Altogether, ANA-IIF and CTD Screen were both positive in 112 (11.3 %) patients, and both negative in 597 (60 %) patients. The results of 257 samples (25.8 %) were conflicting between the two methods: 216 had ANA-IIF+ and CTD Screen <0.7 and 41 were ANA-IIF− and CTD Screen ≥1.

A small proportion of patients (n=29) had CTD Screen results between 0.7 and 1 (i.e. borderline), of which about half were ANA-IIF+; when tested for the CTD Reflex panel, 7/14 of the ANA-IIF+ and 10/15 of the ANA-IIF− had a specific antibody positivity, mostly anti-dsDNA (Table 2).

ANA-IIF+/CTD Screen− samples with a single antibody positivity were 13 (Table 2), corresponding to 6 % of the conflicting group (13/216) and 1.3 % of the entire cohort (13/995).

Table 1: Total results for IIF and SPA CTD Screen (percentages on the total number of patients, n = 995).

	ANA-IIF+	ANA-IIF−	Total
CTD Screen ≥1	112 (11.3 %)	41 (4.1 %)	153 (15.4 %)
CTD Screen ≥0.7 and <1	14 (1.4 %)	15 (1.5 %)	29 (2.9 %)
CTD Screen <0.7	216 (21.7 %)	597 (60 %)	813 (81.7 %)
Total	342 (34.4 %)	653 (65.6 %)	995 (100 %)

Additional testing for anti-DFS70 was performed in 214 patients, with a positive result in 17, namely 7.9 % of the conflicting group (17/214) and 1.7 % of the entire cohort, corresponding to 251.14 € per positive patient.

CTD Screen+/ANA-IIF− patients with a single antibody positivity were 33 (Table 2, of note some patients had multiple antibody positivity), corresponding to 21.6 % (33/153) of the entire SPA positive group and an additional 3.3 % of the entire cohort (33/995). The resulting 8 patients CTD Screen+/ANA-IIF− had a CTD Screen result range of 1–1.6.

For the cost-effective assessment the comparison of the number of tests that would have been included for the three approaches is summarized in Table 3. The traditional approach encompasses the lowest figure (1,337 total tests performed), with 995 IIF tests plus 342 CTD Reflex panels, while the combined show the highest number of 2,172 tests (995 IIF plus 995 CTD Screen tests plus 182 CTD Reflex panels).

Accordingly, the latter approach identifies the highest number of patients with at least a defined antibody positivity (Table 4, top panel), with a diagnostic gain of 23.8 % vs. the traditional approach and 38.1 % vs. the progressive one. Although the lowest number of tests, the traditional approach also involves a highest cost per each "diagnosis" (658 €, Table 4, bottom panel), while the lowest is for progressive (351€).

Discussion

In this study we analysed the potential gain of combining IIF and SPA for any laboratory request of ANA testing both for antibody findings and economic advantages. In this unselected cohort of 995 outpatients, the agreement between IIF and SPA occurred overall in 71.3 % requests (112 both positive and 597 both negative), while for 257 requests (25.8 %) the results were conflicting and 29 (3 %) had a SPA borderline result, of which 14 were IIF positive and 15 negative; thus, in almost one third of requests a single method would not be enough to guarantee an accurate autoantibody result.

Table 2: Number of samples positive for single antibody specificity in subgroup of conflicting results between the two methods; IIF patterns of conflicting results ANA-IIF+ and specific concentrations of conflicting results CTD Screen borderline (≥0.7 and <1) or + (≥1).

	CTD Screen borderline/ANA-IIF+		CTD Screen borderline/ANA-IIF−		CTD Screen−/ANA-IIF+		CTD Screen+/ANA-IIF−	
	IIF pattern (n.)		Specific concentrations		IIF pattern (n.)		Specific concentrations	
ds-DNA	4	AC-1 (2); AC-4 (2)	6	10–18 kU/L	2	AC-4 (1); AC-5 (1)	14	10–101 kU/L
Sm			1	16 kU/L				
Rib-P	1	AC-4 (1)						
PCNA								
RNP (70 kDa, A, C)					3	AC-1 (3)	4	6–43 kU/L
SS-A/Ro60			2	24 kU/L	1	AC-4	5	7–24 kU/L
SS-A/Ro52	1	AC-4 (1)					6	7–65 kU/L
SS-B/La					1			
Scl-70					2	AC-1 (2)	1	7 kU/L
CENP-B							2	15 kU/L
Fibrillarin	1	AC-9	1	11 kU/L	1	AC-9	1	7 kU/L
RNA Polymerase III							2	7–9 kU/L
Jo-1							1	75 kU/L
PM/Scl					3	AC-1 (3)		
Mi2								
Total positive	7		10		13		33[a] (patients)	

[a]Number of total positive patients, as some samples had multiple antibody positivity.

Table 3: Number of tests that would have been performed for the three different diagnostic approaches and number of patients with a single defined antibody positivity that would have been diagnosed.

Diagnostic approach	No. of ANA-IIF	No. of CTD Screen	No. of CTD reflex	No. of patients with antibody positivity
Traditional	995	0	342	126
Progressive	995	342	126	113
Combined	995	995	182	156

Table 4: Cost-effectiveness assessment.

Patients with single antibody positivity				
Approach	n	Progressive vs. traditional	Combined vs. traditional	Combined vs. progressive
Traditional	126	−10.3 %	23.8 %	38.1 %
Progressive	113			
Combined	156			
Tariff per each "diagnosis" based on single antibody positivity				
Approach		Progressive vs. traditional	Combined vs. traditional	Combined vs. progressive
Traditional	658 €	−46.7 %	−42.6 %	7.8 %
Progressive	351 €			
Combined	378 €			

Various studies have recently analysed clinical usefulness and diagnostic efficacy of SPA, and its agreement with the gold standard test, and different research groups have demonstrated the added value of the combination of both assays (IIF plus SPA) [7], though the algorithm for the combination of IIF and SPA testing for ANA screening should consider the specific AARD clinical suspect [17].

We observed a higher diagnostic accuracy when IIF and CTD Screen were combined, as previously suggested. The combination of the two assays has potentials benefits not only for the increased sensitivity and specificity of concordant results (positive or negative), but also for conflicting results; in these cases a pragmatical approach would be a stepwise algorithm with progressive specific testing based on the fluorescence pattern on HEp-2 following the ICAP (Figures 1–3), to be applied differently by each laboratory based on the available CTD Screen assay, due to different antigen composition for each manufacturer. Moreover, even the analytical method used to confirm the specific antibody positivity might change the workflow of each laboratory, as showed by Infantino et al. that evaluated a new diagnostic algorithm based on customized immunoblot SPA that grouped most of the antigens related to a specific IIF pattern, which resulted in a higher number of AARD [18]. The simplest approach is for double positivity on both assays: next step after IIF and CTD Screen would be to identify the antibody specificity with SPA for anti-ENA based on IIF pattern and anti-dsDNA with CLIFT or other quantitative or semiquantitative specific immunoassays (e.g. immunoblotting) (Figure 1).

If ANA-IIF+/CTD Screen−: we suggest confirming IIF positivity with a different SPA method (e.g. ELISA, immunoblotting) according to fluorescence pattern as seen in

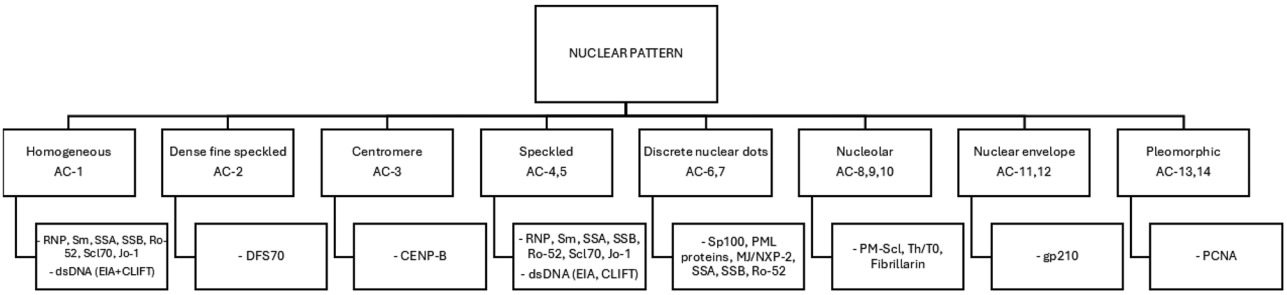

Figure 1: ANA-IIF+/CTD Screen+ confirm algorithm to identify the antibody specificity based on IIF pattern with SPA; anti-dsDNA should also be tested with CLIFT. Modified from ICAP https://www.anapatterns.org/trees-full.php. EIA, enzyme immuno assay; CLIFT, crithidia luciliae immunofluorescence test.

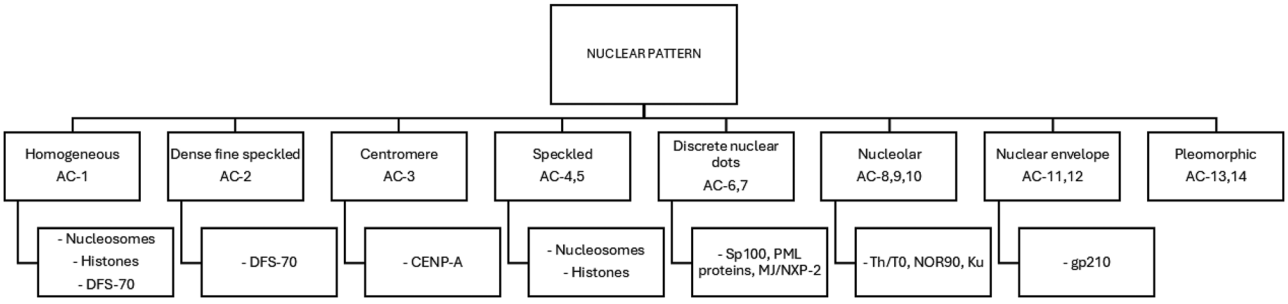

Figure 2: ANA-IIF+/CTD Screen− confirm algorithm to identify a potential specific antibody towards antigens not included in the CTD Screen with other SPA. Modified from ICAP https://www.anapatterns.org/trees-full.php. EIA, enzyme immuno assay; CLIFT, crithidia luciliae immunofluorescence test.

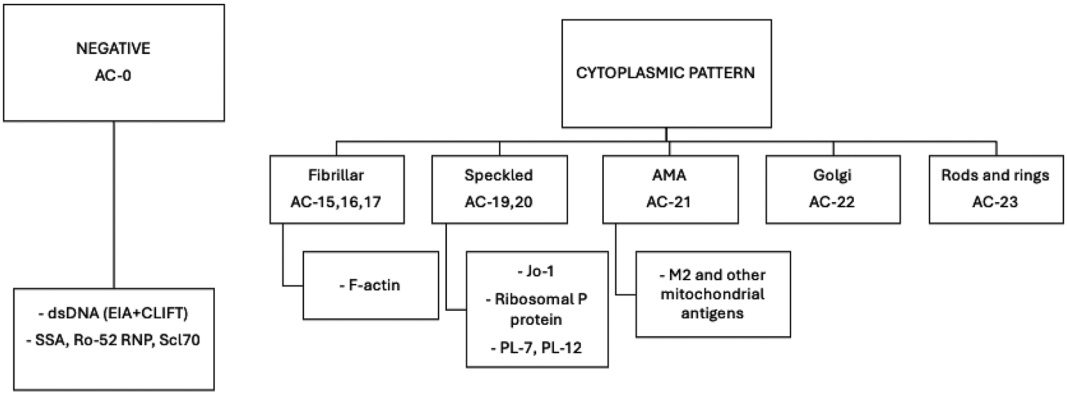

Figure 3: ANA-IIF−/CTD Screen+ confirm algorithm for anti-dsDNA and other SPA for cytoplasmic pattern. Modified from ICAP https://www.anapatterns.org/trees-full.php. EIA, enzyme immuno assay; CLIFT, crithidia luciliae immunofluorescence test; SMA, smooth muscle antibody.

Figure 2, in order to identify a potential specific autoantibody towards other antigens not included in the CTD Screen.

If ANA-IIF−/CTD Screen+: consider confirmatory testing for anti-dsDNA, quantitative determination of anti-ENA and evaluation of possible cytoplasmic pattern with other SPA confirmatory testing (Figure 3).

However, it should be mentioned in the limitations of this study that cytoplasmic positive patterns in IIF without nuclear staining were considered negative, while the updated ICAP consensus encompasses different of such patterns. Given the low probability of developing an autoimmune disease in patients with an isolated DFS70 positivity [10], some authors demonstrated that including DFS70 testing can reduce unnecessary costs of clinical follow-up of these patients [19]; however, the higher costs calculated in our study suggest, that even in this case, a wise approach should be to test only when ANA-IIF pattern is morphologically suggestive of DFS70 positivity. This, in fact, would

ensure a reduction of not-appropriated costs. Anti-dsDNA was the most frequent single antibody positivity found in our study for the conflicting results between IIF and SPA, as 20 patients had this specific antibody in FEIA while ANA IIF negative. This is not surprising, as HEp2 IIF is not useful to detect anti-dsDNA, whose reference method is still today considered the Farr assay (i.e. radioimmunoassay method), which, however, cannot be widely adopted in clinical practice due to its complexity. Valid alternatives are Crithidia luciliae immunofluorescence test (CLIFT), but with low sensitivity, and the SPAs (FEIA, CLIA, ELISA and multiplex assay), which are less specific, however with variable sensitivity, mostly because of a different antigenic source (i.e. plasmide derived for FEIA, synthetic, recombinant or purifies for the others) [20]. Rojo recommended, for anti-dsDNA testing, the double screening strategy, starting with a last generation SPA, and subsequently, the CLIFT as the confirmation test [20]. This sequential approach minimizes false positive results obtained with the SPA, however it should be reserved to specific requests with high pre-test probability being too time-consuming and costly for initial screening requests.

When considering the entire cohort, 3.3 % diagnosis of antibody positivity in ANA-IIF−/CTD Screen+ and 1.3 % diagnosis in ANA-IIF+/CTD Screen− would have been made with the combined approach, which could be considered a potential diagnostic gain with respect to the traditional and the progressive approaches. This potential diagnostic gain is higher than what reported by a recent article, where in a large cohort of 58,627 patients, the rate of ANA-IIF− and anti-ENA positive findings with subsequent diagnosis of autoimmune connective tissue disease was calculated in 0.37 % only [21]. Moreover, Robier et al. found that ANA-IIF−/CTD Screen+ were infrequently observed and mostly did not contribute to the diagnosis (with the exception of dsDNA and SSA/Ro) [17]. Accordingly, the positive predictive value for AARD of anti-ENA positive finding in 1,728 ANA IIF negative patients was calculated in 6.09 % by other authors in a retrospective multicenter study [22], concluding that concurrent anti-ENA testing in this setting has a limited diagnostic yield. The lack of clinical data in our study, which represents the main limitation of this work, does not allow to compare the positive predictive value, which is a relevant issue when considering the high sensitivity of the current analytical technologies.

In their work, through a Health Economic analysis Ethington et al. demonstrated relevant cost savings when strictly applying a progressive approach of ANA-IIF testing followed by anti-ENA testing only in ANA-IIF+ [21], reducing cost of $645,741.60 for unnecessary anti-ENA testing in ANA-IIF− patients that turned out to be negative. In our study the cost for a single antibody positivity would have resulted in 378 € with the combined approach, for a total cost of 58,992 €, and 351 € with the progressive approach, corresponding to a total cost of 39,645 €. This relevant cost gap is, however, balanced by a plus 38.1 % of specific antibody findings with the combined approach, that might lead to a timely diagnosis.

As the cost assessment in this study considers the Italian context, and might change in other countries, each clinical laboratory should carefully evaluate its diagnostic algorithm for ANA testing on the volume and type of requests (e.g. rheumatologic requests vs. general practitioners) and its own economic model, to decide whether to prefer cost savings or case finding.

The combined approach of two different methods ensures the highest reliability, while with the progressive approach the exclusion of negative patients relies only on IIF testing, that might suffer from different analytical variables. However, the most important strategy to optimize cost-effectiveness of ANA screening test would be a high pre-test probability, as Otten HG et al. in their analysis of over 1,000 requests for ANA screening found that 83 % had no fitting clinical signs or symptoms, corresponding to an increase of about 6-fold of costs for single SPA analytes without diagnostic gain [23].

The clinical autoimmunology laboratories have nowadays a wide range of possible methods to fulfill both systemic and organ-specific autoantibodies requests, thus each laboratory should define its own simplified workflow that maximize the diagnostic power and minimize the costs, avoiding useless testing and time wasting respecting the five rights of Laboratory Medicine [24], and overall achieving a high level of efficiency, along with assuring patient safety. The analysis of patient outcomes related to costs is pivotal in the current concept of value-based laboratory medicine [25], where a higher expense in diagnostic services might often turn into reduced overall clinical management costs [26], particularly when a diagnosis of a chronic rheumatic disease is early established before the onset of serious and costly conditions. Finally, thorough economic assessment should also be applied to the autoimmunology specialist section leading to new cost-effective reimbursement models [26].

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References

1. Pérez D, Gilburd B, Azoulay D, Shovman O, Bizzaro N, Shoenfeld Y. Antinuclear antibodies: is the indirect immunofluorescence still the gold standard or should be replaced by solid phase assays? Autoimmun Rev 2018;17:548–52.
2. Bizzaro N. Can solid-phase assays replace immunofluorescence for ANA screening? Ann Rheum Dis 2020;79:e32.
3. Infantino M, Manfredi M, Soda P, Merone M, Afeltra A, Rigon A. ANA testing in 'real life'. Ann Rheum Dis 2020;79:e3.
4. Bonroy C, Vercammen M, Fierz W, Andrade LE, Van Hoovels L, Infantino M, et al. Detection of antinuclear antibodies: recommendations from EFLM, EASI and ICAP. Clin Chem Lab Med 2023;61:1167–98.
5. Aringer, Costenbader, K, Daikh, D, Brinks, R, Mosca, M, Ramsey-Goldman, R, et al., 2019 European league against rheumatism/ American College of rheumatology classification criteria for systemic lupus erythematosus. Arthritis Rheumatol 2020;71:1400–12.
6. Pisetsky DS, Spencer DM, Lipsky PE, Rovin BH. Assay variation in the detection of antinuclear antibodies in the sera of patients with established SLE. Ann Rheum Dis 2018;77:911–13.
7. Orme ME, Andalucia C, Sjölander S, Bossuyt X. A comparison of a fluorescence enzyme immunoassay versus indirect immunofluorescence for initial screening of connective tissue diseases: systematic literature review and meta-analysis of diagnostic test accuracy studies. Best Pract Res Clin Rheumatol 2018;32:521–34.
8. Damoiseaux J. The international consensus on ANA patterns (ICAP): from conception to implementation. Clin Chem Lab Med 2024;62:789–92.
9. Andrade LEC, Klotz W, Herold M, Musset L, Damoiseaux J, Infantino M, et al. Reflecting on a decade of the international consensus on ANA patterns (ICAP): accomplishments and challenges from the perspective of the 7th ICAP workshop. Autoimmun Rev 2024;23:103608.
10. Infantino M, Carbone T, Manfredi M, Grossi V, Benucci M, Casiano CA, et al. Dense fine speckled (DFS) immunofluorescence pattern and anti-DFS70 antibodies: cleaning up the current concepts. Clin Chim Acta 2020;510:157–9.
11. Bossuyt X, De Langhe E, Borghi MO, Meroni PL. Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. Nat Rev Rheumatol 2020;16:715–26.
12. Claessens J, Belmondo T, De Langhe E, Westhovens R, Poesen K, Hüe S, et al. Solid phase assays versus automated indirect immunofluorescence for detection of antinuclear antibodies. Autoimmun Rev 2018;17:533–40.
13. Bizzaro N, Brusca I, Previtali G, Alessio MG, Daves M, Platzgummer S, et al. The association of solid-phase assays to immunofluorescence increases the diagnostic accuracy for ANA screening in patients with autoimmune rheumatic diseases. Autoimmun Rev 2018;17:541–7.
14. Bossuyt X, Fieuws S. Detection of antinuclear antibodies: added value of solid phase assay? Ann Rheum Dis 2014;73:e10.
15. Bonaguri C, Melegari A, Ballabio A, Parmeggiani M, Russo A, Battistelli L, 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.
16. Tonutti E, Bizzaro N, Morozzi G, Radice A, Cinquanta L, Villalta D, et al. The ANA-reflex test as a model for improving clinical appropriateness in autoimmune diagnostics. Autoimmun Highlights 2016;7:9.
17. Robier C, Amouzadeh-Ghadikolai O, Stettin M, Reicht G. Comparison of the clinical utility of the Elia CTD Screen to indirect immunofluorescence on Hep-2 cells. Clin Chem Lab Med 2016;54: 1365–70.
18. Infantino M, Carbone T, Manfredi M, Grossi V, Antico A, Panozzo MP, et al. A new diagnostic algorithm for pattern-oriented autoantibody testing according to the ICAP nomenclature: a pilot study. Autoimmun Rev 2020;19:102588.
19. Moroni L, Restovic G, Cervera R, Espinosa G, Viñas O, García M, et al. Economic analysis of the use of anti-DFS70 antibody test in patients with undifferentiated systemic autoimmune disease symptoms. J Rheumatol 2020;47:1275–84.
20. Rojo R, Calvo Alén J, Prada Á, Valor S, Roy G, López-Hoyos M, et al. Recommendations for the use of anti-dsDNA autoantibodies in the diagnosis and follow-up of systemic lupus erythematosus – a proposal from an expert panel. Autoimmun Rev 2023;22:103479.
21. Ethington E, Melrose E, Stratman EJ. The relative timing, outcomes, and economic impact of anti-nuclear antibody (ANA) and extractable nuclear antigen (ENA) laboratory ordering. Clin Med Res 2024;22: 123–6.
22. Yeo AL, Ojaimi S, Le S, Leech M, Morand E. Frequency and clinical utility of antibodies to extractable nuclear antigen in the setting of a negative antinuclear antibody test. Arthritis Care Res (Hoboken) 2023;75: 1595–601.
23. Otten HG, Brummelhuis WJ, Fritsch-Stork R, Leavis HL, Wisse BW, van Laar JM, et al. Measurement of antinuclear antibodies and their fine specificities: time for a change in strategy? Clin Exp Rheumatol 2017;35:462–70.
24. Plebani M. Towards a new paradigm in laboratory medicine: the five rights. Clin Chem Lab Med 2016;54:1881–91.
25. Plebani M, Cadamuro J, Vermeersch P, Jovičić S, Ozben T, Trenti T, et al. A vision to the future: value-based laboratory medicine. Clin Chem Lab Med 2024;62:2373–87.
26. Trenti T, Petrini AM, Plebani M. New reimbursement models to promote better patient outcomes and overall value in laboratory medicine and healthcare. Clin Chem Lab Med 2024;62:1795–803.